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Sex-specific constitutive expression of the pre-neoplastic marker glutathione *S*-transferase, YfYf, in mouse liver

Lesley I. McLELLAN and John D. HAYES
University Department of Clinical Chemistry, The Royal Infirmary, Edinburgh EH3 9YW, Scotland, U.K.

Hepatic glutathione *S*-transferase isoenzyme content has been investigated in both sexes of three inbred strains of mice (DBA/2, C3H/He, C57BL6). A polypeptide (M_r 24 800), which is immunologically related to Yf purified from rat lung, was found to be expressed as a major form in all male mouse livers but represented only a minor enzyme form in female mouse liver. Glutathione *S*-transferases comprising subunits with molecular masses of 25800 (Ya) or 26400 (Yb) were present in males and females of the three strains under investigation. Cytosolic isoenzymes from all strains and sexes were purified to apparent homogeneity and no significant inter-strain differences in the properties of the individual forms were observed. In addition, no differences were detected in the microsomal glutathione *S*-transferase content of the different strains or sexes.

INTRODUCTION

The glutathione *S*-transferases (GST) catalyse the conjugation of reduced glutathione (GSH) with a large number of electrophilic metabolites (Boyland & Chasseaud, 1969; Mannervik, 1985). Multiple GST forms have been described in the rat (Habig *et al.*, 1974; Hayes, 1984, 1986; Kitahara *et al.*, 1984; Tu & Reddy, 1985; Guthenberg *et al.*, 1985), the mouse (Lee *et al.*, 1981; Pearson *et al.*, 1983; Warholm *et al.*, 1986; Hayes *et al.*, 1987) and man (Awasthi *et al.*, 1980; Stockman *et al.*, 1985). Both cytosolic and microsomal GST exist. The cytosolic GST each comprise two subunits and the multiple forms that exist arise from dimeric combinations of a limited number of subunits (Habig *et al.*, 1976; Mannervik & Jensson, 1982; Hayes & Chalmers, 1983; Hayes, 1984, 1986; Meyer *et al.*, 1984) that are thought to be the products of three gene families (Mannervik *et al.*, 1985; Li *et al.*, 1986; Rothkopf *et al.*, 1986). Rat cytosolic enzymes have been shown to be composed of Yf (M_r 24800), Yk (M_r 25000), Ya (M_r 25500), Yn (M_r 26000), Yb (M_r 26300) and Yc (M_r 27500) polypeptides (Hayes, 1986; Hayes & Mantle, 1986*b*). Microsomal GST is distinct in that it is composed of three identical polypeptides (M_r 17000) which have little or no similarity to cytosolic subunits (Morgenstern *et al.*, 1985).

Several biological mechanisms exist that control the expression of GST, but their significance is not understood. Differences in hepatic GST activity have been observed in male and female rats (Kaplowitz *et al.*, 1975; Hales & Neims, 1976; Igarashi *et al.*, 1985). Normal rat liver, from either male or female, contains significant levels of all subunits except Yf, but this subunit is found in high concentrations in hepatic pre-neoplastic nodules. Wide interest exists in the use of the Yf monomer as a tumour marker and it has variously been referred to as Yp (Kitahara *et al.*, 1984; Suguoka *et al*, 1985), P21 (Farber, 1984), or subunit 7 (Meyer *et al.*, 1985).

Cytosolic and microsomal GST from the mouse have been studied in several laboratories; the physical properties of the enzymes isolated by separate groups of workers differ (Lee *et al.*, 1981; Lee & McKinney, 1982; Pearson *et al.*, 1983; Morgenstern *et al.*, 1984; Warholm *et al.*, 1986: Hayes *et al.*, 1987). The reasons for the lack of consistency of results from different research groups are not clear, but they suggest that strain-specific variations in mouse GST might occur. In this context, Wheldrake *et al.* (1981) reported that inter-strain variations exist in mouse liver GST activity and, in recognition of this possibility, Warholm *et al.* (1986) proposed a mouse GST nomenclature that incorporates a strain designation. Alternatively, or in addition to inter-strain variation, sex-specific expression of GST isoenzymes could account for the apparently anomalous results reported for the mouse enzymes.

In the present study, we have investigated sex and strain differences associated with both cytosolic and microsomal GST in mouse liver. We describe the identification, purification and characterization of a GST which is present as the major enzyme form in the livers of male mice, but is essentially absent from the livers of female mice.

EXPERIMENTAL PROCEDURES

Animals

Male and female mice of strains DBA/2, C3H/He and C57BL6 were purchased from Bantin and Kingman, Hull, U.K. The mice were fed *ad libitum* until killed at 13 weeks old. Livers were stored at −85 °C for 1 month before use.

Chemicals

All chemicals were of analytical grade and readily available commercially.

Buffers

These were prepared at 20 °C and pH values were measured at the working temperature. Buffer A,

Abbreviations used: GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; PAGE, polyacrylamide-gel electrophoresis.

Table 1. Levels of GST activity in hepatic cytosol from different strains and sexes of mouse

All analyses were performed in duplicate at 37 °C.

Strain	Sex	Specific activity (μmol·min^{-1}·mg^{-1})			
		CDNB	DCNB	Ethacrynic acid	Cumene hydroperoxide
DBA/2	Male	14.1	0.10	0.12	1.04
	Female	9.7	0.12	0.05	1.30
C3H/He	Male	17.4	0.13	0.20	1.48
	Female	8.9	0.12	0.03	1.66
C57BL6	Male	12.8	0.13	0.14	1.12
	Female	7.1	0.10	0.04	1.50

20 mM-Tris/HCl, pH 7.8, containing 200 mM-NaCl; buffer B, 150 mM-Tris/HCl, pH 8.0; buffer C, 12 mM-potassium phosphate, pH 7.0, containing 250 mM-sucrose, 1 mM-GSH and 0.1 mM-EDTA; buffer D, 10 mM-sodium phosphate buffer, pH 6.7, containing 2-mercaptoethanol (2 mM).

Analytical

Enzyme assays. All enzyme assays were carried out at 37 °C. Assays for cytosolic GST, with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate, were carried out using a Cobas Fara centrifugal analyser essentially as described by Hayes & Clarkson (1982). Assays for microsomal GST, with CDNB as substrate, were performed manually as described by Morgenstern *et al.* (1980). Assays with either 1,2-dichloro-4-nitrobenzene (DCNB), ethacrynic acid or *p*-nitrophenyl acetate as substrates were performed as described by Habig & Jakoby (1981). Selenium-independent glutathione peroxidase activity for organic hydroperoxide was determined by using the coupled assay system described by Reddy *et al.* (1981).

Electrophoresis and immunoblotting. The methods used have been described in previous publications from this laboratory (Hayes & Mantle, 1986*a*).

Preparation of hepatic cytosols and microsomes

Ten mouse livers (10–18 g) from each strain and sex were separately homogenized with 3 vol. of ice-cold buffer A. Liver homogenates were centrifuged at 10000 *g* (4 °C) for 20 min and the resulting supernatants were centrifuged at 100000 *g* (4 °C) for 60 min. The 100000 *g* supernatants were retained as the cytosolic fractions. In each case, the sediment that could easily be loosened by gentle agitation (using a rotary mixer) from the glycogen, which had formed a pellet at the bottom of the centrifuge tube, was retained as the microsomal fraction; the glycogen pellet was discarded. The microsomes were washed by re-suspending the protein sediment in approx. 10 vol. of ice-cold buffer B; the particulate material was harvested by centrifugation. The washing procedure was repeated and, following the third 100000 *g* (4 °C) centrifugation, the pellet was suspended in buffer C.

Enzyme purification

Cytosol preparations were dialysed at 4 °C for 18 h against two changes of 2 litres of butter A before being applied to columns of *S*-hexylglutathione–Sepharose 6B (1.6 cm × 12 cm), equilibrated at 4 °C with the same buffer. The affinity matrices were washed with buffer A until all non-specifically bound material had been removed. The eluate was monitored at 280 nm and, when A_{280} was < 0.02, the GST were eluted with a solution of 5 mM-*S*-hexylglutathione in buffer A. The GST-containing fractions were combined and the individual GST isoenzymes were resolved using hydroxyapatite h.p.l.c.; this was performed at 20 °C as described elsewhere (Hayes *et al.*, 1987).

Treatment of microsomes with *N*-ethylmaleimide

Activation of microsomal GST activity towards

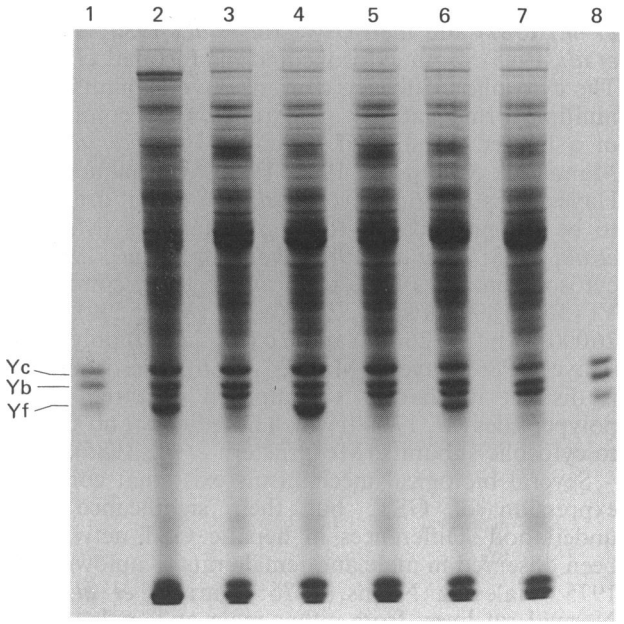

Fig. 1. Electrophoretic analysis of cytosol preparations from both sexes of three stains of mice

Cytosols from mouse livers (30 μg of protein each) were analysed by SDS/PAGE by the method of Laemmli (1970) using a 12% (w/v) resolving gel. The gel was loaded as follows: lanes 1 and 8, rat lung GST isoenzyme mixture [Yf (M_r 24800), Yb (M_r 26300) and Yc (M_r 27500)]; lane 2, male DBA/2 hepatic cytosol; lane 3, female DBA/2 hepatic cytosol; lane 4, male C3H/He hepatic cytosol; lane 5, female C3H/He hepatic cytosol; lane 6, male C57BL6 hepatic cytosol; lane 7, female C57BL6 hepatic cytosol.

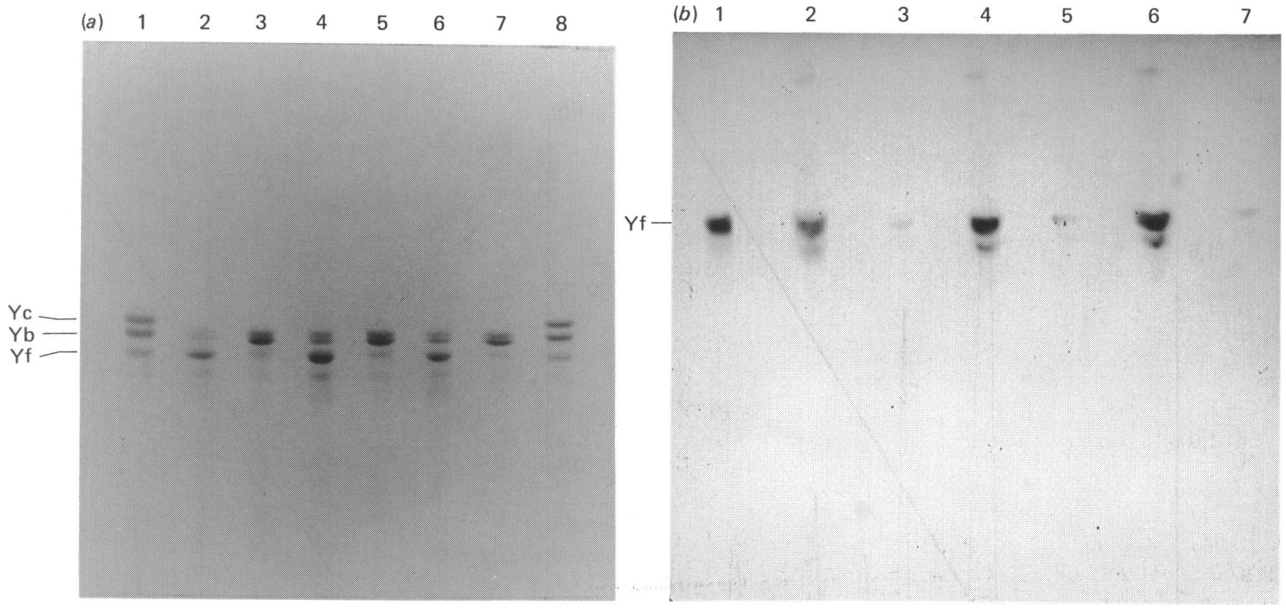

Fig. 2. SDS/PAGE and immunoblotting of purified hepatic isoenzyme mixtures from different strains and sexes of mouse

Mouse GST from liver cytosols of each strain and sex were purified by *S*-hexylglutathione–Sepharose 6B affinity chromatography and the subunit content was analysed by SDS/PAGE in 12% polyacrylamide resolving gels. The gel was loaded with 4 μg of GST in each lane as follows: lanes 1 and 8, rat lung GST (Yf, Yb and Yc subunits); lane 2, male DBA/2 GST; lane 3, female DBA/2 GST; lane 4, male C3H/He GST; lane 5, female C3H/He GST; lane 6, male C57BL6 GST; lane 7, female C57BL6 GST. Panel (*a*) shows the polyacrylamide gel stained with Coomassie Blue R250 for protein; panel (*b*) shows the immunoblot developed with anti-Yf IgG. Lane 8 of the gel was omitted from the immunoblot.

CDNB as substrate (Morgenstern *et al.*, 1980) was carried out by incubation of microsomes (2–3 mg of protein/ml) with 2 mM-*N*-ethylmaleimide for 2 min at room temperature (20 °C). Treatment was terminated by the addition of an equimolar amount of GSH and returning to 4 °C.

RESULTS

Table 1 shows the level of GST activity in the hepatic cytosols from male and female mice of the strains DBA/2, C3H/He and C57BL6 using a number of different substrates. In all strains specific activities towards both ethacrynic acid and CDNB as substrates are considerably higher in male mice than in females. GST activity for DCNB was similar in both sexes, whereas activity for cumene hydroperoxide was significantly higher in females than in males. The C3H/He strain appeared to possess greater GST activity for CDNB and ethacrynic acid than either DBA/2 or C57BL6 mice. Moreover, C3H/He mice exhibited a marginally higher glutathione peroxidase activity (using cumene hydroperoxide as a substrate) than the other mice studied.

Analysis of the hepatic cytosolic protein from both sexes of the three strains by SDS/PAGE demonstrated that all the male mice contain a polypeptide of M_r about 24800. This apparently male-specific polypeptide co-migrated during SDS/PAGE with the Yf GST subunit obtained from rat lung (Fig. 1).

The GST pools purified from the mouse livers by *S*-hexylglutathione–Sepharose 6B were analysed by SDS/PAGE and immunoblotting using antisera raised against rat cytosolic GST L (YaYa), GST A (Yb_1Yb_1) and GST H (YfYf). Fig. 2 demonstrates that, in all strains, only male mouse liver contained the Yf subunit in significant amounts. All strains and sexes contained similar levels of Ya and Yb subunits (results not shown).

Isoelectric focusing of the purified GST mixtures

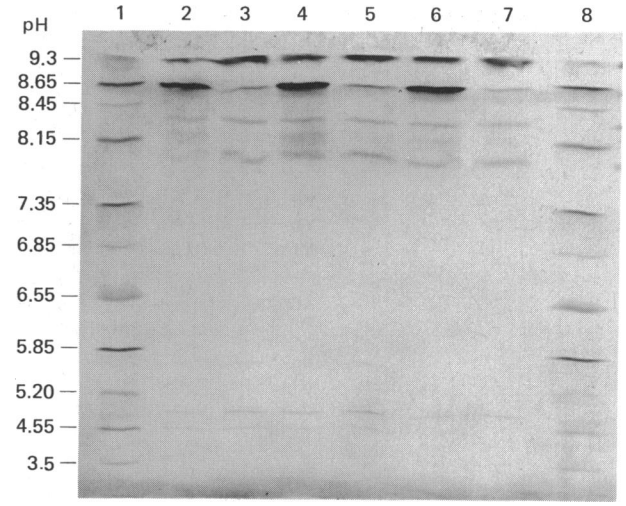

Fig. 3. Isoelectric focusing of purified hepatic GST from various strains and both sexes of mouse

Analytical isoelectric focusing with a pH gradient of 3–10 was performed in thin-layer 4.85% (w/v) polyacrylamide gel using a LKB Multiphor apparatus according to the manufacturer's instructions. The gel was loaded with 25 μg of purified GST mixtures from each sex and strain and protein pI markers. Loadings were as follows: lanes 1 and 8, marker proteins (pI values indicated); lane 2, male DBA/2 GST; lane 3, female DBA/2 GST; lane 4, male C3H/He GST; lane 5, female C3H/He GST; lane 6, male C57BL6 GST; lane 7, female C57BL6 GST.

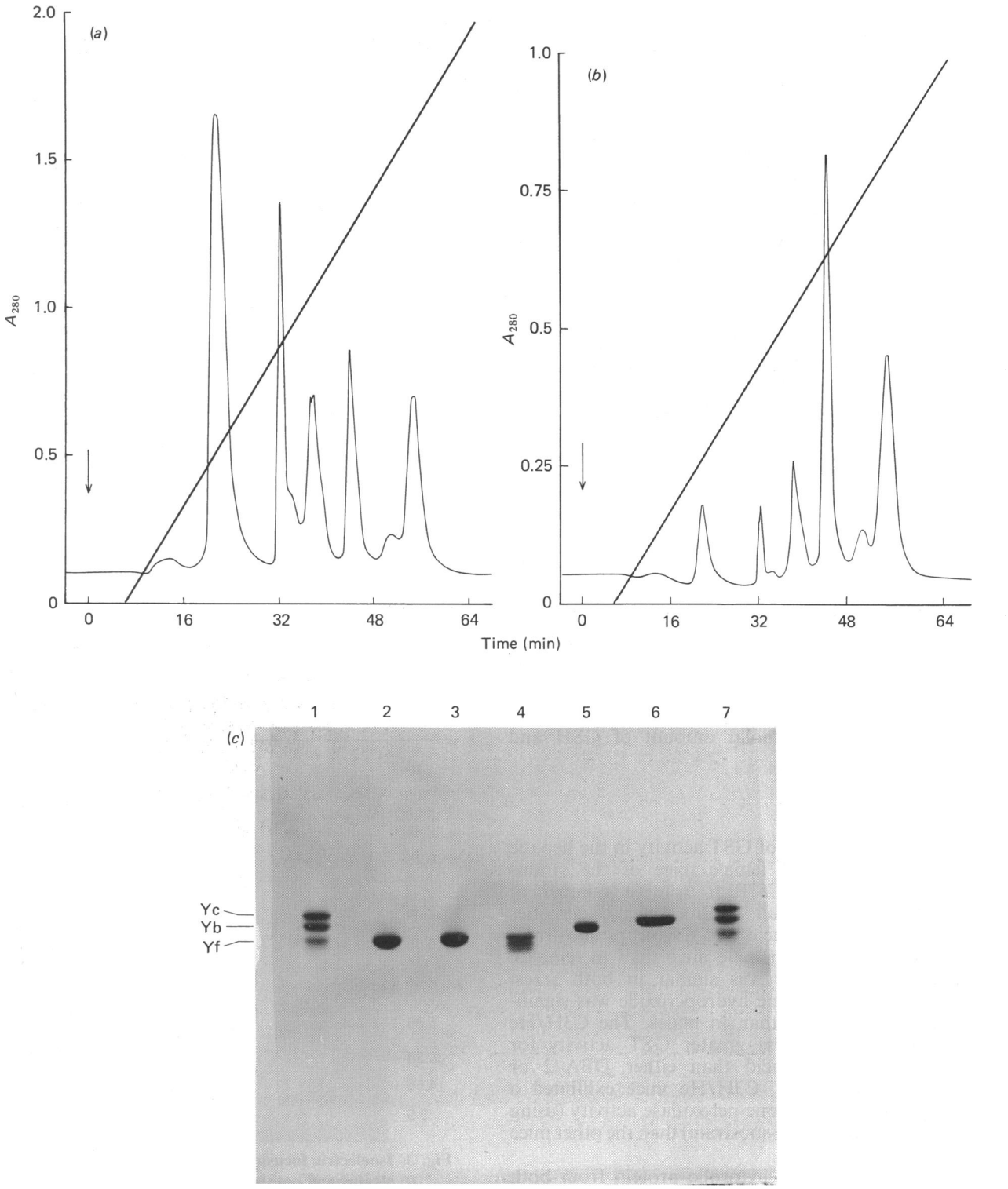

Fig. 4. Use of hydroxyapatite h.p.l.c. to resolve mouse liver GST from males and females of the strain C3H/He

Portions (2 ml; 6.4 mg and 2.4 mg of protein for male and female, respectively) of affinity-purified GST were dialysed against 10 mM-sodium phosphate buffer, pH 6.7, containing 2-mercaptoethanol (2 mM) before being subjected to chromatography on Bio-Gel HPHT. GST were eluted from the hydroxyapatite at 0.5 ml/min with a linear gradient of 10–350 mM-sodium phosphate with contained 2-mercaptoethanol (2 mM) and $CaCl_2$ (0.4 mM). Protein peaks were detected by monitoring absorbance of the eluate at 280 nm. The GST peaks that were eluted from 17 to 39 min were designated PIa, b and c, and those that were eluted at 45 and 55 min were named PII and PIII respectively. Panels (*a*) and (*b*) show elution profiles of hepatic GST from male and female C3H/He respectively. Panel (*c*) shows SDS/PAGE analyses of purified GST from male C3H/H3; lanes 1 and 7, rat lung GST markers (Yf, Yb and Yc subunits); lane 2, PIa; lane 3, PIb; lane 4, PIc; lane 5, PII; lane 6, PIII.

Table 2. Proportions of individual isoenzymes recovered from h.p.l.c.

Strain	Sex	Level of GST as a percentage of total cytosolic protein	Yields of GST subunit types from h.p.l.c. as a percentage of total protein recovered			Levels of Yf expressed as a percentage of Ya+Yb	Levels of Ya expressed as a percentage of Ya+Yb
			Yf	Ya	Yb		
DBA/2	Male	3.4	51	14	35	104	28
	Female	1.4	15	37	48	18	44
C3H/He	Male	4.0	70	10	20	228	34
	Female	1.8	22	35	43	29	45
C57BL6	Male	3.8	52	18	30	110	37
	Female	1.9	15	32	53	18	38

Table 3. Specific activities of the individual isoenzymes from both sexes of the strain C3H/He for a variety of substrates

Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; CH, cumene hydroperoxide; DCNB; 1,2-dichloro-4-nitrobenzene; BSP, bromosulphophthalein; EA, ethacrynic acid; pNPA, *p*-nitrophenyl acetate; ND, not detectable.

Sex	GST fraction	Specific activity (μmol·min^{-1}·mg^{-1})					
		CDNB	DCNB	EA	CH	BSP	pNPA
Male	Purified isoenzyme mixture applied to column	61.4	0.8	2.6	1.9	0.09	0.23
	PIa (Yf)	75.4	0.1	3.7	0.2	ND	0.26
	PIb (Yf)	85.2	ND	3.6	ND	ND	0.23
	PIc (Yf+Ye)	27.6	0.1	1.7	0.2	ND	0.06
	PII (Ya)	15.1	0.1	0.1	12.7	0.01	0.01
	PIII (Yb)	175.5	7.9	0.1	0.8	0.68	0.79
Female	Purified isoenzyme mixture applied to column	43.2	1.4	0.6	4.3	0.18	0.20
	PIa (Yf)	78.5	0.1	3.2	0.1	ND	0.24
	PIb (Yf)	77.9	ND	3.1	0.1	ND	0.24
	PIc (Yf+Ye)	9.1	0.1	1.2	0.4	ND	0.07
	PII (Ya)	12.2	0.1	0.1	13.9	0.01	ND
	PIII (Yb)	171.3	7.0	0.3	0.8	0.69	0.75
Coefficient of variation		6%	11.4%	8.5%	7.6%	13.5%*	13.7%*

* Coefficient of variation values are higher with BSP and pNPA than normally found in this laboratory owing to low specific activities of mouse GST for these substrates.

revealed the existence of a band with pI 8.7 that appeared to be a major protein in all male strains, but only weakly represented in females (Fig. 3). It is highly probable that this protein of pI 8.7 is the GST YfYf, since previous work in this laboratory has shown that GST YfYf from male LACA mice has a closely similar isoelectric point (Hayes *et al.*, 1987). The focusing gel also showed that all the GST bands from the three strains co-focused, indicating that all the GST in the DBA/2, C3H/He and C57BL6 strains possess similar isoelectric points.

To investigate sex-specific differences in mouse GST, the individual isoenzymes were prepared. This was achieved by using hydroxyapatite h.p.l.c. and resulted in the resolution of three subunit types, Yf, Ya and Yb. The Yf-containing GST was found to be the major enzyme in males of all strains investigated, but was only present as a minor form in females. Fig. 4 shows h.p.l.c. traces from hydroxyapatite chromatography of hepatic GST isoenzymes from males and females of the strain C3H/He. Peaks eluted between 17 and 39 min were named PIa–c (since they are closely related; see below); at 45 min, PII, and at 55 min, PIII. SDS/PAGE analysis was carried out on all peaks from all strains and sexes.

In every case, PIa–c comprised Yf subunits with a contaminating lower-M_r subunit in PIc, named Ye; Ye may represent glyoxalase I subunits. PII comprised Ya subunits and PIII Yb subunits. The retention time of

Table 4. GST activity of microsomes before and after treatment with N-ethylmaleimide

Strain	Sex	Activity with CDNB (μmol·min^{-1}·mg^{-1})		
		Untreated	Treated	Elevation of GST activity
DBA/2	Male	0.247	0.592	0.345
	Female	0.151	0.465	0.314
C3H/He	Male	0.210	0.432	0.222
	Female	0.131	0.341	0.210
C57BL6	Male	0.305	0.593	0.288
	Female	0.173	0.344	0.171

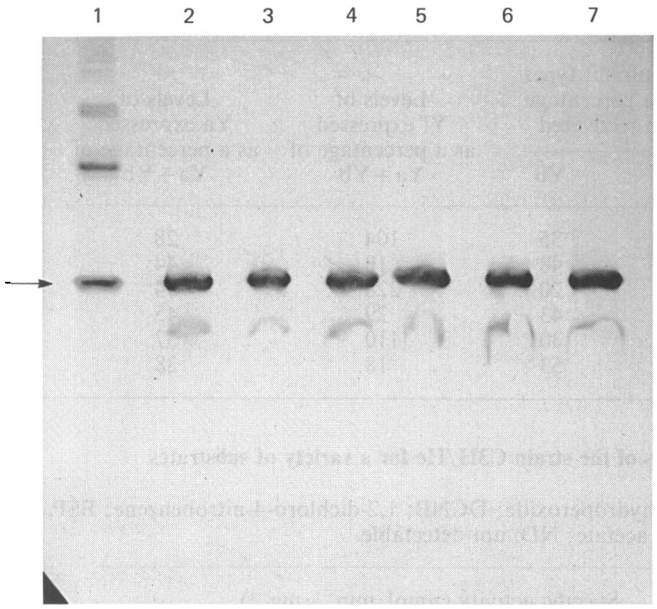

Fig. 5. Immunoblotting of microsomes from three strains of mouse and both sexes, with antisera raised to rat microsomal GST

Microsomes were prepared as described in the text and 150 μg of microsomal protein from each strain and sex was subjected to SDS/PAGE (Laemmli, 1970) using a 15% (w/v) polyacrylamide resolving gel before transfer to nitrocellulose paper. Immunoblotting was performed as described by Hayes & Mantle (1986*a*). The gel was loaded as follows; lane 1, purified rat microsomal GST; lane 2, male DBA/2 microsomes; lane 3, female DBA/2 microsomes; lane 4, male C3H/He microsomes; lane 5, female C3H/He microsomes; lane 6, male C57BL6 microsomes; lane 7, female C57BL6 microsomes. The arrow marks the position of rat microsomal GST.

GST YfYf varied between 17 and 39 min, but the variability was not thought to reflect any covalent differences in the YfYf forms that were eluted at various positions, since individual YfYf peaks appeared to interconvert when re-applied to hydroxyapatite. The retention times for mouse YaYa and YbYb were 45 and 55 min respectively in all cases. The relative yields from h.p.l.c. of the individual forms from all strains and sexes are shown in Table 2.

Specific activities of peaks from hydroxyapatite chromatography for all strains and sexes for a range of electrophilic substrates were determined. Within strains, peaks I, II and III all have distinct catalytic activities (Table 3). Peaks Ia and Ib are similar in specific activities, whereas Ic has significantly lower levels of activity, probably due to the presence of Ye that may represent contamination by glyoxalase I. Between strains and sexes, the catalytic properties of the individual peaks show only minor differences.

Microsomal GST of the three strains and sexes were examined for differences by treatment of microsomes with *N*-ethylmaleimide (Table 4) and immunoblotting with antisera raised against rat microsomal GST (Fig. 5). No significant differences were observed in the microsomal GST content between strains and sexes.

DISCUSSION

Cytosolic GST enzymes in the mouse have been studied in several laboratories (Lee *et al.*, 1981; Pearson *et al.*, 1983; Warholm *et al.*, 1986; Hayes *et al.*, 1987). Microsomal GST in mouse liver have also been studied (Lee & McKinney, 1982; Morgenstern *et al.*, 1984). Significant differences in the characteristics of individual isoenzymes have been reported by the various groups. It is not known whether the apparently discrepant results have a biological basis (sex or strain differences) or are due to methodological differences. To investigate the former possibility we have studied the hepatic GST in both sexes of three inbred strains of mice, DBA/2, C3H/He and C57BL6.

We have demonstrated a major difference in the content of the Yf GST subunit (M_r 24800) as between male and female livers. Analysis of cytosolic GST by a variety of techniques, including SDS/PAGE, immunoblotting and hydroxyapatite h.p.l.c., showed that Yf was conspicuous in males of all strains, but present in much smaller amounts in females. This finding accounts for the marked difference in the specific activities of hepatic cytosol from male and female mice (Table 1). In addition to the Yf subunit, polypeptides with molecular masses of 25800 (Ya) and 26400 (Yb) were identified in all strains and sexes. Female mouse livers contained a slightly greater concentration of Ya subunits than did male. This difference may also be reflected in the greater glutathione peroxidase II activity found in whole liver cytosols from females (Table 1). Minor differences were found in specific activities of cytosol between strains. Although variations in the comparative yields of GST isoenzymes were observed from the hydroxyapatite h.p.l.c. columns, no detectable difference was perceived in either subunit size, charge or catalytic properties between equivalent GST from different strains. Our data, therefore, suggest that the levels of GST subunits expressed in the liver vary from strain to strain, but that the properties are constant.

Comparisons between data from various laboratories are fraught with difficulty as differences in the standards used to calibrate analytical techniques give widely different results (Hayes & Mantle, 1986*b*). Moreover, it is not always possible to use a correction factor to identify unambiguously the GST forms described, since some purification schemes are not comprehensive and do not result in the isolation of all three subunit types. However, we shall now briefly review our data in relation to previous reports.

The individual forms resolved by hydroxyapatite h.p.l.c. in this study (PI, YfYf; PII, YaYa; PIII, YbYb) have subunit molecular masses of 24800, 25800 and 26400, respectively, determined using GST purified from rat lung as standards. The pI values for PI, PII and PIII of 8.6, $\geqslant$ 9.2 and 7.8–8.2, respectively, have been determined previously (Hayes *et al.*, 1987).

Warholm *et al.* (1986) used NMRI mice and characterized three distinct isoenzyme forms with subunit molecular mass and pI values in reasonable accord with those described from male strains of mice in this laboratory.

Pearson *et al.* (1983) characterized only two GST forms from CD-1 mice; these were designated according to their pI values as GT 8.7 and GT 9.3. Both were thought to be homodimers of equal size; a subunit M_r

of 24000 was calculated. Since these workers used female mice as the tissue source, it is unlikely that GT 8.7 or GT 9.3 represent Yf subunits. Indeed, the amino acid sequences deduced from cDNA encoding these polypeptides suggested that they both represent Yb-type subunits (Ding *et al.*, 1985; Mannervik, 1985). It is likely that GST PIII corresponds to GT 8.7, since Pearson *et al.* (1983) reported that GT 8.7 is expressed at much greater levels than GT 9.3 in the livers of normal, untreated, mice; the latter enzyme is only detected in significant amounts following treatment with *t*-butyl-4-hydroxyanisole.

Lee *et al.* (1981) described three major forms, FI, FII and FIII, from DBA/2 mice of which FI and FII were reported to be charge isomers (pI values 6.5 and 8.2) with subunit molecular masses of 22000, whereas FIII was reported to have a pI of 8.8 and a subunit molecular mass of 27000. It would seem that FI and FII correspond to GST comprising Yf-type subunits and FIII to the YbYb-type GST, judging by their order of elution from hydroxyapatite, mobility of subunits relative to each other on SDS/PAGE, and specific activity towards CDNB as a substrate. Neither Lee *et al.* (1981) nor Pearson *et al.* (1983) appear to have described a YaYa-type GST, but the fact that mouse YaYa has low specific activity towards CDNB as a substrate means that it could easily have escaped detection.

Initial attempts by Lee & McKinney (1982) to isolate a unique GST from the microsomal fraction of mouse liver were unsuccessful; these workers used male DBA/2J mice. Later, Morgenstern *et al.* (1984) described the presence of a specific microsomal GST that can be activated by *N*-ethylmaleimide treatment. Morgenstern and his colleagues used C57BL and NMR1 mice as tissue source. Our data show that DBA/2 as well as C3H/He and C57BL mice contain the microsomal *N*-ethylmaleimide-activatable GST. This enzyme has a polypeptide of M_r 17000 and is distinct from the cytosolic Ya-, Yb- and Yf-type subunits. The mouse therefore contains at least four separate families of GST.

It has previously been shown in the rat that livers from females contain higher levels of the Ya subunit than do those of males (Hales & Neims, 1976; Igarashi *et al.*, 1985). Likewise, in the mouse, we have found that livers from females contain marginally higher levels of the Ya subunit than do those of males (Table 2). Sex-specific regulation of the Yf subunit has not been described in rats. The physiological implication of sex-specific expression of Yf in the mouse is unclear, but is likely to be of pharmacological significance. Its existence in the livers of male mice is of particular interest as this subunit, which is not normally expressed in rat liver, is conspicuous in livers bearing pre-neoplastic foci and nodules, with particular localization in γ-glutamyltranspeptidase-positive foci (Kitahara *et al.*, 1984). Spontaneous hepatomas are known to occur with a much higher frequency in inbred male mice than in females (Smith *et al.*, 1973). Whether the presence of YfYf in male mouse liver is a consequence or cause of this phenomenon, or merely coincidental, remains to be determined.

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