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A Sex Difference in Hepatic Glutathione *S*-Transferase B and the Effect of Hypophysectomy

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The glutathione *S*-transferases are a group of proteins with overlapping substrate specificities and ligand-binding capacities. This report examines certain approaches to the measurement of transferase B (ligandin) in rat liver. The ratio of catalytic activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene gives some indication of the relative proportions of the various transferases present in 100000*g* supernatants. The fraction of catalytic activity towards 1-chloro-2,4-dinitrobenzene, due to transferase B, was best measured by immunoprecipitation with anti-(transferase B). Male rat liver exhibited three times more activity towards 1,2-dichloro-4-nitrobenzene than female tissue; however, the activities towards 1-chloro-2,4-dinitrobenzene were almost identical. By assuming a specific activity of 11 μmol/min per mg, immunoprecipitable transferase B comprised 4.5$\pm$0.2% of total protein in the 100000*g* supernatant of female rat liver, and 70% of the transferase activity towards 1-chloro-2,4-dinitrobenzene. The amount of transferase B in the 100000*g* supernatant from male rat liver is significantly lower with respect to both fraction of total protein (3.3$\pm$0.2%) and overall transferase activity towards 1-chloro-2,4-dinitrobenzene (48%). Hypophysectomy eliminated this sex difference in the hepatic concentration of glutathione *S*-transferase B.

Levi *et al.* (1969) have emphasized the potential physiological significance in hepatic function of a group of ligand-binding proteins in the 100000*g* supernatant fraction of liver. These proteins, which were observed to interact with bilirubin, sulphobromophthalein and Indocyanine Green, were distinguished from each other by gel filtration. Most attention has been given to the fraction (Y) that is eluted in the molecular-weight region of 45000. The ligand-binding component of this fraction, Y protein, has been reported to comprise 2–5% of hepatic cytosol protein. Chemical, physical and immunological observations later suggested the identity of Y protein with cortisol metabolite binder I (Morey & Litwack, 1969) and an azocarcinogen binder (Ketterer *et al.*, 1967); the protein was named ligandin (Litwack *et al.*, 1971).

In 1974, it was recognized that ligandin also exhibited glutathione *S*-transferase activity (Habig *et al.*, 1974*b*). Glutathione *S*-transferases (EC 2.5.1.18) catalyse nucleophilic attack by the thiol group of glutathione on xenobiotics such as epoxides, halogenonitrobenzenes and sulphobromophthalein. The various glutathione *S*-transferases are lettered according to the reverse order of their elution from a CM-cellulose column (Habig *et al.*, 1974*a*). The transferases that have been purified to homogeneity (AA, A, B, C, E) are all of the same molecular weight, all have overlapping substrate specificities, and all bind non-substrate ligands (Fjellstedt *et al.*, 1973; Habig *et al.*, 1974*a*; Pabst *et al.*, 1974; Ketley *et al.*, 1975). Two of this family of transferases, A and C, are immunologically indistinguishable. Ligandin has been identified as glutathione *S*-transferase B, the predominant glutathione *S*-transferase in liver (Habig *et al.*, 1974*b*). Measurements of either glutathione *S*-transferase activity or ligand-binding capacity reflect the cumulative activity of each of these related proteins.

The conjugation with glutathione of 1-chloro-2,4-dinitrobenzene proceeds rapidly in the presence of glutathione *S*-transferase B (specific activity 11 μmol/min per mg). The turnover rate with 1,2-dichloro-4-nitrobenzene as substrate is substantially slower (0.003 μmol/min per mg). Although absolute turnover rates vary widely among the other transferases, none is as discriminatory between these two substrates as is glutathione *S*-transferase B. The ratio of rates for these substrates thus might provide some indication of transferase B concentration relative to the other transferases. A second approach that might also prove useful in this regard involves chromatography on CM-cellulose columns. The third, and possibly most definitive method for

quantification of transferase B is immunological. This report examines these three approaches to the measurement of hepatic glutathione *S*-transferase in male and female rats and the effect of hypophysectomy thereon. Sex differences have been observed in hepatic transferase activity toward certain substrates (Darby & Grundy, 1972; Kaplowitz *et al.*, 1975), but ligandin concentrations have been reported to be the same in male and female rats (Reyes *et al.*, 1971). Hypophysectomy has been noted to increase the concentration of ligandin in male rat liver (Reyes *et al.*, 1971).

Experimental

Animals

Male and female Sprague–Dawley rats were hypophysectomized surgically by the Canadian Breeding Laboratory (St. Constant, Quebec, Canada) at the age of 40 days. They were maintained on 5% (w/v) dextrose for 14 days and then on Purina lab chow *ad libitum*. These animals, as well as normal controls, were killed by decapitation at the age of 60 days. Excised livers were homogenized (1:3, w/v) in ice-cold 1.15% (w/v) KCl containing 0.3M-sodium phosphate buffer, pH7.4. The homogenates were centrifuged at 10000*g* for 15min, and the resulting supernatants were stored at −20°C after freezing with liquid N_2. Before enzyme assay, the 10000*g* supernatants were thawed and centrifuged at 105000*g* for 60min. The '100000*g*' supernatants were used for all assays.

Enzyme assays

Assay conditions for measurement of the conjugation of 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene (both from Eastman Kodak Co., Rochester, NY, U.S.A.) were identical with those of Habig *et al.* (1974*a*). The reactions were followed on a Beckman Acta III double-beam recording spectrophotometer. Enzyme activities are expressed in μmol of substrate conjugated/min per mg of protein in the 100000*g* supernatant. Storage at −20°C for several months has no effect on catalytic activity.

Protein concentration was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Purification of glutathione S-transferase B

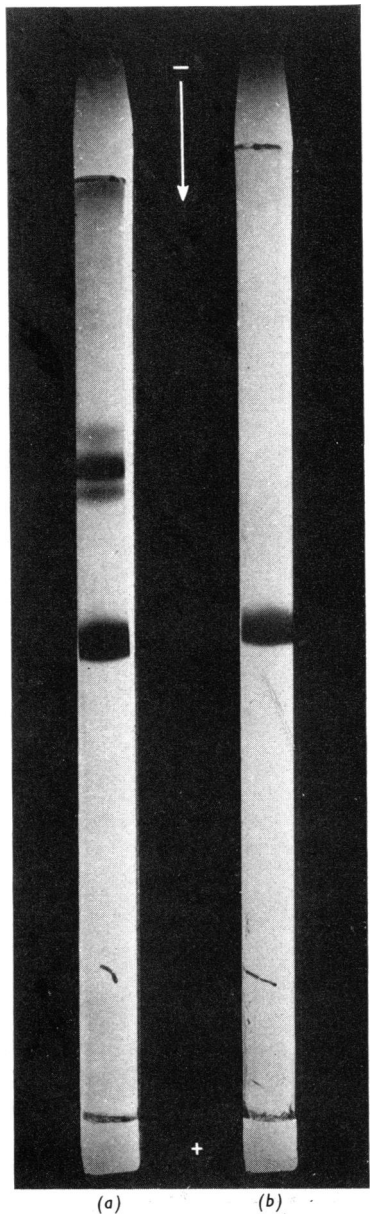
Glutathione *S*-transferase B was purified by the procedure of Habig *et al.* (1974*a*) with some modifications. Livers were excised from five male Sprague–Dawley rats (200–225g) under diethyl ether anaesthesia. All subsequent steps were performed at 4°C. Hepatic tissue (50g) was homogenized for 1min in a Waring Blendor in 150ml of 10mM-Tris/HCl, pH8.0, and the homogenate was centrifuged for 30min at 10000*g*. The supernatant was collected and re-centrifuged at 105000*g* for 60min to yield '100000*g*' supernatant.

This 100000*g* supernatant (110ml) was applied to a column (2.5cm×100cm) of DEAE-cellulose (Whatman DE-52) previously equilibrated with 10mM-Tris/HCl, pH8.0. The column was eluted with the same buffer, and 7.5ml fractions were collected until the eluate was devoid of transferase activity towards 1-chloro-2,4-dinitrobenzene. Fractions 26 (the first fraction with activity and protein)–80 were pooled and concentrated to 100ml by ultrafiltration of solvent through a Diaflo PM-10 membrane. The concentrated solution was dialysed for 2 days against four changes of a total volume of 4 litres of 10mM-potassium phosphate buffer, pH6.7, containing 30% (v/v) glycerol (buffer A).

The dialysed preparation was applied to a column (2.6cm×40cm) of CM-cellulose (Whatman CM-52) equilibrated with buffer A. After elution with 220ml of buffer A, a 2-litre linear salt gradient composed of 1 litre of buffer A and 1 litre of buffer A containing 75mM-KCl was initiated; fractions (7.5ml) were collected. The five expected peaks of transferase activity were eluted from the column and were designated alphabetically following the nomenclature of Habig *et al.* (1974*a*).

Fractions 154–173 (transferase B) from the CM-cellulose column were pooled and concentrated to 12ml by ultrafiltration of solvent through a Diaflo PM-10 membrane. The concentrated material was dialysed against 1 litre of 10mM-potassium phosphate buffer, pH6.7, containing 30% glycerol, 2mM-GSH (reduced glutathione) and 0.1mM-EDTA (buffer B). This solution was applied to a column (0.9cm×30cm) of hydroxyapatite (Bio-Rad Bio-Gel HT) equilibrated with buffer B. After washing with 12ml of buffer B, the column was developed with a 200ml linear gradient of 10–200mM-potassium phosphate buffer, pH6.7, containing 30% (v/v) glycerol, 2mM-GSH and 0.1mM-EDTA; fractions (3.5ml) were collected. Only fractions 24–27 contained conjugating activity towards 1-chloro-2,4-dinitrobenzene. These fractions were pooled, concentrated and dialysed as above, and the hydroxyapatite chromatography was repeated.

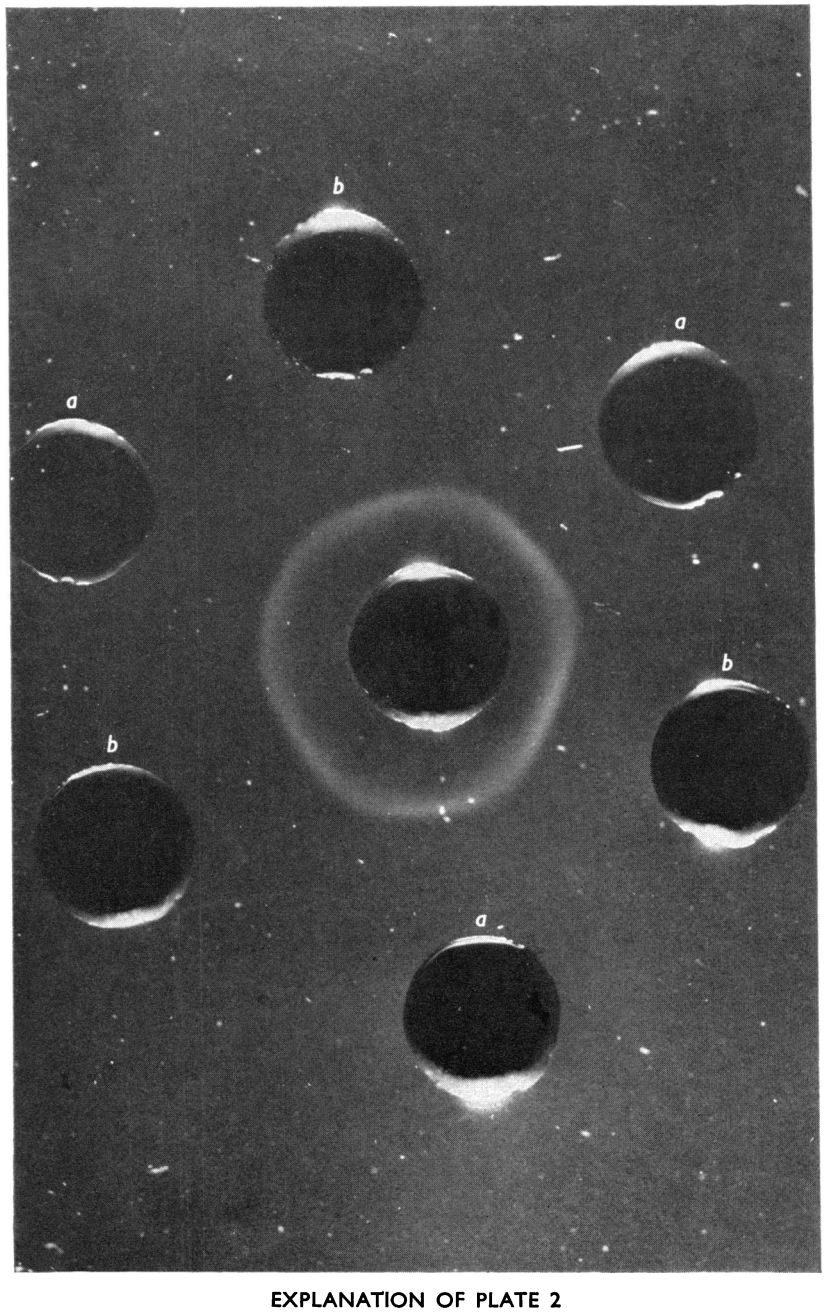
Fractions 23–26 from the second hydroxyapatite column were pooled, concentrated to 10ml and dialysed against 1 litre of 10mM-sodium phosphate buffer, pH7.4. The dialysed solution was applied to a column (2.5cm×40cm) of Sephadex G-75 equilibrated and developed with the same buffer; fractions (3.5ml) were collected. The catalytic activity toward 1-chloro-2,4-dinitrobenzene was eluted between fractions 25 and 31. These fractions were pooled, concentrated by dialysis against crystalline sucrose, and dialysed for 1 day against 1 litre of buffer B before storage at −20°C.



EXPLANATION OF PLATE 1

Sodium dodecyl sulphate/polyacrylamide gel electrophoresis of glutathione S-transferase B

Electrophoretic patterns on 10% (w/v) acrylamide gels of (*a*) transferase B fraction after CM-cellulose chromatography, and (*b*) purified transferase B after elution from Sephadex G-75.



EXPLANATION OF PLATE 2

Immunodiffusion pattern of anti-(transferase B) with glutathione S-transferase B and 100000 *g supernatant from male rat liver*

The Ouchterlony plate contained 1% (w/v) agar and 0.1% (w/v) NaN_3 in 0.9% NaCl. Antiserum (25 μl) was placed in the centre well, and outside wells contained alternately 100000*g* supernatant fraction (*a*) or transferase B (*b*) from liver of male rats.

Gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed as described by Weber & Osborn (1969). Lysozyme (mol.wt. 14300), myoglobin (17200), chymotrypsinogen (25700) and ovalbumin (43000) were used as standard molecular-weight marker proteins. Gel electrofocusing was performed as described by Wrigley (1972) by using 67% ampholytes, pH range 8–10 (Bio-Rad Laboratories) and 33% ampholytes, pH range 3–10.

Antibody production

One young male New Zealand white rabbit received a total of 0.8 mg of purified transferase B over a period of 3 months. The initial injection contained 100 μg of antigen mixed with complete Freund's adjuvant and was administered in several intramuscular sites. Booster injections were administered both intramuscularly and intravenously. When immunodiffusion indicated the presence of antiserum to transferase B, the rabbit was bled from the marginal ear vein. The anti-(transferase B) thus obtained was stored in 2–3 ml batches at −20°C.

Immunotitration of enzyme activity

The precipitin curves with antiserum prepared to transferase B were obtained by incubating antigen (either purified transferase B or 100000*g* hepatic supernatant) with increasing amounts of anti-(transferase B) overnight in 0.1 M-potassium phosphate/0.9% NaCl, pH 7.4, in a total volume of 300 μl at 4°C. At 4h after the addition of 30 μl of 22% (w/v) polyethylene glycol 6000 (Union Carbide Corp., New York, NY, U.S.A.) in phosphate-buffered saline, pH 7.4, the precipitate was removed by centrifugation at 5000*g* for 30 min. The supernatant was assayed for glutathione-conjugating activity towards 1-chloro-2,4-dinitrobenzene. The precipitin curves have been corrected both for the minimum catalytic rate observed in the presence of control serum, and for the consistent loss of enzymic activity (17–20%) observed when purified transferase B or any of the 100000*g* supernatants were incubated with control serum from a non-immunized rabbit.

Results

Properties of purified transferase B and antiserum

Transferase B was eluted as a single symmetrical peak from a Sephadex G-75 column. On electrophoresis on 10% (w/v) acrylamide gels in the presence of sodium dodecyl sulphate, the preparation exhibited homogeneity; a single protein band with a molecular weight of about 25000 was found (Plate 1). On polyacrylamide-gel isoelectric focusing, one band with 1-chloro-2,4-dinitrobenzene-conjugating activity was observed (Fig. 1). The specific activities obtained for this preparation of transferase B with 1-chloro-2,4-dinitrobenzene (11.82 μmol/min per mg), 1,2-dichloro-4-nitrobenzene (0.005 μmol/min per mg) and ethacrynic acid {[2,3-dichloro-4-(2-methylenebutyryl)phenoxy]acetic acid; Merck, Sharp and Dohme, Kirkland, Quebec, Canada} (0.264 μmol/min per mg) were similar to those reported by Habig *et al.* (1974*a*).

Rabbit antiserum to transferase B [anti-(transferase B)] generated a single line of precipitin with 100000*g* liver supernatant when examined by Ouchterlony immunodiffusion (Munoz, 1971). When purified transferase B and 100000*g* hepatic supernatant were placed in alternate wells around the antiserum, the line of precipitin fused (Plate 2).

Immunotitration

Antiserum had no inhibitory effect on transferase B activity when added just before the enzyme assay. However, when conditions allowed for immunoprecipitation, anti-(transferase B) completely eliminated the catalytic activity of purified transferase B (Fig. 2*a*); 100 μl of antiserum immunoprecipitated approx. 10 nmol of activity toward 1-chloro-2,4-dinitrobenzene/min. The antiserum precipitated about 50% of the conjugating activity of 100000*g* supernatant of male rat liver toward 1-chloro-2,4-dinitrobenzene. On the basis of a specific activity for transferase B of 11 μmol/min per mg, this transferase can be calculated to comprise 3.3±0.2% of the protein in the 100000*g* supernatant from male rat liver. This is consistent with the percentage of ligandin reported in 100000*g* supernatant from liver (Litwack *et al.*, 1971).

A typical elution profile from a CM-cellulose column of the various transferase activities toward 1-chloro-2,4-dinitrobenzene is shown in Fig. 3.

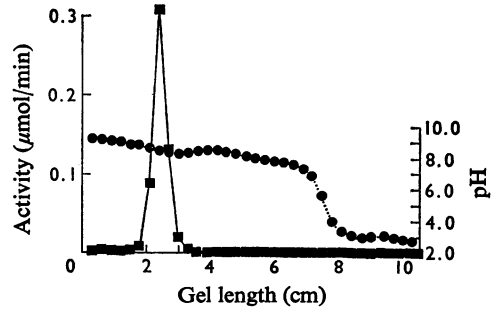


Fig. 1. *Gel isoelectric focusing of purified glutathione S-transferase B*

Conjugating activity towards 1-chloro-2,4-dinitrobenzene was eluted into 500 μl of 0.1 M-potassium phosphate, pH 6.5, from 0.3 mm gel slices after focusing. ■, Activity; ●, pH. Isoelectric focusing and assay for catalytic activity are described in the text.

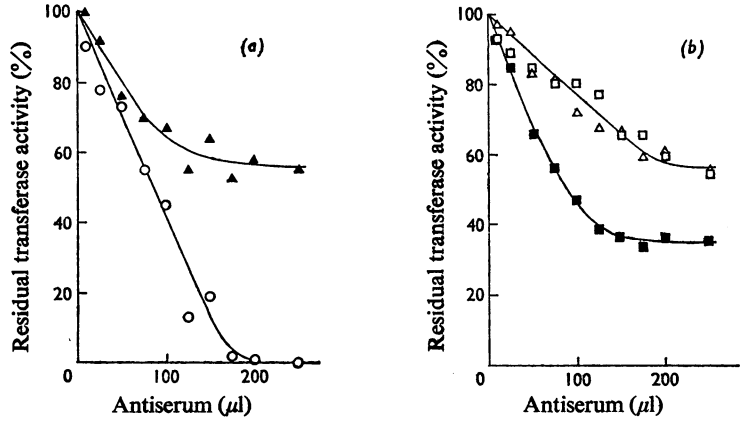


Fig. 2. *Immunotitration of (a) purified glutathione S-transferase B and 100000g supernatant from male rat liver or (b) 100000g supernatants from livers of female and hypophysectomized male and female rats*

Conjugating activity towards 1-chloro-2,4-dinitrobenzene was assayed as described in the text after immunoprecipitation with increasing amounts of anti-(transferase B). (*a*) Purified transferase B (○) activity is completely removed, whereas less then 50% of the activity from the 100000*g* supernatant of male rat liver (▲) is precipitated by anti-(transferase B). (*b*) About 70% of the activity from the 100000*g* supernatant of female rat liver (■) is precipitated. Preparations from female (□) and male (△) hypophysectomized rats are not significantly different from those of control males.

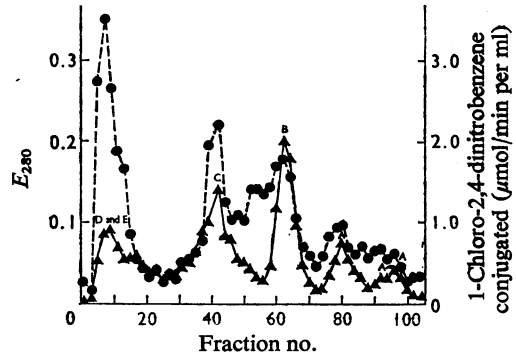


Fig. 3. *CM-cellulose chromatography of glutathione S-transferases*

Livers from two male Sprague–Dawley rats (100g each) were excised and homogenized (1:3, w/v) in ice-cold 10mM-Tris/HCl, pH8.0. The 100000*g* supernatant obtained from centrifugation of this homogenate was applied to a column (2.6cm×40cm) of DEAE-cellulose equilibrated with 10mM-Tris/HCl, pH8.0, and eluted with the same buffer. This eluate was concentrated by ultrafiltration of solvent through a Diaflo PM-10 membrane and dialysed for 24h against four 1-litre changes of 10mM-potassium phosphate, pH6.7. The CM-cellulose column (0.9cm×30cm) on which this preparation was chromatographed had been equilibrated with 10mM-potassium phosphate, pH6.7. A continuous linear gradient of 250ml portions of 10mM-potassium phosphate, pH6.7, with and without 75mM-KCl was started at fraction 20. Fractions (3.5ml) were collected. ●, E_{280} (protein); ▲, 1-chloro-2,4-dinitrobenzene conjugated. For details of labelled peaks see the text and Table 1.

The ratios of catalytic activity with 1-chloro-2,4-dinitrobenzene to that with 1,2-dichloro-4-nitrobenzene for each pooled transferase peak (Table 1) differ markedly from those reported by Habig *et al.* (1974*a*) for the purified transferases (AA, 1750; A, 14; B, 3667; C, 5). Thus each peak of transferase activity from the CM-cellulose column seems to be only 'enriched' in the transferase for which it is named. This supposition was supported by the observation that catalytic activity toward 1-chloro-2,4-dinitrobenzene was removed from each of the transferase peaks by immunoprecipitation with excess of anti-(transferase B). The fraction of activity so removed varied between peaks, the largest being found in the B peak (Table 1). Interestingly, the fraction of activity towards 1-chloro-2,4-dinitrobenzene attributable to glutathione *S*-transferase B in the entire hepatic 100000*g* supernatant (48%) is the same as that computed from the catalytic activities in each peak from the CM-cellulose column before and after immunoprecipitation.

Sex differences and the effect of hypophysectomy

The transferase activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene in control and hypophysectomized male and female rat liver 100000*g* supernatants are presented in Table 2. Male rat livers exhibited three times more 1,2-dichloro-4-nitrobenzene activity per mg of protein than did female rat livers; however, the activities towards 1-chloro-2,4-dinitrobenzene were almost identical. Hypophysectomy of the male did not

Table 1. *Removal of glutathione S-transferase activity toward 1-chloro-2,4-dinitrobenzene in the various CM-cellulose transferase peaks by immunoprecipitation with anti-(transferase B)*

The peaks of glutathione S-transferase activity from the CM-cellulose column illustrated in Fig. 3 were pooled. Enzyme activities toward 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene were assayed as described in the text. Samples of each peak were incubated with excess of anti-(transferase B), and activity remaining after immunoprecipitation was assayed.

Glutathione S-transferase peak	CM-cellulose column fractions	Ratio of conjugating activity towards 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene	Total 1-chloro-2,4-dinitrobenzene activity (μmol/min)	Activity precipitable by anti-(transferase B) (μmol/min)
D and E	5–20	330	48.05	19.70
C	35–45	78	42.12	21.48
B	58–67	135	47.85	30.15
A	76–85	12	18.80	4.51
AA	90–100	75	13.59	5.71

Table 2. *Sex differences and effect of hypophysectomy on glutathione S-transferase B*

Enzyme activities assayed as described in the text are expressed in μmol/min per mg of 100000*g*-supernatant protein. Values are means±S.E.M. for eight to ten animals, with evaluation by Student's *t* test. Determinations of activity toward 1-chloro-2,4-dinitrobenzene precipitable by anti-(transferase B) involved four animals randomly chosen from each group: male, female, hypophysectomized male and hypophysectomized female. Calculations of the percentage of total protein in the 100000*g* supernatant attributable to transferase B are based on a specific activity for this protein of 11 μmol/min per mg with 1-chloro-2,4-dinitrobenzene as substrate.

Substrate	Male	Male hypophysectomized	Female	Female hypophysectomized
1-Chloro-2,4-dinitrobenzene*	0.981±0.049	1.115±0.075	0.901±0.028	1.160±0.066
1,2-Dichloro-4-nitrobenzene†	0.044±0.002	0.046±0.004	0.016±0.001	0.045±0.004
Ratio	22.3	24.2	56.3	25.8
% of 1-chloro-2,4-dinitrobenzene-conjugating activity precipitated by anti-(transferase B)†	48±2	44±4	70±1	39±1
% of transferase B in 100000*g* supernatant‡	3.3±0.2	3.6±0.3	4.5±0.2	3.5±0.3

* Comparison of female and female hypophysectomized, $P<0.01$.
† Comparison of female with any of the other groups, $P<0.01$.
‡ Comparison of female and male, $P < 0.01$. Comparison of female and female hypophysectomized, $P<0.05$.

significantly alter activity towards either substrate. In female rats, hypophysectomy significantly increased conjugating activity towards both substrates: by about 180% with 1,2-dichloro-4-nitrobenzene and 30% with 1-chloro-2,4-dinitrobenzene. By increasing the catalytic activity of female liver towards 1,2-dichloro-4-nitrobenzene relative to that towards 1-chloro-2,4-dinitrobenzene, hypophysectomy eliminated the sex differences in substrate specificity and activity per mg of 100000*g*-supernatant protein.

When the 100000*g* supernatants from the livers of control male and female rats were examined by immunotitration with anti-(transferase B), the fraction of the overall activity toward 1-chloro-2,4-dinitrobenzene attributable to transferase B specifically was significantly greater in the female (0.70) than in the male (0.48) (Fig. 2). With the assumption of a specific activity of 11 μmol/min per mg, transferase B comprises 4.5±0.2% of the protein in 100000*g* supernatant of female rat liver. This is significantly greater than the percentage (and amount) of transferase B in male rat liver (3.3±0.2%). After hypophysectomy, the percentage of transferase B in female hepatic 100000*g* supernatant decreases to 3.5±0.3%, similar to that of the male; hypophysectomy of male rats had no significant effect on transferase B.

Discussion

The quantification of ligandin or glutathione S-transferase B in the presence of an unknown mixture of the other glutathione S-transferases is not simple. Although it may be more appropriate in some circumstances to delineate total ligand-binding capacity or total glutathione S-transferase

activity, other studies require assessment of discrete proteins for interpretation. A number of possible assay methods for glutathione *S*-transferase B have been suggested. Assessment of ligand-binding capacity by gel filtration cannot distinguish the various transferases, all of which are of about the same molecular size and exhibit overlapping binding specificities (Ketley *et al.*, 1975). Even if there were but one binding protein, quantitative application of this non-equilibrium procedure can be questioned. The purification procedure for glutathione *S*-transferase B is laborious and impractical for routine measurement of this protein.

The ratio of transferase activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene gives some indication of the relative proportions of the various transferases present. Although transferase B is most discriminatory with respect to these two substrates, wide variation in the absolute activity toward each substrate by the individual transferases (Habig *et al.*, 1974*a*) precludes use of the ratio for quantitative purposes. Separation of the transferases by chromatography on CM-cellulose is inadequate, since immunoprecipitable transferase B was found in every peak; substrate specificities also suggested that each transferase peak contained other transferases. These results are compatible with the findings of Habig *et al.* (1974*a*) that many of the transferase peaks from CM-cellulose chromatography were resolved into two or more catalytically active components during subsequent purification on hydroxyapatite.

The results of the present study suggest the value of an immunological approach. The antiserum was produced against a preparation of glutathione *S*-transferase B that had exhibited a specific activity equivalent to that reported by Habig *et al.* (1974*a*) and homogeneity on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and isoelectric focusing. A single precipitin line was obtained on immunodiffusion of the antiserum against 100000*g* supernatant of male rat liver. Immunoprecipitation removed all of the catalytic activity of the purified transferase B, but only a fraction of that present in liver 100000*g* supernatant.

With use of these immunological techniques, as well as the ratio of activity toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene, the glutathione *S*-transferase activities in livers from control and hypophysectomized male and female rats were compared. Both Darby & Grundy (1972) and Kaplowitz *et al.* (1975) have reported a sex difference in glutathione *S*-transferase activity with 1,2-dichloro-4-nitrobenzene as substrate. In the present paper, the ratio of activities toward 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene exhibited by the 100000*g* supernatant from female liver is twice that of preparations from the male.

Without application of immunological techniques, it is difficult to define this sex difference in terms of the relative proportions of the specific transferases. Transferase B was originally purified as glutathione *S*-alkyltransferase with methyl iodide as substrate (Habig *et al.*, 1974*a*). Kaplowitz *et al.* (1975) reported no sex difference in alkyltransferase activity. However, a significant difference in transferase B concentration might not be observed by using the methyl iodide assay, since the specific activity for transferase B is 0.59 μmol/min per mg, whereas that for transferase E, for example, is 8.9 μmol/min per mg. Ligandin concentrations have been reported to be the same in male and female rats (Reyes *et al.*, 1971).

With the assumption that the specific activity characteristic of glutathione *S*-transferase B from male rat liver applies also to enzyme from the female, immunoprecipitable transferase B is found to comprise $4.5 \pm 0.2\%$ of the total protein in 100000*g* supernatant of female rat liver; 70% of the transferase activity toward 1-chloro-2,4-dinitrobenzene is attributable to transferase B. The amount of transferase B in the 100000*g* supernatant from male rat liver is significantly lower both with respect to fraction of total protein ($3.3 \pm 0.2\%$) and overall transferase activity toward 1-chloro-2,4-dinitrobenzene (48%). The difference in the substrate specificity of the 100000*g* supernatant from male and female rat livers seems to reflect an increased content of transferase B (and a decreased content of one or more of the other transferases) in female liver.

Hypophysectomy eliminated these sex differences in glutathione *S*-transferase B concentration; delineation of the mechanism(s) of this effect was not the purpose of this investigation. Although Reyes *et al.* (1971) have reported that hypophysectomy of male rats doubled the ligand-binding capacity of Y protein (or ligandin), our data do not reveal a specific effect on glutathione *S*-transferase B.

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References

Darby, F. J. & Grundy, R. K. (1972) *Biochem. J.* **128**, 175–177

Fjellstedt, T. A., Allen, R. H., Duncan, B. K. & Jakoby, W. B. (1973) *J. Biol. Chem.* **248**, 3702–3707

Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974*a*) *J. Biol. Chem.* **249**, 7130–7139

Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M. & Jakoby, W. B. (1974*b*) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3879–3882

Kaplowitz, N., Kuhlenkamp, J. & Clifton, G. (1975) *Biochem. J.* **146**, 351–356
Ketley, J. N., Habig, W. H. & Jakoby, W. B. (1975) *J. Biol. Chem.* **250**, 8670–8673
Ketterer, B., Ross-Mansell, P. & Whitehead, J. K. (1967) *Biochem. J.* **103**, 316–324
Levi, A. J., Gatmaitan, Z. & Arias, I. M. (1969) *J. Clin. Invest.* **48**, 2156–2167
Litwack, G., Ketterer, B. & Arias, I. M. (1971) *Nature (London)* **234**, 466–467
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Morey, K. S. & Litwak, G. (1969) *Biochemistry* **8**, 4813–4821
Munoz, J. (1971) in *Methods in Immunology and Immunochemistry* (Williams, C. A. & Chase, M. W., eds.), vol. 3, pp. 146–160, Academic Press, New York
Pabst, M. J., Habig, W. H. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7140–7150
Reyes, H., Levi, A. J., Gatmaitan, Z. & Arias, I. M. (1971) *J. Clin. Invest.* **50**, 2242–2252
Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412
Wrigley, C. W. (1972) *Methods Enzymol.* **22**, 559–564