Bile acid inhibition of basic and neutral glutathione *S*-transferases in rat liver

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A purification scheme is described for the neutral glutathione *S*-transferases of rat liver. Discontinuous sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed that one of these enzymes contains a previously unidentified subunit, which has a molecular mass of 23 000 Da and has been designated Yn. Bile acids inhibited the activity of all the basic and neutral transferases investigated, but marked differences in the effects of bile acids on individual enzymes were observed. The activity of each transferase was inhibited more by lithocholate 3-sulphate than by chenodeoxycholate, which in turn was more inhibitory than cholate. The enzymes that were most sensitive to cholate inhibition were not found to be as readily inhibited as other transferases by chenodeoxycholate or lithocholate 3-sulphate. Conversely, the activity of transferase AA was more resistant to cholate, chenodeoxycholate and lithocholate 3-sulphate inhibition than was any of the other enzymes studied.

The glutathione *S*-transferases not only catalyse the conjugation of GSH with a large number of electrophiles but also bind hydrophobic compounds that are not substrates (Jakoby & Habig, 1980). The transferases can be divided into two groups: the basic enzymes and the neutral/acidic enzymes.

In the rat, the basic transferases (D, X, C, B, A and AA) are dimeric proteins of molecular mass 44 000, 47 000 or 50 000 Da and comprise subunits of molecular mass 22 000 Da (Ya), 23 500 Da (Yb) and 25 000 Da (Yc) (Hayes *et al.*, 1980; Scully & Mantle, 1981). These enzymes are best understood in terms of their subunit compositions, since transferases B and C are hybrid enzymes (Hayes *et al.*, 1981; Beale *et al.*, 1982; Hayes, 1983) and the individual subunits possess characteristic activities (Mannervik & Jensson, 1982).

In contrast with the detailed information describing the basic transferases, little is known about the neutral/acidic enzymes in the rat, as no purification scheme has been devised for these enzymes.

The removal of bile acids from the blood, the synthesis and repair of bile acids, and the excretion of bile acids into bile, are important aspects of liver function. Little is known about the proteins involved in these processes, but several groups of workers have demonstrated that bile acids serve as ligands for certain basic transferases (Strange *et al.*, 1977; Hayes *et al.*, 1979, 1980; Pattinson, 1981; Vessey &

Zakim, 1981). The interaction between bile acids and the glutathione *S*-transferases is poorly understood, as no quantitative systematic study has been reported. This information would be valuable for a number of reasons. Firstly, it would allow the importance of individual transferases in bile acid transport and/or metabolism to be assessed. Secondly, knowledge of the inhibitory effects of bile acids on individual enzymes would help to determine which electrophiles would prove more toxic during cholestasis.

In the present study, we describe the purification and characterization of several neutral transferases, one of which contains a previously unidentified subunit. The inhibitory effects of cholate, chenodeoxycholate and lithocholate 3-sulphate on the neutral enzymes are compared with the effects on the basic enzymes.

Materials and methods

Chemicals

These were of analytical grade and were readily available commercially.

Buffers

These were prepared at 20°C and were as follows: buffer A, 10 mM-Tris/HCl, pH 7.6; buffer B, 25 mM-sodium phosphate, pH 7.0; buffer C, 20 mM-Tris/HCl, pH 7.3, containing 200 mM-NaCl; buffer D, 25 mM-ethanolamine/acetate, pH 8.6; buf-

Abbreviation used: GSH, reduced glutathione.

fer E, aq. 10% (v/v) Polybuffer 96 adjusted to pH 6.4 with acetic acid; buffer F, 100mM-sodium phosphate, pH 7.5.

Analytical methods

Protein determinations were performed by the method of Bradford (1976), with bovine serum albumin as calibration standard.

Glutathione *S*-transferase activity was measured at 37°C as described previously (Jakoby & Habig, 1980; Hayes & Clarkson, 1982).

Bile acid inhibition of transferase activity

The effects of cholate, chenodeoxycholate and lithocholate 3-sulphate on transferase activity were investigated at pH 6.5 in 0.5 ml reaction mixtures that contained GSH (2mM) and various concentrations of 1-chloro-2,4-dinitrobenzene (0.1, 0.2, 0.4, 0.7 and 1.0mM). The activity of portions of individual enzymes (1–6 µg) was measured at seven different bile acid concentrations (cholate, 0, 0.1, 0.2, 0.3, 0.5, 0.7 and 1.0 µM; chenodeoxycholate, 0, 10, 20, 40, 70, 112.5 and 173 µM; lithocholate 3-sulphate, 0, 0.25, 0.5, 1.0, 2.5, 5.0 and 10mM, 0, 1, 2.5, 5, 10, 20 and 40 µM or 0, 5, 10, 25, 50, 75 and 100 µM) for each of the five 1-chloro-2,4-dinitrobenzene concentrations. These determinations were each performed in quintuplicate, and the mean velocity [µmol of *S*-(2,4-dinitrophenyl)glutathione formed/min] was determined and used for subsequent calculations. Matrices, comprising 35 experimental points, were constructed for each transferase; these allowed the K_m (for 1-chloro-2,4-dinitrobenzene) and V_{max} values to be calculated

(Wilkinson, 1961) in the presence of the stated concentrations of the three bile acids. The data in each matrix were plotted by using three independent methods (Lineweaver & Burk, 1934; Dixon, 1953; Cornish-Bowden, 1974), which enabled the type of inhibition to be determined and provided an estimate of the K_i value.

Preparation of liver extract

The livers were from male Sprague–Dawley rats (250–350g) and were stored (2–10 days) at –70°C before use. Soluble liver extracts were prepared as previously described (Habig *et al.*, 1974; Hayes, 1983). All purification steps were performed at 4°C.

Experimental and results

Separation of basic transferases from neutral transferases

The soluble liver extract (545 ml, 13 g of protein) was applied to DEAE-cellulose columns (bed volume 1.5 litres), which were eluted (48 ml/h) with buffer A. The basic transferases were eluted in the 'flow-through' fractions from DEAE-cellulose whereas the neutral transferases were retained by the exchanger.

The basic enzymes were purified by a combination of CM-cellulose, hydroxyapatite, chromatofocusing and *S*-hexylglutathione affinity chromatography (Habig *et al.*, 1974; Mannervik & Guttenberg, 1981; Hayes, 1983). Five of the basic enzymes (transferases D, X, C, A and AA) were prepared to allow their interaction with bile acids to be studied.

Fig. 1. Elution of neutral glutathione *S*-transferases from DEAE-cellulose

The soluble extract (545 ml, 13 g of protein) from 16 rat livers was eluted from DEAE-cellulose (bed volume approx. 1.5 litres) as described in the text. The flow rate was 48 ml/h, and 9.6 ml fractions were collected. The basic transferases were eluted in the 'flow-through' fractions (65–150). The Na^+ concentrations (■) were determined by flame photometry, and transferase activity was measured with GSH and 1-chloro-2,4-dinitrobenzene (▲). The horizontal bars indicate the fractions that were combined.

The neutral transferases were eluted from DEAE-cellulose as three peaks of activity (with 1-chloro-2,4-dinitrobenzene as substrate) by a 0–100 mM NaCl gradient in buffer A. These were separately combined and were designated pools 1–3 (Fig. 1).

Purification of the neutral transferases

The three pools of activity from DEAE-cellulose were purified by *S*-hexylglutathione affinity chromatography (Mannervik & Guthenberg, 1981). Pools 1–3 were applied to 2.2 cm × 11.0 cm columns of *S*-hexylglutathione–Sepharose 6B and eluted (18 ml/h) with buffer B.

The gel was washed with 70–100 ml of buffer C before the transferases were eluted with a 5 mM solution of *S*-hexylglutathione in the same buffer. The three pools of purified transferase activity were each dialysed for 9 h against 2 litres of buffer D and applied to PBE 94 chromatofocusing columns (1.6 cm × 36.0 cm), which were also equilibrated with buffer D. The transferases in each of the pools were subsequently resolved by using a pH 8.2–6.5 gradient, produced by eluting (19.2 ml/h) the column with buffer E (Fig. 2). The fractions that contained the five major forms of activity (peaks Q–U) were separately combined as indicated, and peaks S, T

Fig. 2. Resolution of neutral glutathione *S*-transferases by chromatofocusing chromatography

The three pools of neutral transferase activity, eluted from DEAE-cellulose, were further purified by *S*-hexylglutathione–Sepharose 6B chromatography and eluted from columns (1.6 cm × 36.0 cm) of chromatography gel PBE 94 as described in the text. Fractions (5 ml) were collected, and transferase activity (▲) and pH (●) were determined. The activity was resolved into five peaks (Q, R, S, T and U), which were separately combined as shown by the horizontal bars.

Table 1. *Purification of neutral glutathione S-transferases*

For full experimental details see the text. Activity was determined by using the standard 1-chloro-2,4-dinitrobenzene-GSH-conjugation assay. Yield is expressed as a percentage of total neutral transferase activity.

Fraction	Volume (ml)	Protein (A_{280} units)	Activity ($\mu\text{mol/min}$)	Yield (%)
1. Total neutral transferases	770	2000	4700	100
2. DEAE-cellulose:				
Pool 1	86	407	1150	24
Pool 2	46	90	645	14
Pool 3	87	522	560	12
3. S-Hexylglutathione chromatography of:				
Pool 1	12	44	1100	23
Pool 2	10	12	600	13
Pool 3	20	11	290	6
4. PBE 94 chromatofocusing peaks:				
Q (from pool 1)	19	22	593	13
R (from pool 1)	15	4.1	310	7
S (from pool 2)	24	3.7	501	11
T (from pool 3)	5	1.0	82	2
U (from pool 3)	5	0.9	42	1

and U were concentrated to about 5 ml each by ultrafiltration.

The results of the purification of the neutral transferases are summarized in Table 1. It should be noted that transferases Q, R and S were recoverable from pools 1, 2 and 3 and that the amount of each therefore represents significantly more than the 13, 7 and 11% respectively of the total neutral transferase activity indicated in Table 1.

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis demonstrated that the neutral enzymes were homogeneous. Transferases Q, R, T and U comprised polypeptides that migrated with the Yb monomer (molecular mass 23 500 Da). In contrast, sodium dodecyl sulphate/polyacrylamide-gel electrophoresis resolved transferase S into two bands (Fig. 3). The larger monomer co-migrated with the Yb monomer, and the smaller monomer migrated between the Ya and Yb monomers. This new subunit had an apparent molecular mass 23 000 Da and was designated Yn. Transferase S appears to be a heterodimer, because the Yb and Yn monomers are present in equal concentrations and the Yb/Yn ratio did not vary with different preparations. Further, cross-linking experiments with dimethyl suberimidate (Davies & Stark, 1970) resulted in the formation of a protein complex of molecular mass 50 000 Da that, during electrophoresis, migrated as a single, but broad, band.

Characterization of the neutral transferases

Transferases Q, R, S, T and U were characterized by determining their Michaelis constants (K_m), for 1-chloro-2,4-dinitrobenzene and GSH, and their specific activities for a variety of substrates (Table 2,

Table 2. *Neutral transferases: specific activities, kinetic properties and cholate inhibition*

For full experimental details see the text. Abbreviations: DCNB, 1,2-dichloro-4-nitrobenzene; ENPOP, 1,2-epoxy-3-(*p*-nitrophenoxy)propane; TPBO, *trans*-4-phenylbut-3-en-2-one; CDNB, 1-chloro-2,4-dinitrobenzene.

(a) Specific activity ($\mu\text{mol/min}$ per mg of protein)				
Transferase	DCNB	ENPOP	TPBO	CDNB
Q	0.07	0.64	4.0	20
R	2.55	0.57	1.9	75
S	3.13	0.56	1.9	135
T	3.30	0.25	1.1	80
U	1.64	0.14	1.1	45
(b) K_m ($\pm$ S.E.M.) (mM)				Concn. of cholate for 50% inhibition (μM)
Transferase	CDNB	GSH		
Q	1.866 ± 0.499	0.157 ± 0.006		500
R	0.165 ± 0.010	0.194 ± 0.003		300
S	0.202 ± 0.036	0.123 ± 0.015		700
T	0.225 ± 0.003	0.155 ± 0.016		1200
U	0.200 ± 0.005	0.186 ± 0.015		850

parts *a* and *b*). The inhibitory effect of cholate, a possible non-substrate ligand, was also investigated. These results are expressed as the concentration of cholate required to inhibit the 1-chloro-2,4-dinitrobenzene-GSH-conjugating activity by 50% under standard assay conditions (Table 1, part *b*). Paucity of material prevented a study of the interaction between transferases T and U and other potential ligands.

Fig. 3. Electrophoresis of transferase *S*

The transferases were purified and analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis as described in the text. Portions of each protein were applied to the gel as follows: (1) 5 μ g of transferase X; (2) 5 μ g of transferase C; (3) 5 μ g of transferase AA; (4) 5 μ g of transferase S; (5) 15 μ g of transferase S. The positions of the Ya, Yb and Yc subunits are indicated.

Behaviour of neutral transferases on cholic acid-aminohexyl-Sepharose 4B

The differences in the inhibitory effect of cholates on transferases Q, R, S, T and U suggested that these enzymes might be resolved by cholic acid affinity chromatography. This hypothesis was investigated by combining the three neutral enzyme pools (from DEAE-cellulose) and, after *S*-hexylglutathione affinity chromatography, eluting the purified transferases from cholic acid-aminohexyl-Sepharose 4B (Pattinson, 1981). The cholic acid affinity column (2.2 cm $\times$ 2.2 cm) was equilibrated with buffer B containing 1 mM-2-mercaptoethanol.

The purified enzymes (30 mg) were dialysed for 18 h against the same buffer before being applied to the column. The gel was then washed with 2 column volumes of the running buffer followed by 10 column volumes of buffer F before a final wash with a 5 mM solution of cholates in buffer F.

All the neutral transferases bound the affinity matrix, but the washing procedure resulted in the resolution of two peaks of activity. The first peak, which was eluted with buffer F, contained an equal concentration of Yb and Yn subunits, and, as it was subsequently eluted from a chromatofocusing column at pH 7.6 as a single symmetrical peak, it was identified as transferase S. The second peak, which was eluted with cholic acid, comprised only Yb monomers, and contained transferases Q, R, T and U.

Fig. 4. Cornish-Bowden plots of the interaction between chenodeoxycholate and transferases R (a) and AA (b). Enzyme activity was measured with 2 mM-GSH and 1.0 mM- (●), 0.7 mM- (▲), 0.4 mM- (■), 0.2 mM- (○) or 0.1 mM-1-chloro-2,4-dinitrobenzene (Δ) in the presence of various concentrations of chenodeoxycholate. Each experimental point represents the mean of five determinations. The inhibition matrices and plots were constructed as described in the text.

Bile acid inhibition of glutathione *S*-transferase activity

The effects of cholate, chenodeoxycholate and lithocholate 3-sulphate on the GSH-1-chloro-2,4-dinitrobenzene-conjugating activity of a number of transferases (Q, R, S, A, C, D, X and AA) were investigated as described. The activity of each transferase was inhibited by the three bile acids studied.

Transferase AA was more resistant to bile acid inhibition than were the other enzymes. Under standard assay conditions (1 mM-1-chloro-2,4-dinitrobenzene, 2 mM-GSH) the activity of transferase AA in the presence of 173 μ M-chenodeoxycholate only decreased to 96% of that obtained in the absence of the bile acid, whereas the activities of transferases Q, R, S, A, C, D and X were decreased to 31, 30, 33, 34, 33, 30 and 20% respectively. Fig. 4

Fig. 5. Cornish-Bowden plots of the interaction between lithocholate 3-sulphate and transferases A (a) and AA (b)

The activities of transferases A and AA were measured with 2 mM-GSH and 1.0 mM- (●), 0.7 mM- (▲), 0.4 mM- (■), 0.2 mM- (○) or 0.1 mM-1-chloro-2,4-dinitrobenzene (△). Each experimental point represents the mean of five determinations. The inhibition matrices and plots were constructed as described in the text.

shows a comparison of the [1-chloro-2,4-dinitrobenzene]/ v -versus-[chenodeoxycholate] plots obtained from transferases AA and R. Similarly, at a lithocholate 3-sulphate concentration of 10 μ M, transferase AA activity was decreased to 98% whereas the activities of transferases Q, R, S, A, C, D and X were decreased to 35, 57, 41, 72, 61, 33 and 11% respectively. Although increasing the lithocholate 3-sulphate concentration to 100 μ M resulted in a significant decrease in the activity of transferase AA (82%), the limited solubility of this bile acid did not permit the use of higher inhibitor concentrations, and therefore prevented an accurate estimate of the K_i value. Dixon plots suggested that the K_i of transferase AA was about 250 μ M for lithocholate

Fig. 6. Cornish-Bowden plots of the interaction between cholate and transferases R (a) and S (b)

Transferase activity was measured with 2 mM-GSH and 1.0 mM- (●), 0.7 mM- (▲), 0.4 mM- (■), 0.2 mM- (○) or 0.1 mM-1-chloro-2,4-dinitrobenzene (△). Each experimental point represents the mean of five determinations. The inhibition matrices and plots were constructed as described in the text.

3-sulphate, and Cornish-Bowden plots indicated that the inhibition was competitive. A comparison of [1-chloro-2,4-dinitrobenzene]/*v*-versus-[lithocholate 3-sulphate] plots from transferases AA and A is shown in Fig. 5.

The enzymes prepared from the neutral transferase pools were all inhibited by cholate (Table 2). Although the effects of chenodeoxycholate and lithocholate 3-sulphate on the activity of transferases Q, R and S were studied, this was not possible with transferases T and U because of their low concentrations in the rat liver extracts. The K_i of transferase S for cholate could not be determined since plots of $1/v$ or [1-chloro-2,4-dinitrobenzene]/*v*-versus-[cholate] were non-linear (Fig. 6). This result probably indicates that the two subunits that transferase S contains (i.e. Yn and Yb) possess distinct affinities for cholate.

Table 3 shows that the calculated K_i values of the transferases (with the exception of transferase AA) for the two primary bile acids are similar. Variations in K_i between transferases Q, R, S, A, C, D and X were approximately 2-fold for cholate and about 3-fold for chenodeoxycholate. However, larger differences (about 20-fold) in the K_i values of the same transferases for lithocholate 3-sulphate were observed. Each transferase studied appeared to bind chenodeoxycholate more avidly than cholate. Likewise, lithocholate 3-sulphate appeared to be bound more tightly than chenodeoxycholate. Table 3 indicates that transferase A, which had the smallest K_i value for cholate, did not exhibit the smallest K_i value for chenodeoxycholate or lithocholate 3-sulphate. In contrast, transferase X, which had the

lowest K_i for lithocholate 3-sulphate also possessed the lowest K_i for chenodeoxycholate.

Discussion

The basic glutathione *S*-transferases in rat liver have been widely studied and six forms (D, X, C, B, A and AA) have been isolated (Hayes, 1983). In the present study a number of neutral transferases have been purified from rat liver. Five peaks of activity were resolved, which were designated transferases Q, R, S, T and U. The properties of transferases R, S, T and U suggest that these enzymes are distinct from the basic transferases. It is concluded that transferase Q is identical with transferase D, as these preparations are co-eluted from chromatofocusing columns, exhibit similar catalytic behaviour and cross-react with antiserum raised against transferase C.

Transferases R, S, T and U all contain Yb subunits (M_r 23 500). Hayes (1983) and Ketterer *et al.* (1983) have reported that transferases A, C and D comprise Yb₁Yb₁, Yb₁Yb₂ and Yb₂Yb₂ dimers respectively. The fact that the neutral enzymes also contain polypeptides that migrate with the Yb₁ and Yb₂ monomers suggests that there is at least one further type of Yb subunit (i.e. Yb₃).

During the present study a new class of transferase subunit (Yn; M_r 23 000) was purified from the neutral transferase pool. Transferase S comprised YbYn subunits. There are therefore a total of four different sizes of transferase subunits (Ya, Yn, Yb and Yc), which can be resolved from rat liver cytosol by using sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (12.5% resolving gel). It should, however, be noted that Tu *et al.* (1982) have also previously observed four bands on electrophoresis of a mixture of purified transferases. However, these workers used a polyacrylamide gradient gel (9–13%) and, although they did not identify which bands corresponded to the Ya, Yb and Yc polypeptides, it is likely, since the preparation studied was not enriched for transferase S, that the gradient electrophoretic system employed split the Yb band into the Yb₁ and Yb₂ monomers (Mannervik & Jensson, 1982; Hayes, 1983; Ketterer *et al.*, 1983).

The importance of the transferases in bilirubin transport has been recognized for a number of years, and is frequently emphasized (Levi *et al.*, 1969), but it has only been shown more recently that certain transferases also bind bile acids (Tipping *et al.*, 1976; Strange *et al.*, 1977; Hayes *et al.*, 1979, 1980; Sugiyama *et al.*, 1983). As the liver handles at least 50 times more bile acid per day than bilirubin, bile acids may be quantitatively the most important group of ligands encountered by the transferases.

Vessey & Zakim (1981) showed that the activity of partially purified preparations of transferases A, B and C are inhibited by physiological concentrations

Table 3. Bile acid inhibition of glutathione *S*-transferases

For full experimental details see the text. Type of inhibition: nc, non-competitive; m, mixed; uc, uncompetitive; c, competitive. Abbreviation: N.D., not determined.

Transferase	10 ⁻³ × Subunit mass (Da)	K_i (μM)		
		Cholate	Chenodeoxycholate	Lithocholate 3-sulphate
Q	23.5	540 nc	54 m	4 m
R	23.5	390 uc	66 uc	15 uc
S	23.5/23	†	60 m	13 m
A	23.5	280 m*	80 uc	26 uc
C	23.5	400 m*	78 uc	20 uc
D	23.5	N.D.	52 m	4 m
X	22	550 c*	25 c	1.4 m
AA	25	2500*	‡	~250 c§

* Values from Hayes & Clarkson (1982).

† K_i could not be estimated (Fig. 6).

‡ K_i could not be estimated (Fig. 4).

§ K_i could not be accurately determined (Fig. 5, see the text).

of bile acids, but no K_i values were calculated. Transferases B and C are hybrid enzymes, and the study by Vessey & Zakim (1981) gave little information about the interaction between bile acids and individual subunits. Further, nothing is known about the inhibitory effect of bile acids with the neutral transferases.

The data contained in Tables 2 and 3 demonstrate that all the neutral as well as the basic transferases bind bile acids. Differences in the specificity of the bile acid-binding activity possessed by individual transferases was observed. For example, transferase A binds the trihydroxy bile acid (cholate) more avidly than do the other transferases, and transferase X appears to possess the greatest affinity for the primary dihydroxy bile acid (chenodeoxycholate). The K_i values exhibited by transferase A for cholate and chenodeoxycholate differ by a factor of only 3.5-fold, whereas those of transferase X differ by a factor of 22-fold. The inhibitory effect of lithocholate 3-sulphate, which possesses no hydroxy groups but contains a negatively charged sulphate group attached to the A steroid ring, was greatest for transferase X. In every instance the transferases possessed lower K_i values for lithocholate 3-sulphate than for chenodeoxycholate, but the magnitude of these differences varied considerably.

The binding properties of the transferases suggests that these enzymes are involved in the hepatic transport and/or storage of bile acids. It is possible their function is to minimize the back-diffusion of bile acids from liver to plasma, as has been proposed by Wolkoff *et al.* (1979) for bilirubin. However, the details of this putative involvement must await further information about the route that bile acids follow during transport across the hepatocyte (Suchy *et al.*, 1983). Further, the report by Redick *et al.* (1982) that the transferases are not distributed uniformly throughout the liver indicates that each isoenzyme may have its own niche. The mathematical models that have been used previously to study bile acid transport are therefore clearly oversimplified.

The marked differences between the inhibitory effects of bile acids on the activities of transferases X and AA are of interest because these enzymes possess substantial sequence homology (Beale *et al.*, 1982; Pickett *et al.*, 1982, 1983). The ability of transferase AA to bind to a cholic acid-amino-hexyl-Sepharose 4B column (Pattinson, 1981; Hayes & Clarkson, 1982) while displaying a relative resistance to bile acid inhibition may indicate the presence of an alternative bile acid-binding site, separate from the active centre. This suggests that Ya and Yc subunits have distinct physiological roles.

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References

- Beale, D., Ketterer, B., Carne, T., Meyer, D. & Taylor, J. B. (1982) *Eur. J. Biochem.* **126**, 459–463
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Cornish-Bowden, A. (1974) *Biochem. J.* **137**, 143–144
- Davies, G. E. & Stark, G. R. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 651–656
- Dixon, M. (1953) *Biochem. J.* **55**, 170–171
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139
- Hayes, J. D. (1983) *Biochem. J.* **213**, 625–633
- Hayes, J. D. & Clarkson, G. H. D. (1982) *Biochem. J.* **207**, 459–470
- Hayes, J. D., Strange, R. C. & Percy-Robb, I. W. (1979) *Biochem. J.* **181**, 699–708
- Hayes, J. D., Strange, R. C. & Percy-Robb, I. W. (1980) *Biochem. J.* **185**, 83–87
- Hayes, J. D., Strange, R. C. & Percy-Robb, I. W. (1981) *Biochem. J.* **197**, 491–502
- Jakoby, W. B. & Habig, W. H. (1980) in *Enzymatic Basis of Detoxication* (Jakoby, W. B., ed.), vol. 2, pp. 63–94, Academic Press, London and New York
- Ketterer, B., Beale, D., Taylor, J. B. & Meyer, D. J. (1983) *Biochem. Soc. Trans.* **11**, 467–468
- Levi, A. J., Gatmaitan, Z. & Arias, I. M. (1969) *J. Clin. Invest.* **48**, 2156–2167
- Lineweaver, H. & Burk, D. (1934) *J. Am. Chem. Soc.* **56**, 658–666
- Mannervik, B. & Guthenberg, C. (1981) *Methods Enzymol.* **77**, 231–235
- Mannervik, B. & Jensson, H. (1982) *J. Biol. Chem.* **257**, 9909–9912
- Pattinson, N. (1981) *Anal. Biochem.* **115**, 424–427
- Pickett, C. B., Donohue, A. M., Lu, A. Y. H. & Hales, B. F. (1982) *Arch. Biochem. Biophys.* **215**, 539–543
- Pickett, C. B., Telakowski-Hopkins, C. A., Donohue, A. M. & Lu, A. Y. H. (1983) *Arch. Biochem. Biophys.* **221**, 89–98
- Redick, J. A., Jakoby, W. B. & Baron, J. (1982) *J. Biol. Chem.* **257**, 15200–15203
- Scully, N. C. & Mantle, T. J. (1981) *Biochem. J.* **193**, 367–370
- Strange, R. C., Cramb, R., Hayes, J. D. & Percy-Robb, I. W. (1977) *Biochem. J.* **165**, 425–429
- Suchy, F. J., Garfield, S. A., Balistreri, W. F. & Miller, P. (1983) *Gastroenterology* **82**, 1246
- Sugiyama, Y., Yamada, T. & Kaplowitz, N. (1983) *J. Biol. Chem.* **258**, 3602–3607
- Tipping, E., Ketterer, B., Christodoulides, L. & Enderby, G. (1976) *Eur. J. Biochem.* **67**, 583–590
- Tu, C.-P. D., Weiss, M. J. & Reddy, C. C. (1982) *Biochem. Biophys. Res. Commun.* **108**, 461–467
- Vessey, D. A. & Zakim, D. (1981) *Biochem. J.* **197**, 321–325
- Wilkinson, G. N. (1961) *Biochem. J.* **80**, 324–332
- Wolkoff, A. W., Goresky, C. A., Sellin, J., Gatmaitan, Z. & Arias, I. M. (1979) *Am. J. Physiol.* **236**, E638–E648