Glutathione *S*-transferases of human brain

Evidence for two immunologically distinct types of 26500-*M_r* subunits

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Human brain contains one cationic (pI 8.3) and two anionic (pI 5.5 and 4.6) forms of glutathione *S*-transferase. The cationic form (pI 8.3) and the less-anionic form (pI 5.5) do not correspond to any of the glutathione *S*-transferases previously characterized in human tissues. Both of these forms are dimers of 26500-*M_r* subunits; however, immunological and catalytic properties indicate that these two enzyme forms are different from each other. The cationic form (pI 8.3) cross-reacts with antibodies raised against cationic glutathione *S*-transferases of human liver, whereas the anionic form (pI 5.5) does not. Additionally, only the cationic form expresses glutathione peroxidase activity. The other anionic form (pI 4.6) is a dimer of 24500-*M_r* and 22500-*M_r* subunits. Two-dimensional gel electrophoresis demonstrates that there are three types of 26500-*M_r* subunits, two types of 24500-*M_r* subunits and two types of 22500-*M_r* subunits present in the glutathione *S*-transferases of human brain.

GSH *S*-transferases (EC 2.5.1.18) are a family of multifunctional enzymes that provide protection to organisms from the toxic effects of xenobiotics (Booth *et al.*, 1961; Jakoby, 1978; Chasseaud, 1979). These enzymes catalyse the conjugation of a large variety of xenobiotics with GSH (Booth *et al.*, 1961; Chasseaud, 1979). GSH *S*-transferases can also bind a wide group of ligands (Litwack *et al.*, 1971; Jakoby, 1978), and several forms of this enzyme express GSH peroxidase activity towards lipid hydroperoxides (Prohaska & Ganther, 1977; Pierce & Tappel, 1978; Awasthi *et al.*, 1980).

In human tissues several forms of GSH *S*-transferases differing in isoelectric points and catalytic abilities have been described (Kamisaka *et al.*, 1975; Marcus *et al.*, 1978; Awasthi *et al.*, 1980; Awasthi & Dao, 1981; Partridge *et al.*, 1984). It was previously suggested that five cationic forms present in human liver are homodimers of a single gene product, and that the charge heterogeneity of these forms is due to deamidation *in vivo* (Kamisaka *et al.*, 1975). Subsequent investigations in this laboratory (Dao *et al.*, 1982), however, have demonstrated that at least two subunits, of *M_r*

26500 and 24500, are present among the cationic GSH *S*-transferases of human liver. The presence of another subunit, of *M_r* 22500, has been demonstrated in the anionic GSH *S*-transferases of human liver, placenta and lung, and it has been suggested that at least three different constituent subunits in their binary combinations give rise to different forms of GSH *S*-transferase of human tissues (Dao *et al.*, 1984; Partridge *et al.*, 1984). Three subunits, however, do not account for the multitude of different GSH *S*-transferases that have been reported so far from human tissues, and the structural basis for the origin of different GSH *S*-transferases still remains to be explained.

In order to understand better the genetic and structural basis for the origin of different GSH *S*-transferases of human tissues, we have studied the structural, kinetic and immunochemical characteristics of three forms of GSH *S*-transferase present in human brain. These enzymes were purified to homogeneity, and their subunit structures, amino acid compositions and substrate specificities were studied. Using two-dimensional gel electrophoresis, we investigated the heterogeneity in the subunits having similar *M_r* values. Antibodies raised against purified GSH *S*-transferases of human liver, lung and placenta were used to in-

Abbreviation used: GSH, reduced glutathione.

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investigate the immunological interrelationships among these enzymes.

Materials and methods

Materials

Human brain samples were obtained at autopsy, and were either processed immediately or frozen at -20°C . The sources of all chemicals used in this study were the same as those reported by us in previous studies (Partridge *et al.*, 1984; Singh *et al.*, 1984). Antibodies raised against the cationic GSH *S*-transferases of liver and those raised against the anionic enzymes of lung and placenta were the same as those used by us previously (Dao *et al.*, 1984).

Purification of human brain GSH *S*-transferases

All purification steps were performed at 4°C , and enzyme activity during the purification was monitored with 1-chloro-2,4-dinitrobenzene as the substrate. Brain tissue was cleaned, washed with distilled water and cut into small pieces. A 10% (w/v) homogenate was prepared in 10mM-potassium phosphate buffer, pH 7.0, containing 1.4mM-2-mercaptoethanol (buffer A) by using a Sorvall Omnimixer at 4000 rev./min. The homogenate was centrifuged at 16000g for 30 min, and most of the GSH *S*-transferase activity was recovered in the supernatant. Solid $(\text{NH}_4)_2\text{SO}_4$ was gradually added to the supernatant with constant stirring to bring about a final saturation of 80%; the pH was maintained at 7.0 throughout the addition. The mixture was stirred overnight and centrifuged at 16000g for 30 min. The supernatant had negligible GSH *S*-transferase activity and was discarded. The pellet was resuspended in buffer A, and dialysed for 48 h against the same buffer (40 vol., four changes). After dialysis, the enzyme solution was centrifuged at 16000g for 2 h and the supernatant was subjected to affinity chromatography over a column (1 cm $\times$ 10 cm) of GSH-linked epoxy-activated Sepharose 6B (Simons & Vander Jagt, 1977). The column was pre-equilibrated with 22mM-potassium phosphate buffer, pH 7.0, containing 1.4mM-2-mercaptoethanol (buffer B) at a flow rate of 10 ml/h. After application of the enzyme, the column was washed thoroughly with buffer B until the eluate had no absorbance at 280 nm. The enzyme was eluted with 10mM-GSH in 50mM-Tris/HCl buffer, pH 9.6, and was dialysed against buffer A. The dialysed enzyme was subjected to isoelectric focusing in an LKB 8100 column in the pH range 3.5–10 in a 0–50% (w/v) sucrose density gradient at 1600 V for 16 h. Three peaks of GSH *S*-transferase activity, having pI values of 8.3, 5.5 and 4.6 (Fig. 1), were pooled

separately and dialysed against distilled water containing 1.4mM-2-mercaptoethanol.

Enzyme assays

GSH *S*-transferase activities with different substrates were determined by using methods described by Habig *et al.* (1974), Fjellstedt *et al.* (1973) and Keen & Jakoby (1978). GSH peroxidase activities with cumene hydroperoxide and H_2O_2 were determined by the method of Awasthi *et al.* (1979). Protein determinations were performed by the method of Bradford (1976), with bovine serum albumin as a standard.

Electrophoretic methods

Urea/sodium dodecyl sulphate/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis was performed by the method of Laemmli (1970) with 12.5%-polyacrylamide gels in a vertical-slab-gel apparatus (LKB model 2001). Two-dimensional gel electrophoresis was performed by the method of O'Farrell (1975) with a 10%-polyacrylamide resolving gel at 40 mA/gel. Gels were stained by the method of Cleveland *et al.* (1977) and destained in 50% (v/v) methanol/10% (v/v) acetic acid.

Amino acid analysis

Amino acid compositions were determined with a Beckman model 121 automatic amino acid analyser by the method described by Kurosky *et al.* (1977).

Immunological studies

Immunodiffusion studies were performed with Ouchterlony plates (Ouchterlony, 1958), and immunotitrations were performed as described previously (Awasthi *et al.*, 1980).

Results

Human brain contains significant amounts (about 3 units/g wet wt.) of GSH *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene. Upon isoelectric focusing of human brain supernatant, three peaks of GSH *S*-transferase activity, with pI values of 8.3, 5.5 and 4.6, were obtained. Enzyme preparations from three different brain samples exhibited similar isoelectric-focusing patterns. When grey matter and white matter of brain were separated, the isoelectric-focusing profiles of GSH *S*-transferases present in both fractions were found to be similar to those obtained from whole brain.

From 200 g of human brain tissue, about 200 units of purified GSH *S*-transferase was obtained by $(\text{NH}_4)_2\text{SO}_4$ fractionation and affinity chromatography (Table 1). Polyacrylamide-disc-gel electrophoresis (Davis, 1964) of this prepara-

Table 1. Purification of human brain GSH S-transferases

For full experimental details see the text. The enzyme activity was monitored with 1-chloro-2,4-dinitrobenzene as substrate. One unit of enzyme activity utilizes 1 μ mol of substrate/min at 25°C.

	Enzyme activity		Protein (mg/ml)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
	(units/ml)	(total units)				
16000g supernatant	0.38	620.1	3.17	0.12	—	100
80%-satn.-(NH ₄) ₂ SO ₄ fraction	1.38	377.9	7.24	0.19	1.6	61
Affinity chromatography	14.76	196.2	0.64	23.06	192.2	32
Isoelectric focusing						
Cationic (pI8.3)	0.06	4.3	0.02	2.78	—	0.7
Anionic (pI5.5)	0.43	15.7	0.03	14.48	—	2.5
Anionic (pI4.6)	1.63	125.6	0.07	23.33	—	20.3

Fig. 1. Isoelectric focusing of purified human brain GSH S-transferases

Details are described in the text. ●, GSH S-transferase activity; ○, pH gradient. Inset: expanded scale of fractions 1-60.

tion as well as of each of the two anionic forms showed the presence of a single protein band (results not shown). Upon isoelectric focusing, GSH *S*-transferase obtained by affinity chromatography resolved into three distinct peaks of enzyme activity, corresponding to pI values of 8.3, 5.5 and 4.6 (Fig. 1). The peak at pI 4.6 contained the major portion (about 85%) of brain GSH *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene, whereas the peaks at pI 5.5 and 8.3 accounted for about 11% and 3% of this activity respectively. The cationic enzyme, which accounted for only about 3% of the total enzyme activity towards 1-chloro-2,4-dinitrobenzene, represented about 20% of total GSH *S*-transferase protein of human brain (Table 1).

Structural studies

Upon Sephadex G-100 gel filtration the M_r value of human brain GSH *S*-transferases was found to be in the range 45000–50000. The urea/sodium dodecyl sulphate/2-mercaptoethanol/polyacrylamide-gel electrophoretogram (Fig. 2a) of total GSH *S*-transferases of brain obtained after affinity chromatography showed the presence of three protein bands corresponding to M_r values of about 26500, 24500 and 22500. When the subunit structures of the individual GSH *S*-transferases of brain were studied, the cationic enzyme (pI 8.3) and the less-anionic enzyme (pI 5.5) both showed the presence of only one band, with an M_r value of about 26500 (Fig. 2b). The more-anionic (pI 4.6) enzyme showed the presence of two

bands, with M_r values of 24500 and 22500 (Fig. 2a).

The subunit structures of human brain GSH *S*-transferases were further examined by two-dimensional electrophoretic analysis by the method of O'Farrell (1975). On two-dimensional electrophoresis of total GSH *S*-transferase of brain, the presence of at least seven distinct polypeptide spots on the slab gel was observed (Fig. 3a). Three of these spots corresponded to M_r 26500, two corresponded to M_r 24500, and the remaining two corresponded to M_r 22500. When the individual enzymes were subjected to two-dimensional electrophoretic analysis, the anionic enzyme (pI 4.6) showed the presence of two spots corresponding to M_r 22500 and two spots corresponding to M_r 24500 (Fig. 3d). The less-anionic form (pI 5.5) resolved into two spots, both corresponding to M_r values of 26500 (Fig. 3c), whereas the cationic enzyme (pI 8.3) indicated the presence of a single spot corresponding to M_r 26500 (Fig. 3b).

The amino acid compositions of the three human brain GSH *S*-transferases are given in Table 2. There appear to be significant amino acid compositional differences among these forms. Most notably, the contents of serine, glycine, leucine and histidine show the maximum variation. In order to assess the amino acid compositional differences among the brain enzymes and those of other human tissues, the difference-index values were calculated by the method of Metzger *et al.* (1968), and these values are presented in Table 3. Difference-index values reveal significant com-

Fig. 2. Urea/SDS/2-mercaptoethanol/polyacrylamide-slab-gel electrophoresis of human brain GSH *S*-transferases (a) Lane 1, protein standards; lane 2, affinity-chromatography pool; lane 3, brain GSH *S*-transferase, pI 4.6. (b) Lane 1, protein standards; lane 2, brain GSH *S*-transferase, pI 8.3; lane 3, brain GSH *S*-transferase, pI 5.5.

Fig. 3. Two-dimensional gel electrophoresis of human brain GSH S-transferases (a) Affinity pool; (b) brain GSH S-transferase, pI8.3; (c) brain GSH S-transferase pI5.5; (d) brain GSH S-transferase, pI4.6.

Table 2. Amino acid compositions of human brain GSH S-transferases

Values are the means $\pm$ s.d. for three samples hydrolysed for 24, 48 and 96 h. Values for serine and threonine were obtained by extrapolation to time zero of hydrolysis. Values are based on an M_r value of 53000 for the pI8.3 and pI5.5 forms and an M_r value of 47000 for the pI4.6 form.

Residue	Composition (mol of residue/mol)		
	Cationic enzyme (pI8.3)	Anionic enzyme (pI5.5)	Anionic enzyme (pI4.6)
Asp	31.3 $\pm$ 0.2	41.4 $\pm$ 1.4	41.3 $\pm$ 0.4
Thr	23.9	21.2	23.1
Ser	72.6	56.5	39.7
Glu	67.8 $\pm$ 0.7	63.6 $\pm$ 2.9	57.3 $\pm$ 3.0
Pro	14.7 $\pm$ 0.0	20.6 $\pm$ 1.9	21.3 $\pm$ 0.0
Gly	76.2 $\pm$ 3.3	43.4 $\pm$ 1.8	47.1 $\pm$ 3.1
Ala	31.8 $\pm$ 0.5	28.1 $\pm$ 1.2	32.3 $\pm$ 1.3
Val	20.4 $\pm$ 0.2	17.8 $\pm$ 2.5	30.3 $\pm$ 2.1
Met	2.4 $\pm$ 0.5	5.5 $\pm$ 0.8	2.4 $\pm$ 0.6
Ile	14.4 $\pm$ 0.3	22.2 $\pm$ 1.3	15.2 $\pm$ 0.8
Leu	23.4 $\pm$ 0.8	38.5 $\pm$ 2.0	62.2 $\pm$ 0.9
Tyr	11.3 $\pm$ 0.8	15.3 $\pm$ 1.8	21.0 $\pm$ 0.6
Phe	9.7 $\pm$ 0.7	16.6 $\pm$ 0.6	14.5 $\pm$ 1.0
Lys	25.4 $\pm$ 0.7	29.9 $\pm$ 1.7	27.1 $\pm$ 1.5
His	50.4 $\pm$ 5.0	29.7 $\pm$ 1.4	14.3 $\pm$ 1.4
Arg	11.5 $\pm$ 1.8	12.6 $\pm$ 0.7	15.4 $\pm$ 0.3

Table 3. Amino acid compositional difference-index values between GSH S-transferases of human brain and other tissues. Difference-index (DI) values were calculated by the method of Metzger *et al.* (1968).

GSH S-transferase of human tissues	Calculated DI value with human brain GSH S-transferases			Reference
	pI8.3 form	pI5.5 form	pI4.6 form	
Liver (pI8.9–7.8)	35.4	22.0	18.4	Kamisaka <i>et al.</i> (1975)
Liver (pI4.6)	24.3	15.6	8.0	Awasthi <i>et al.</i> (1980)
Lung (pI4.9)	24.9	16.4	7.4	Partridge <i>et al.</i> (1984)
Placenta (pI4.5)	26.3	16.6	6.7	Dao <i>et al.</i> (1984)
Brain (pI8.3)	—	14.2	20.7	Present study
Brain (pI5.5)	14.2	—	11.8	Present study
Brain (pI4.6)	20.7	11.8	—	Present study

Fig. 4. Double immunodiffusion of human brain GSH S-transferases

Centre wells: box A, antibodies raised against cationic GSH S-transferases of liver; box B, antibodies raised against anionic GSH S-transferase of lung; box C, antibodies raised against GSH S-transferase of placenta. Outer wells in boxes A, B and C: a, brain GSH S-transferase, pI5.5; b, brain GSH S-transferase, pI8.3; c, brain GSH S-transferase, pI4.6.

Table 4. Immunological cross-reactivities of human brain GSH S-transferases with antibodies against placental, liver and lung enzymes

For experimental details see the text. The subunit structures designated by Dao *et al.* (1984) for the placental, lung and liver enzymes are given in parentheses: A, M_r 26 500; B, M_r 24 500; C, M_r 22 500.

Antibodies raised against GSH S-transferases of other human tissues	Cross-reactivity of brain GSH S-transferases		
	pI8.3 form (M_r 26 500/26 500)	pI5.5 form (M_r 26 500/26 500)	pI4.6 form (M_r 24 500/22 500)
Placental enzyme (CC)	—	—	+
Lung enzyme (CC)	—	—	+
Cationic enzymes of liver (AB)	+	—	+

positional differences among the three brain enzymes. Additionally, the cationic enzyme of brain (pI8.3) has significant compositional differences from the cationic enzymes of liver (Kamisaka *et al.*, 1975). These differences, along with the observed differences in subunit structures, indicate that the cationic enzyme of brain is different from the cationic enzymes of liver. The amino acid composition of the anionic enzyme (pI4.6) is similar to the anionic enzymes of liver, placenta and lung.

Immunological properties

Results of immunological studies obtained by immunodiffusion (Fig. 4) and immunotitration are summarized in Table 4. The cationic GSH S-transferase (pI8.3) of brain cross-reacted with antibodies raised against the cationic GSH S-transferases of human liver, but not with the antibodies raised against the anionic GSH S-transferases of human lung or placenta. The anionic GSH S-trans-

Table 5. *Specific activities of human brain GSH S-transferases towards different substrates*
Experimental details are indicated in the text. Abbreviation: N.D., not detected.

	GSH S-transferase activity (units/mg of protein)		
	Cationic enzyme (pI8.3)	Anionic enzyme (pI5.5)	Anionic enzyme (pI4.6)
1-Chloro-2,4-dinitrobenzene	2.78	14.48	23.33
<i>p</i> -Nitrobenzyl chloride	0.07	1.18	0.45
1,2-Epoxy-3-(<i>p</i> -nitrophenoxy)propane	0.07	0.08	0.61
Ethacrynic acid	0.10	0.09	0.45
1,2-Dichloro-4-nitrobenzene	0.02	0.05	0.23
<i>p</i> -Nitrophenyl acetate	0.06	0.06	0.08
Sulphobromophthalein	N.D.	N.D.	0.11
Cumene hydroperoxide*	0.77	N.D.	N.D.

* GSH peroxidase activity at 37°C.

ferase (pI4.6) cross-reacted with antibodies raised against the anionic GSH S-transferases of human lung and placenta, as well as with the antibodies raised against the cationic GSH S-transferases of liver. The less-anionic enzyme (pI5.5) did not cross-react with antibodies raised against the anionic GSH S-transferases of lung and placenta, nor with the antibodies raised against the cationic GSH S-transferases of liver, indicating that this enzyme is immunologically different from the other two enzymes of brain.

Kinetic properties

The specific activities of the three GSH S-transferases of human brain were determined towards a variety of substrates (Table 5). 1-Chloro-2,4-dinitrobenzene appears to be the preferred substrate for all three forms of brain enzyme. The anionic enzyme (pI4.6) had higher specific activities towards all of the substrates examined, except for *p*-nitrobenzyl chloride. The less-anionic form (pI5.5) had higher specific activity for this substrate. The cationic enzyme (pI8.3) had much lower specific activity for most of the substrates tested, and was the only GSH S-transferase of brain that expressed GSH peroxidase activity.

Discussion

Human brain has three forms of GSH S-transferase that may provide protection to this organ from the toxic effects of a wide variety of xenobiotics. The cationic (pI8.3) enzyme expresses GSH peroxidase activity, and may play a role in the protection of brain tissue from oxidative stress. This enzyme accounts for only a small portion (<5%) of GSH S-transferase activity of brain, yet a significant amount (about 20%) of total brain GSH S-transferase protein exists in this form. It is

possible that this protein may be involved in non-catalytic functions suggested for GSH S-transferases in detoxication processes. The subunit composition of the cationic form (pI8.3) indicates that this enzyme is different from any other GSH S-transferase previously reported from human tissues. Cationic GSH S-transferases having similar pI values have been reported previously from lung (Partridge *et al.*, 1984) and liver (Kamisaka *et al.*, 1975); however, the cationic form of brain is a dimer of 26 500- M_r subunits whereas the cationic forms of liver are heterodimers of 26 500- M_r and 24 500- M_r subunits (Dao *et al.*, 1982), and the cationic form of lung is a homodimer of 24 500- M_r subunits (Partridge *et al.*, 1984). The enzyme form with pI5.5 also does not correspond to any previously characterized GSH S-transferase of human tissues. Even though the M_r value of the subunits of this form is similar to that of the cationic enzyme of brain (pI8.3), these two enzymes are different from each other. This is indicated by the differences in their immunological properties, amino acid compositions, catalytic properties and isoelectric points, which provide evidence for the presence of more than one type of 26 500- M_r subunit in human brain GSH S-transferases.

Two-dimensional gel-electrophoresis studies reveal the presence of three charge isomers of 26 500- M_r subunits and two each of 24 500- M_r and 22 500- M_r subunits in the GSH S-transferases of human brain. The enzyme form with pI8.3 is a homodimer of 26 500- M_r subunits, and the enzyme form with pI5.5 is a dimer of two different 26 500- M_r subunits, both of which are immunologically distinct from the 26 500- M_r subunits present in the cationic enzyme of brain. The presence of two 24 500- M_r subunits and two 22 500- M_r subunits in the more anionic form (pI4.6) raises the possibility that this form may actually be a mixture of several possible binary combinations of these four sub-

units, which may not have separated during the protocol used in this study.

We have previously suggested (Dao *et al.*, 1982, 1984) that GSH *S*-transferases of human liver, placenta and lung arise from the binary combinations of three different sizes of subunits designated as A (M_r 26 500), B (M_r 24 500) and C (M_r 22 500). The present studies indicate that the brain GSH *S*-transferases have considerable structural and immunological similarities with the GSH *S*-transferases of other human tissues. Immunological cross-reactivity of the cationic enzyme of brain (pI 8.3) with the cationic enzymes of liver (AB) may indicate that the 26 500- M_r subunit of the cationic enzyme of brain is similar to the A subunits of liver enzymes. The brain enzyme form with pI 5.5 is also composed of 26 500- M_r subunits, but these subunits are immunologically distinct from the A subunit of liver enzymes, and we have designated these subunits as A₁ and A₂ in order of increasing pI value as seen on two-dimensional gels. The immunological similarities of the pI 4.6 enzyme with the anionic enzymes of placenta and lung (CC) as well as with the cationic enzymes of liver (AB) may suggest its subunit structure to be BC. More probably, this form may be a mixture of several possible dimeric combinations of two B-type and two C-type subunits. Thus brain GSH *S*-transferases may originate from the dimeric combination of at least five and up to seven different subunits. Three of these subunits are similar to the A, B and C subunits of liver, placental and lung enzymes. The other two subunits, A₁ and A₂, present in the enzyme form with pI 5.5, are immunologically distinct from A and the rest of the subunits.

Heterogeneity in the similar-size subunits of rat GSH *S*-transferases has been described in several previous studies (Mannervik & Jensson, 1982; Frey *et al.*, 1983; Singh *et al.*, 1984). The present studies for the first time provide evidence for heterogeneity in the similar-size subunits of human GSH *S*-transferases. The underlying structural and genetic basis for the heterogeneity in the A-type, B-type and C-type subunits of human GSH *S*-transferases remains obscure. Whereas these studies provide the evidence for two immunologically distinct A-type subunits, the interrelationship between A₁ and A₂, between the two B-type subunits and that between the two C-type subunits remains to be elucidated. Deamidation *in vivo* of a single gene product has been suggested to be the cause of heterogeneity among the GSH *S*-transferases of human liver (Kamisaka *et al.*, 1975), and it might be tempting to suggest that the charge heterogeneity seen among the subunits of human brain GSH *S*-transferases could arise by this process. However, the existence of at least two immunologically distinct A-type subunits in brain

GSH *S*-transferases clearly indicates that deamidation *in vivo* alone cannot explain this heterogeneity.

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References

- Awasthi, Y. C. & Dao, D. D. (1981) *Placenta Suppl.* **3**, 289–301
- Awasthi, Y. C., Dao, D. D., Lal, A. K. & Srivastava, S. K. (1979) *Biochem. J.* **177**, 471–476
- Awasthi, Y. C., Dao, D. D. & Saneto, R. P. (1980) *Biochem. J.* **191**, 1–10
- Booth, J., Boyland, E. & Sims, P. (1961) *Biochem. J.* **79**, 516–524
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Chasseaud, L. F. (1979) *Adv. Cancer Res.* **29**, 175–274
- Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106
- Dao, D. D., Partridge, C. A. & Awasthi, Y. C. (1982) *IRCS Med. Sci. Libr. Compend.* **10**, 175
- Dao, D. D., Partridge, C. A., Kurosky, A. & Awasthi, Y. C. (1984) *Biochem. J.* **221**, 33–41
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
- Fjellstedt, T. A., Allen, R. H., Duncan, B. K. & Jakoby, W. B. (1973) *J. Biol. Chem.* **248**, 3702–3707
- Frey, A. B., Friedberg, T., Oesch, F. & Kreibich, G. (1983) *J. Biol. Chem.* **258**, 11321–11325
- Habig, W. H., Pabst, M. J. & Jakoby, W. B. (1974) *J. Biol. Chem.* **249**, 7130–7139
- Jakoby, W. B. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* **46**, 383–414
- Kamisaka, K., Habig, W. H., Ketley, J. N., Arias, I. M. & Jakoby, W. B. (1975) *Eur. J. Biochem.* **60**, 153–161
- Keen, J. H. & Jakoby, W. B. (1978) *J. Biol. Chem.* **253**, 5654–5657
- Kurosky, A., Markel, D. E. & Peterson, J. W. (1977) *J. Biol. Chem.* **252**, 7257–7264
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Litwack, G., Ketterer, B. & Arias, I. M. (1971) *Nature (London)* **234**, 466–467
- Mannervik, B. & Jensson, H. (1982) *J. Biol. Chem.* **257**, 9909–9912
- Marcus, C. J., Habig, W. H. & Jakoby, W. B. (1978) *Arch. Biochem. Biophys.* **188**, 287–293
- Metzger, H., Shapiro, M. B., Mosimann, J. E. & Vinton, J. E. (1968) *Nature (London)* **219**, 1166–1168
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Ouchterlony, Ö. (1958) *Prog. Allergy* **5**, 1–78
- Partridge, C. A., Dao, D. D. & Awasthi, Y. C. (1984) *Lung* **162**, 27–36
- Pierce, S. & Tappel, A. L. (1978) *Biochim. Biophys. Acta* **523**, 27–36
- Prohaska, J. R. & Ganther, H. E. (1977) *Biochem. Biophys. Res. Commun.* **76**, 437–445
- Simons, P. C. & Vander Jagt, D. L. (1977) *Anal. Biochem.* **82**, 334–341
- Singh, S. V., Partridge, C. A. & Awasthi, Y. C. (1984) *Biochem. J.* **221**, 609–615