The effects of selenium and copper deficiencies on glutathione *S*-transferase and glutathione peroxidase in rat liver

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Selenium (Se) deficiency in rats produced significant increases in the activity of hepatic glutathione *S*-transferase (GST) with 1-chloro-2,4-dinitrobenzene as substrate and in various GST isoenzymes when determined by radioimmunoassay. These changes in GST activity and concentration were associated with Se deficiency that was severe enough to provoke decreases of over 98 % in hepatic Se-containing glutathione peroxidase activity (Se-GSHpx). However, decreases in hepatic Se-GSHpx of 60 % induced by copper (Cu) deficiency had no effect on GST activity or concentration. Increased GST activity in Se deficiency has previously been postulated to be a compensatory response to loss of Se-GSHpx, since some GSTs have a non-Se-glutathione peroxidase (non-Se-GSHpx) activity. However, the GST isoenzymes determined in this study, GST Yb₁Yb₁, GST YcYc and GST YaYa, are known to have up to 30-fold differences in non-Se-GSHpx activity, but they were all significantly increased to a similar extent in the Se-deficient rats.

INTRODUCTION

Selenium (Se) deficiency in animals initially causes a decrease in the cytosolic activity of the seleno-enzyme glutathione peroxidase (Se-GSHpx), thus compromising the cell's antioxidant systems (Hoekstra, 1975; Reiter & Wendel, 1984). More prolonged Se deficiency increases glutathione *S*-transferase (GST) activity in liver, kidney and duodenal mucosa of rats and mice (Lawrence *et al.*, 1978; Reiter & Wendel, 1983, 1985; Masukawa *et al.*, 1984). Some GSTs, in addition to their ability to conjugate GSH with electrophiles such as 1-chloro-2,4-dinitrobenzene (CDNB), have a glutathione peroxidase activity (non-Se-GSHpx). This alternative peroxidase pathway requires organic hydroperoxides as substrate, but, in contrast with Se-GSHpx, not H₂O₂ (Prohaska & Ganther, 1977). This elevation of GST activity by Se deficiency has been postulated to be a compensatory response that helps correct for the decreased Se-GSHpx activity by provision of non-Se-GSHpx activity (Lawrence *et al.*, 1978; Masukawa *et al.*, 1984; Mehlert & Diplock, 1985). The effects on Se-GSHpx and GST activity are not inextricably linked, since induced hepatic GST activity in Se-deficient mice can be restored to normal values by small doses of Se that do not repair cytosolic Se-GSHpx activity. Severe Se deficiency may therefore affect GST activity by a mechanism independent of changes in cytosolic Se-GSHpx activity (Reiter & Wendel, 1984).

GSTs exist in multiple forms in rat liver (Habig *et al.*, 1974; Mannervik, 1985). The major cytosolic forms consist of homo- or hetero-dimers of the subunits Ya (*M_r* 25 500), Yc (*M_r* 27 500), Yb₁ and Yb₂ (both *M_r* 26 300) (Hayes & Mantle, 1986). These subunits have also been named with, respectively, the arabic numerals 1, 2, 3 and 4 (Mannervik, 1985). Heterodimers can only be formed between subunits that have extensive sequence

homology, and the subunits that can hybridize are thought to arise from the same gene family (Ketterer *et al.*, 1983; Hayes, 1984). The major GST isoenzymes are GST YaYa, GST YaYc, GST YcYc, GST Yb₁Yb₁, GST Yb₁Yb₂ and GST Yb₂Yb₂ (Hayes, 1983). The GSTs have differing non-Se-GSHpx activities, dependent upon the activity of their individual subunits, with the Ya and Yc subunits having the highest activity (Meyer *et al.*, 1985; Mannervik, 1985).

Lawrence *et al.* (1978) demonstrated induction of GST YaYc activity in rat liver in response to prolonged Se deficiency. Mehlert & Diplock (1985), using SDS/polyacrylamide-gel electrophoresis and densitometric measurements, have shown induction of GSTs YaYc and YcYc by Se deficiency with a preferential increase in the Yc subunit relative to Ya. However, it is still not clear (a) whether the induction of GST activity in Se deficiency is due to activation of the enzymes or an increase in the mass of enzyme protein, and (b) if the increase is a consequence of or independent of changes in cytosolic Se-GSHpx activity. In the present paper we report the use of specific radioimmunoassays (Beckett *et al.*, 1986) to determine GST YaYa, Yb₁Yb₁ and YcYc in livers from rats consuming diets deficient or adequate in Se and/or Cu.

MATERIALS AND METHODS

Chemicals

The sources of chemicals and proteins for the GST radioimmunoassays have been described previously (Beckett *et al.*, 1986); antisera were raised against GST isolated from Wistar rats (Hayes, 1984). NADPH, GSH, cumene hydroperoxide and vitamins were obtained from the Sigma Chemical Co., Poole, Dorset, U.K. CDNB was purchased from the Aldrich Chemical Co., Gilling-

Abbreviations used: GST, glutathione *S*-transferase; GSHpx, glutathione peroxidase; CDNB, 1-chloro-2,4-dinitrobenzene.

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Table 1. Composition of synthetic diet

Constituent	Composition (g/100 g of diet)
Sucrose	71.8
Amino acids*	18.0
Lard	3.5
Cod liver oil BP	1.5
Vitamins, minerals and trace elements†	5.2

* The amino acid mixture contained the following per 113.0 g of mix: L-alanine, 5.0 g, L-arginine hydrochloride, 6.0 g, L-asparagine, 4.0 g, L-aspartic acid, 5.0 g, L-cystine, 2.0 g, glycine, 5.0 g, L-histidine, 3.0 g, L-isoleucine, 5.0 g, L-leucine, 7.5 g, L-lysine hydrochloride, 7.0 g, L-methionine, 4.0 g, L-monosodium glutamate, 30.0 g, L-phenylalanine, 5.0 g, L-proline, 4.0 g, L-serine, 5.0 g, L-threonine, 5.0 g, L-tryptophan, 1.5 g, L-tyrosine, 3.0 g, and L-valine, 6.0 g.

† The vitamins, minerals and trace elements used were as described by Abdel-Rahim *et al.* (1986), and Na₂SeO₃ or CuSO₄·5H₂O was omitted where appropriate.

ham, Dorset, U.K. Purified amino acids were supplied by Forum Chemicals, Reigate, Surrey, U.K. All other reagents were from BDH Chemicals, Poole, Dorset, U.K., and were of AnalaR grade or better.

Animals and diets

Weanling male Hooded Lister rats of the Rowett strain were used in all experiments except one in which Wistar rats (Bantin and Kingman, Hull, U.K.) were used. The animals were group-housed in plastic cages with stainless-steel grid tops and floors; food and distilled water were available *ad libitum*. Diet (Table 1) based on purified amino acids was provided for the rats from weaning. Se-deficient groups (–Se) received the basal diet containing less than 0.005 mg of Se/kg, and the control groups (+Se) the same diet supplemented with 0.1 mg of Se/kg as Na₂SeO₃. All diets contained 200 mg of α -tocopherol acetate/kg and 10 mg of Cu/kg as CuSO₄·5H₂O, except –Cu diets (no CuSO₄·5H₂O added), which contained less than 0.15 mg of Cu/kg. The experimental rats were obtained from dams that consumed standard commercial rat diet (Labsure, Cambridge, U.K.), except for the Cu-deficiency experiment, when the dams consumed a *Torula*-yeast-based diet (Abdel-Rahim *et al.*, 1986) containing 0.02 mg of Se/kg and 2 mg of Cu/kg to enhance subsequent effects of the Se- and Cu-deficient diets. The Se and Cu concentrations in the diets were just sufficient to prevent reproductive problems in the rats (Underwood, 1977).

At 4 or 6 weeks from weaning the experimental animals were anaesthetized with diethyl ether and bled by cardiac puncture into heparinized Vacutainers (Beckton Dickson, Oxford, U.K.). Thereafter livers were perfused via the hepatic portal vein with 0.15 M-KCl (4 °C) to wash out residual blood, frozen in liquid N₂ and stored at –85 °C.

Radioimmunoassay of GST in liver cytosols

Samples of individual livers were thawed, chopped into small pieces and homogenized in a Teflon-pestle/

glass-body homogenizer, with ice-cold 20 mM-sodium phosphate buffer, pH 7.4, to give a 25% (w/v) homogenate. Cytosols were prepared by centrifugation of the homogenates at 100 000 *g* for 1 h at 4 °C. The concentrations of GST YaYa, Yb₁Yb₁ and YcYc were measured by using a double-antibody radioimmunoassay. The details of the assay for GST YaYa have been published previously (Beckett *et al.*, 1986), and GST Yb₁Yb₁ and YcYc concentrations were measured in a similar fashion by using antisera, specific for GSTs Yb₁Yb₁ and YcYc, raised in rabbits. The antisera had cross-reactivities of less than 4% with GSTs other than the immunogen, with the exception of the Yb₁Yb₂ heterodimer, which showed 23% cross-reactivity in the GST Yb₁Yb₁ assay.

Enzyme and other analyses

Total GST activity with CDNB as substrate was determined at 25 °C in the presence of 5 mM-GSH (Habig *et al.*, 1974). GSHpx activity was assayed in the presence of 5 mM-GSH with 0.25 mM-H₂O₂ or 1.5 mM-cumene hydroperoxide as substrate at 25 °C. CuZn superoxide dismutase activity was determined by using an inhibition assay (Arthur & Boyne, 1985). Protein concentrations in cytosols were assayed by the biuret method, and Se and Cu concentrations in acid digests of diets were monitored by methods described previously (Abdel-Rahim *et al.*, 1986). Statistical comparisons between –Se and +Se groups were made by using Student's *t* test. Comparisons in the trial examining the effects of Cu and Se deficiency were made by analysis of variance and test of least significant difference.

RESULTS

Glutathione peroxidase and superoxide dismutase activities

Table 2 shows hepatic GSHpx activities of rats in four experiments. Consumption of –Se diet by Hooded Lister rats for 4 or 6 weeks caused at least a 98% decrease in hepatic Se-GSHpx activity determined with H₂O₂ as substrate (Expts. 1 and 2). In Expt. 3, the effects of both Se and Cu deficiency in Hooded Lister rats were studied. In this case Se-GSHpx activity in +Se rats was found to be lower than in other experiments owing to the restricted Se intake of their dams. Low dietary Cu caused a significant decrease in liver CuZn superoxide dismutase in rats consuming both –Se and +Se diets. Cu deficiency also significantly decreased Se-GSHpx activity, confirming the observations made by Jenkinson *et al.* (1982). In all experiments GSHpx activity with cumene hydroperoxide as substrate was higher in –Se rats than the activity with H₂O₂ as substrate (Table 2). Expt. 4 indicated that the effects of Se deficiency on GSHpx activity were the same in Wistar and Hooded Lister rats.

Glutathione S-transferases

Total hepatic GST activity determined with CDNB as substrate was increased by Se deficiency in all experiments (Table 3). Increased total GST activity was reflected in significant increases in the masses of GSTs YaYa, Yb₁Yb₁ and YcYc determined by radioimmunoassay (Table 4). Although the Wistar rats had higher concentrations of GSTs than did the Hooded Lister rats when receiving adequate supplies of Se, GST mass and

Table 2. Glutathione peroxidase activity and superoxide dismutase activity in livers from Se- and/or Cu-deficient and -sufficient rats

Rats were offered +Se and -Se diets as described in the Materials and methods section. GSHpx activity was measured in Expts. 1-4 with either H₂O₂ (Se-GSHpx) or cumene hydroperoxide (Se-GSHpx and non-Se-GSHpx). Superoxide dismutase was only determined in Expt. 3 since in this instance dietary Cu deficiency was induced. Results are means $\pm$ S.E.M. with numbers of animals used in parentheses. Significantly different from corresponding +Se groups: *** P < 0.001; ** P < 0.005. Significance of difference between +Cu and -Cu groups: † P < 0.005; †† P < 0.001.

Expt. no.	Rat strain	Treatment time (weeks)	Substrate ...	GSHpx activity (units/mg of protein)			
				H ₂ O ₂		Cumene hydroperoxide	
				+Se	-Se	+Se	-Se
1	Lister	4		1.158 $\pm$ 0.070 (8)	0.008 $\pm$ 0.002 (10)***	1.226 $\pm$ 0.054	0.056 $\pm$ 0.007***
2	Lister	6		1.365 $\pm$ 0.068 (7)	0.006 $\pm$ 0.002 (7)***	1.586 $\pm$ 0.079	0.133 $\pm$ 0.017***
3	Lister (+Cu)†	6		0.627 $\pm$ 0.063 (5)	0.003 $\pm$ 0.001 (5)***	0.643 $\pm$ 0.063	0.112 $\pm$ 0.011***
	Lister (-Cu)†§	6		0.266 $\pm$ 0.036 (6)†	0.005 $\pm$ 0.002 (6)***	0.271 $\pm$ 0.037†	0.124 $\pm$ 0.007**
4	Wistar	6		1.549 $\pm$ 0.045 (6)	0.010 $\pm$ 0.006 (6)***	1.776 $\pm$ 0.035	0.261 $\pm$ 0.041***

Expt. no.	Rat strain	Treatment time (weeks)	Superoxide dismutase (μ g/mg of protein)	
			+Se	-Se
3	Lister (+Cu)†	6	8.55 $\pm$ 1.60 (5)	13.02 $\pm$ 1.38 (5)
	Lister (-Cu)†§	6	0.99 $\pm$ 0.12 (6)††	1.31 $\pm$ 0.17 (6)††

† Rats derived from dams receiving diets with 0.02 mg of Se/kg and 2 mg of Cu/kg.

§ Experimental diets contained less than 0.15 mg of Cu/kg.

Table 3. Cytosolic GST activity in livers from Se- and/or Cu-deficient and -sufficient rats

Animal numbers and dietary treatments are as described in Table 2. GST activity was measured at 25 °C with CDNB as substrate, and results are expressed as μmol of conjugate/min per mg of protein. Significantly different from corresponding +Se group: ** $P < 0.005$; *** $P < 0.001$.

Expt. no.	Rat strain	Treatment time (weeks)	Total GST activity ($\mu\text{mol}/\text{min}$ per mg of protein)	
			+ Se	– Se
1	Lister	4	0.351 ± 0.022	$0.529 \pm 0.045^{**}$
2	Lister	6	0.281 ± 0.013	$0.624 \pm 0.032^{***}$
3	Lister (+ Cu)	6	0.281 ± 0.017	$0.687 \pm 0.026^{***}$
	Lister (– Cu)	6	0.234 ± 0.010	$0.726 \pm 0.029^{***}$
4	Wistar	6	0.400 ± 0.017	$0.961 \pm 0.075^{***}$

activity were increased to a similar extent in Se-deficient rats of the two strains.

DISCUSSION

Low hepatic GSHpx activity with H_2O_2 as substrate in –Se rats was due to loss of Se-GSHpx activity (Table 2). Some GSHpx activity remained in –Se rats when cumene hydroperoxide was used as substrate. Previous work by Lawrence *et al.* (1978) has shown this is probably due to non-Se-GSHpx activity of GSTs. Total hepatic cytosolic GST activity (with CDNB as substrate) was significantly increased in the rats by consumption of –Se diets for 4 or 6 weeks (Table 3). This increase in GST provided more non-Se-GSHpx in Se deficiency (Lawrence *et al.*, 1978; Mehler & Diplock, 1985). However, despite a substantial decrease in hepatic Se-GSHpx activity in Cu-deficient rats (Expt. 3) there was no significant change in total hepatic GST activity or of the hepatic concentrations of GSTs YaYa, Yb₁Yb₁ and YcYc compared with the +Cu rats.

Reiter & Wendel (1985) demonstrated that when Se deficiency was severe enough to decrease hepatic Se-GSHpx activity by over 90% from levels found in Se-adequate mice the deficiency 'modulated' the activity of several enzymes involved in drug metabolism, including GSTs, by a mechanism independent of effects of Se on Se-GSHpx activity. Prolonged Se deficiency affects, either indirectly or directly, other enzyme and metabolic activities such as haem oxygenase activity, glucose and phospholipid metabolism and neutrophil function (Fischer & Whanger, 1977; Burk, 1983; Arthur *et al.*, 1986). Thus the effects of severe Se deficiency on hepatic GST concentration reported in the present paper may be related to a more general effect of the deficiency on cell metabolism, rather than a specific response to compensate for loss of Se-GSHpx activity.

With the possible exception of induction of GST Yb₁Yb₁ in –Cu rats (Expt. 3), the hepatic concentration of each of the GST isoenzymes was increased to a similar extent by Se deficiency. The mean ratios hepatic GST in –Se group/hepatic GST in +Se group for all experiments (Table 4) were respectively 1.93, 2.14 and 1.83 for GSTs YaYa, Yb₁Yb₁ and YcYc. This occurred despite the individual GSTs having up to 30-fold differences in non-Se-GSHpx activity, e.g. GST YcYc has 30 times more activity than GST Yb₁Yb₁ with cumene hydroperoxide as substrate (Mannervik & Jensson, 1982;

Meyer *et al.*, 1985). Hence the induction of GSTs by Se deficiency (Table 4) was not a specific increase of GST isoenzymes with high non-Se-GSHpx activity.

Mehler & Diplock (1985), using conventional chromatographic techniques and densitometric measurements, demonstrated a selective induction of the Yc subunit of GSTs by Se deficiency, and preferential induction of its hetero- and homo-dimers GST YaYc and GST YcYc was reported. Our findings, when radioimmunoassays were used to demonstrate increases of three GST isoenzymes to a similar extent, do not confirm these observations. Chromatographic procedures, as pointed out by Mehler & Diplock (1985), may introduce errors by causing variable losses of GST isoenzymes. The use of radioimmunoassays in our study did not involve any chromatographic steps and thus probably did not introduce variable recovery of GST isoenzymes. This is a plausible explanation for the conflicting results of the two studies, but there is not absolute proof that this is the case.

The extent of induction of GSTs was the same in Wistar and Hooded Lister rats despite the latter strain having lower hepatic GST concentrations in +Se rats (+Se groups, Tables 3 and 4). Thus initial hepatic GST concentrations and presumably total non-Se-GSHpx activity had no influence on the ability of Se deficiency to increase total GST protein.

The three GST subunit types that are recognized by the radioimmunoassays probably account for up to 60% of total GST in the liver cytosol of normal rats (Hayes, 1983). It seems unlikely that the concentrations of the GSTs that were not determined in this study were preferentially increased by Se deficiency. A plot of total GST activity against the sum of GSTs YaYa, Yb₁Yb₁ and YcYc/mg of protein for all experimental groups (Fig. 1) gives a linear relationship: $y = 0.0171x + 0.0408$, with $r = 0.9381$ and $P < 0.001$, where y is total GST activity (μmol of CDNB conjugated/min per mg of protein) (Table 3) and x is GST YaYa + Yb₁Yb₁ + YcYc ($\mu\text{g}/\text{mg}$ of protein). If Se deficiency caused preferential induction or no change in the amount of any of the GSTs that were not determined, such a linear relationship would be unlikely to occur. Moreover, since GST YaYa and GST YcYc were similarly induced by Se deficiency, it is highly unlikely that the heterodimer GST YaYc would be preferentially induced.

Under certain circumstances different GST subunits can be induced independently of each other (Schramm

Table 4. Concentrations of GST YaYa, GST Yb₁Yb₁ and GST YcYc in livers from Se- and/or Cu-deficient and -sufficient rats

The concentrations of GST were determined by radioimmunoassay and are expressed as $\mu\text{g}/\text{mg}$ of cytosolic protein. Animal numbers and dietary treatments are described in Table 2. Significantly different from corresponding +Se groups: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; **** $P < 0.001$.

Expt. no.	Rat strain	Treat-ment time (weeks)	GST ($\mu\text{g}/\text{mg}$ of protein)			
			GST YaYa		GST Yb ₁ Yb ₁	
			+Se	-Se	+Se	-Se
1	Lister	4	13.49 $\pm$ 1.47	20.50 $\pm$ 1.22**	1.89 $\pm$ 0.07	2.22 $\pm$ 0.13*
2	Lister	6	11.46 $\pm$ 0.91	20.33 $\pm$ 0.53***	1.41 $\pm$ 0.06	2.17 $\pm$ 0.16***
3	Lister (+Cu)	6	12.45 $\pm$ 0.92	25.75 $\pm$ 1.68***	2.36 $\pm$ 0.49	5.68 $\pm$ 0.38**
3	Lister (-Cu)	6	11.83 $\pm$ 1.26	29.22 $\pm$ 1.62***	1.82 $\pm$ 0.22	6.48 $\pm$ 0.75***
4	Wistar	6	19.55 $\pm$ 2.89	35.71 $\pm$ 3.93*†	5.02 $\pm$ 0.38	10.04 $\pm$ 0.71***
					GST YcYc	
					+Se	-Se
					0.89 $\pm$ 0.07	1.07 $\pm$ 0.07
					1.54 $\pm$ 0.11	2.91 $\pm$ 0.19***
					1.72 $\pm$ 0.54	4.24 $\pm$ 0.20***
					2.27 $\pm$ 0.29	4.25 $\pm$ 0.20***
					5.22 $\pm$ 0.37	9.11 $\pm$ 0.74***

Fig. 1. Regression of total hepatic GST activity (with CDNB as substrate) against sum of concentrations GST YaYa + Yb₁Yb₁ + YcYc determined by radioimmunoassay

Total GST activity is taken from Table 3 and the sum of GST YaYa + Yb₁Yb₁ + YcYc was calculated from data in Table 4. ▲, Expt. 1; △, Expt. 2; ○, Expt. 3; ●, Expt. 4.

et al., 1985). Phenobarbital preferentially induces Ya and Yb₁ subunits of GST (Hayes *et al.*, 1979), but will also to a lesser extent induce Yb₂ and Yc (Ding *et al.*, 1985, 1986). Subunits with high non-Se-GSHpx activity could therefore have been preferentially induced by Se deficiency. However, in the present study hepatic GSTs YaYa, Yb₁Yb₁ and YcYc and probably other GSTs were increased to a similar extent by Se deficiency. Thus the results support the hypothesis that increases in hepatic GST activity in Se deficiency are as a result of a general effect of the deficiency on drug-metabolizing enzymes and other metabolic pathways in the cells (Reiter & Wendel, 1984) rather than a specific response to correct for loss of Se-GSHpx activity in Se deficiency. However, this does not exclude a functional role for non-Se-GSHpx activity of GSTs in normal metabolism.

REFERENCES

- Abdel-Rahim, A. G., Arthur, J. R. & Mills, C. F. (1986) *J. Nutr.* **116**, 403–411
- Arthur, J. R. & Boyne, R. (1985) *Life Sci.* **36**, 1569–1575
- Arthur, J. R., Boyne, R., Morrice, P. C. & Nicol, F. (1986) *Proc. Nutr. Soc.* **45**, 63A
- Beckett, G. J., Hunter, J. E. & Hayes, J. D. (1986) *Clin. Chim. Acta* **161**, 69–79
- Burk, R. F. (1983) *Annu. Rev. Nutr.* **3**, 53–70
- Ding, G. J.-F., Lu, A. Y. H. & Pickett, C. B. (1985) *J. Biol. Chem.* **260**, 13268–13271
- Ding, G. J.-F., Ding, V. S.-H., Rodkey, J. A., Bennett, C. D., Lu, A. Y. H. & Pickett, C. B. (1986) *J. Biol. Chem.* **261**, 7952–7957
- Fischer, W. C. & Whanger, P. D. (1977) *J. Nutr.* **107**, 1493–1501
- 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. (1984) *Biochem. J.* **224**, 839–852

- Hayes, J. D. & Mantle, T. J. (1986) *Biochem. J.* **237**, 731–740
- Hayes, J. D., Strange, R. C. & Percy-Robb, I. W. (1979) *Biochem. J.* **181**, 699–708
- Hoekstra, W. G. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2083–2089
- Jenkinson, S. G., Lawrence, R. A., Burk, R. F. & Williams, D. M. (1982) *J. Nutr.* **112**, 197–204
- Ketterer, B., Beale, D., Taylor, J. B. & Meyer, D. J. (1983) *Biochem. Soc. Trans.* **11**, 466–467
- Lawrence, R. A., Parkhill, L. K. & Burk, R. F. (1978) *J. Nutr.* **108**, 981–987
- Mannervik, B. (1985) *Adv. Enzymol. Relat. Areas Mol. Biol.* **57**, 357–417
- Mannervik, G. & Jensson, H. (1982) *J. Biol. Chem.* **257**, 9909–9912
- Masukawa, T., Nishimura, T. & Iwata, H. (1984) *Biochem. Pharmacol.* **33**, 2635–2639
- Mehlert, A. & Diplock, A. T. (1985) *Biochem. J.* **227**, 823–831
- Meyer, D. J., Beale, D., Tan, K. H., Coles, B. & Ketterer, B. (1985) *FEBS Lett.* **184**, 139–143
- Prohaska, J. R. & Ganther, H. E. (1977) *Biochem. Biophys. Res. Commun.* **76**, 437–445
- Reiter, R. & Wendel, A. (1983) *Biochem. Pharmacol.* **32**, 3063–3067
- Reiter, R. & Wendel, A. (1984) *Biochem. Pharmacol.* **33**, 1923–1928
- Reiter, R. & Wendel, A. (1985) *Biochem. Pharmacol.* **34**, 2287–2290
- Schramm, H., Robertson, L. W. & Oesch, F. (1985) *Biochem. Pharmacol.* **34**, 3735–3739
- Underwood, E. A. (1977) *Trace Elements in Human and Animal Nutrition*, pp. 83–84 and 321–322, Academic Press, New York and London

Received 25 February 1987/27 July 1987; accepted 11 August 1987