The glutathione S-transferases in selenium and vitamin E deficiency

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1. Preliminary experiments confirmed the work of others showing that the total glutathione peroxidase (GSH-px) activity of rat liver supernatant fraction may be resolved into two peaks of activity (peaks I and II) by gel filtration, and that peak I is the selenium-containing enzyme and peak II is another peroxidase indistinguishable from glutathione S-transferase (GST). 2. In selenium and vitamin E deficiency, the total activity of the GSH-px became very low, and the total activity of GST with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate was enhanced. Study of the time course of these changes as deficiency progressed indicated that the stimulus for the rise in GST(CDNB) activity was the fall in GSH-px activity which preceded it. 3. The peroxidase activity of GST was found to reside only in the GST AA, B and B₂ forms of the enzyme, which were shown to be respectively a homodimer of the Yc subunit, a homodimer of the Ya subunit and a heterodimer of the YaYc subunit. 4. As vitamin E and selenium deficiency progressed, the B₂ and AA forms of the enzyme showed enhanced activity, which was interpreted as implying that the Yc subunit of the enzyme becomes enriched as a consequence of the withdrawal of selenium from the animal's diet. Densitometric measurements of the Yc and Ya subunits confirmed that the amount of the Yc subunit was nearly doubled in selenium deficiency, relative to the Ya subunit.

The only mammalian enzyme that has been shown to be dependent for its activity on selenium is glutathione peroxidase (GSH-px) (Hoekstra *et al.*, 1974). The function of the enzyme *in vitro* is to catalyse the reduction of H₂O₂ and lipid hydroperoxides so that the proliferation of oxygen-derived radical species is prevented (Diplock, 1981). It was shown by Lawrence & Burk (1976) that the liver of selenium-deficient rats, although lacking GSH-px, contained another peroxidase that was capable of catalysing the reduction of lipid hydroperoxides but not of H₂O₂. Prohaska & Ganther (1977) demonstrated that the selenium-independent GSH-px activity was associated in chromatographic fractions with a GST, and it was presumed that a form, or forms, of GST was also capable of exhibiting this lipid peroxidase activity. The GSTs are widely distributed in nature as a

group of enzymes that play an important role in the detoxification of chemicals alien to the organism concerned, as well as being involved in the elimination of endogenous substrates such as steroids (Jakoby & Habig, 1980). The most extensively studied enzymes are those of the rat liver soluble fraction, from which more than six GSTs have been isolated.

The nomenclature of the GSTs is at present under revision because of difficulties arising from the complexity of the enzyme structure involved and their overlapping substrate specificities. The previously isolated six transferases were designated AA, A, B, C, D and E (Jakoby & Habig, 1980); all the transferases are dimers and their monomeric constituents fall into three categories, defined by their apparent-*M_r* values determined by SDS/polyacrylamide-gel electrophoresis: the Ya subunit has an *M_r* of 22000, the Yb subunit an *M_r* of 23500, and the Yc subunit an *M_r* of 25000. GSTs A, C, D and E are homodimers of Yb, and GST AA is a homodimer of Yc (Ketterer *et al.*, 1982). GST B has been further resolved into two forms, one of which is a Ya homodimer and the other a

Abbreviations used : GSH-px, glutathione peroxidase; GST, glutathione S-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 3,4-dichloronitrobenzene; SDS, sodium dodecyl sulphate.

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heterodimer of Ya and Yc (called respectively B₁ and B₂) (Carne *et al.*, 1979; Hayes *et al.*, 1979). The B transferase is remarkable because it occurs in high concentration (calculated to be 0.1 mM) in the liver soluble fraction (Tipping & Ketterer, 1981) and because it possesses properties other than its GST activity. Thus the selenium-independent GSH-px activity is associated with both the Ya and Yc monomers in the B₁ and B₂ forms of the transferase (Ketterer *et al.*, 1982), and the physiologically and pharmacologically important high-affinity binding properties of the transferases for substrates with lipophilic moieties (Ketterer *et al.*, 1978) are also associated with the B-form of the enzyme, hence its earlier name 'ligandin' (Litwack *et al.*, 1971).

The present paper describes a study of the changes in activity and subunit composition of the GSTs during the onset of selenium deficiency, and demonstrates an apparent increase in the activity of the Yc subunit, shown by an increase in the activity of transferases A and B₂ relative to the other transferases, as the selenium deficiency became more severe. A preliminary report of this work has already been published (Diplock, 1984).

Experimental

Chemicals

CDNB and DCNB were obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K. Cumene hydroperoxide and iodoheptane were purchased from Koch-Light Laboratories, Colnbrook, Bucks., U.K. The biochemicals used were obtained from Sigma Chemical Co., Poole, Dorset, U.K., or from BDH Biochemicals, Poole, Dorset, U.K. Sephadex G-25 and epoxy-activated Sepharose 6B were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden, and the hydroxyapatite Bio-Gel HTP from Bio-Rad Laboratories, Watford, Herts., U.K. S-Hexylglutathione was synthesized by method A of Vince *et al.* (1971).

Animals and diets

Weanling male Wistar rats were given the selenium- and vitamin E-deficient diet based on dried torula yeast (Lake States Yeast Corp., Rhinelander, WI, U.S.A.) (Diplock *et al.*, 1971), or that diet supplemented with all-*rac*- α -tocopheryl acetate (100 mg/kg). Half the rats in each dietary group were given distilled water to drink and half were given distilled water containing 0.1 p.p.m. of selenium as Na₂SeO₃. The rats were killed at the time stated after the beginning of the dietary regimen and their livers were removed and placed at once in ice-cold 0.25M-sucrose solution. No gross pathology could be discerned in the livers of the vitamin E- and selenium-deficient rats, and

experience with this diet and this strain of rats has indicated that, after 26–28 days of the experimental diet, all the livers can be expected to show the respiratory decline lesion first described by Schwarz & Folz (1957). In other experiments (J. Buttriss & A. T. Diplock, unpublished work) the mean α -tocopherol concentration in the livers of similar rats was $0.34 \pm 0.07 \mu\text{g/g}$ of wet tissue, measured by the h.p.l.c. method of Buttriss & Diplock (1983), and the selenium concentration was $90 \pm 15 \text{ ng/g}$ of wet tissue, as determined by the fluorimetric method of Olson *et al.* (1975); in corresponding rats given the selenium and vitamin E supplements, the liver α -tocopherol concentration was $9.67 \pm 1.63 \mu\text{g/g}$ of wet tissue and the selenium concentration was $350 \pm 10 \text{ ng/g}$ of wet tissue.

Enzyme assays

GST activities were measured by the method of Habig *et al.* (1974). Two transferase activities were measured; the first, with CDNB [GST(CDNB)] as substrate gave a measure of the total transferase activity, since all the forms of the enzyme catalyse the conjugation of CDNB. The second, with DCNB [GST(DCNB)] gave a measure of GSTs A and C. The assays were carried out at 30°C in a thermostatically controlled cuvette, and enzyme activities were expressed as nkat/ml or nkat/mg of protein as indicated in the individual experiments. GSH-px activity was measured by the coupled assay of Paglia & Valentine (1967), using H₂O₂ [GSH-px(H₂O₂)] and/or cumene hydroperoxide [GSH-px(Cum)] as substrate, and enzyme activities were expressed as nkat/ml or nkat/mg of protein. The protein content of the fractions was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard, or, where an indication of the protein content of column eluates was required, the A₂₈₀ was measured.

Separation methods

Livers were dried rapidly on tissue, and weighed portions were homogenized in 0.25M-sucrose solution using the homogenizer described by Caygill *et al.* (1971), which ensured reproducibility of the homogenization procedure, to give a 40% (w/v) homogenate. The homogenate was centrifuged for 1 h at 100000g in the Beckman S-70 centrifuge; floating lipids were removed from the surface of the fluid in the tube by suction, and the supernatant fraction was used in the experiments.

In a preliminary experiment, liver supernatant fractions were prepared from rats given the four dietary treatments, and 10 ml portions were applied to a column (4.4 cm $\times$ 30 cm) of Sephadex G-150 which had been equilibrated with 50 mM-Tris/HCl buffer, pH 8.6, containing 1 mM-EDTA and 0.1 M-K₂HPO₄. The column was eluted

with 400ml of the same buffer at a flow rate of 60ml/h and 5ml fractions were collected. GSH-px(H_2O_2), GSH-px(Cum) and GST(CDNB) activities were measured in all the fractions.

In the first major group of experiments, the progressive changes in the activities of GSH-px(H_2O_2), GSH-px(Cum) and GST(CDNB) in the liver were studied during the onset of selenium and vitamin E deficiency and compared with selenium-supplemented, vitamin E-deprived animals. Pairs of rats were killed at 0, 10, 15, 20, 25 and 35 days after commencement of the experimental diets,

and liver supernatant fractions from three pairs of rats were subjected to Sephadex G-150 chromatography as described above. The supernatant fraction derived from 4g of liver was applied to the column in each case, and the amount of protein in the fraction was shown to be similar irrespective of the dietary treatment that the rats had received. Fig. 1 shows the means for the three elution profiles for the three enzymes; total activities of the enzymes were assessed by taking the integral of the areas under the curves shown in the elution profiles, and the values obtained are shown in Fig.

Fig. 1. Sephadex G-150 gel filtration of rat liver supernatant fractions at six stages during vitamin E and selenium deprivation. Six weanling male Wistar rats were given the selenium- and vitamin E-deficient diet and killed in pairs at the times stated. Livers of pairs of rats were homogenized in 0.25M-sucrose solution (40%, w/v) and the 100000g (1 h) supernatant solution subjected to gel filtration on Sephadex G-150 as described in the text. Fractions (5ml) were assayed for GSH-px activity with H_2O_2 as substrate (○), GSH-px activity with cumene hydroperoxide as substrate (●), and GST with CDNB as substrate (---). The values plotted are mean values derived from three pairs of rats; standard deviations were little larger than the thickness of the symbols and are omitted for clarity.

2. The experiment was repeated twice and the results led to the same conclusion on each occasion. It was not possible to combine the results together, however, because the results were numerically different each time the experiment was done.

In the second major group of experiments the increase in GST(CDNB) activity in selenium deficiency was investigated further to determine which transferase was giving rise to the increase observed. A method, based on published methods, was evolved that could be applied routinely to duplicate samples of liver supernatant fractions

Fig. 2. GSH-px and GST activities at six stages during vitamin E and selenium deprivation

Experimental details are given in Fig. 1. The total activities of the enzymes were obtained by taking the integral of the areas under the curves shown in Fig. 1. (a) ●, GSH-px(Cum); (b) ○, GSH-px(H₂O₂); (c) □, GST(CDNB). Also given are results obtained at three times (10, 15 and 25 days) after commencement of feeding a selenium-supplemented diet: (a) ▲, GSH-px(Cum); (b) △, GSH-px(H₂O₂); (c) ■, GST(CDNB).

derived from triplicate pairs of rats given each of the four experimental diets for 26–28 days. The supernatant fraction was first desalted by applying 10ml fractions batchwise to a column (3.2cm × 34cm) of Sephadex G-25 which had been equilibrated with 10mM-Tris/HCl buffer, pH 7.8. Fractions (10ml) were eluted with the same buffer and the protein content of each assessed by measuring A_{280} . Those fractions that contained the protein were pooled for affinity chromatography, which was based on the method of Guthenberg & Mannervik (1979), with Sepharose 6B to which hexyl-GSH was linked by the method recommended by the manufacturers (Pharmacia Fine Chemicals, 1979). A column (2.5cm × 6cm) of the hexyl-GSH-treated Sepharose 6B was equilibrated with 10mM-Tris/HCl buffer, pH 7.8, and the column was eluted, after the application of the sample, with 10mM-Tris/HCl buffer, pH 7.8, containing 0.2mM-NaCl until the eluate showed no absorbance at 280nm. A 10ml portion of 10mM-Tris/HCl buffer, pH 7.8, containing 5mM-hexyl-GSH was then applied to the column, followed by a further 10ml of the buffer without hexyl-GSH; 2ml fractions were collected, and those containing protein were pooled. The crude GST fraction was again desalted with a Sephadex G-25 column and the column was equilibrated and eluted with 10mM-Na₂HPO₄ buffer, pH 6.8. The void volume contained the desalted protein, which was again pooled for adsorption chromatography on hydroxyapatite.

A column (1.6cm × 36cm) of Bio-Gel HTP was equilibrated with 10mM-K₂HPO₄ buffer, pH 6.8, and the pooled desalted GST fraction applied to the column; when the sample had entered the gel, about 20ml of 10mM-K₂HPO₄ buffer, pH 6.8, was applied, followed by a 250ml linear gradient of 10–400mM-K₂HPO₄ buffer, pH 6.8. Fractions (4ml) were collected and assayed for GSH-px(Cum), GST(CDNB) and GST(DCNB) activities; A_{280} and the conductivity of each fraction were also monitored.

A sample of the crude desalted GST fraction and the contents of the peak tube of the respective GST activities were tested for purity and homogeneity by discontinuous-slab gel electrophoresis by an adaptation of the method of Laemmli (1970). The acrylamide was used at a total concentration of 4% (w/v) for the stacking gel and 12.5% for the resolving gel and both gels had *NN'*-methylenebisacrylamide added at a concentration of 2.5% (w/w) as cross-linker. Samples were prepared for electrophoresis by boiling them for about 3min with 0.05M-Tris/HCl buffer, pH 6.7, containing 1% (w/v) SDS, 15% (v/v) glycerol, 1% (v/v) mercaptoethanol and 0.1% (w/v) Bromophenol Blue as tracker dye. Samples (0.02ml) of the resultant mix-

ture were added to a slot in the stacking gel. The gels measured 17 cm × 17 cm, and the electrode buffer was 0.4 M-Tris/HCl, pH 8.5, containing 0.52 M-glycine and 1% SDS. Electrophoresis was started at about 15 mA and the current was increased to 25 mA as soon as the samples had entered the resolving gel; electrophoresis was continued until the tracker dye had reached the end of the gel. The gel was stained in a warm solution of 0.05% (w/v) Coomassie Blue in 9% (v/v) acetic acid in aq. 45% (v/v) methanol for 1 h. Destaining was carried out in a solution containing 7.5% (v/v) acetic acid in aq. 5% (v/v) methanol.

This experiment was repeated once and representative results are given in Figs. 3(a)–3(d), which also contains an example of the SDS/polyacrylamide-gel electrophoresis for each dietary condition. The mean value of six duplicate determinations is plotted in each case; the s.d. values have been omitted for the sake of clarity, and generally these were little larger than the breadth of the symbols used. Examination of all the results obtained in this experiment, both those given in Fig. 3 and the duplicate results that are not given, suggested that the activity of GST-B₁ appeared fairly stable in selenium deficiency (as indeed did the activity of GST-C and GST-A), but that the activities of GST-B₂ and GST-AA were enhanced. In order to test this possibility we calculated peak/height ratios for the GSH-px(Cum) activity in GST-B₂/GST-B₁, and in GST-AA/GST-B₁, as well as the GST(CDNB) activity in GST-B₂/GST-B₁ and in GST-AA/GST-B₁. The results are given in Tables 1 and 2.

Results

The results of the preliminary experiment (details not given here) showed essentially the two-peak distribution, called 'peak I' and 'peak II', described for the GSH-px(Cum) activity by Lawrence & Burk (1976), in selenium-supplemented rat liver supernatant fractions. The GSH-px(H₂O₂) activity was restricted to peak I and in selenium-deficient rats the peak I activity of GSH-px with both substrates almost completely disappeared, whereas the GSH-px(Cum) peak II activity remained. Peak II was in the same position in the eluate as the GST(CDNB) activity, confirming the view of Prohaska & Ganther (1977) that the peak II activity is due to GST. The total activity of the GST(CDNB) appeared to be enhanced in selenium deficiency, in confirmation of the results obtained by Lawrence & Burk (1978) and by Lawrence *et al.* (1978). Vitamin E had no apparent effect on the GST activities, but the activity of both GSH-px(Cum) and GSH-px(H₂O₂) was slightly lower in the selenium-deficient rats given

vitamin E than in the rats deprived of both vitamin E and selenium.

The results of the first group of experiments (Fig. 1) demonstrate a complex interplay of factors the understanding of which is facilitated by consideration of Fig. 2. The activity of GSH-px(Cum) (Fig. 2a) fell sharply to a minimum value after only 15 days of selenium deprivation and thereafter it rose steadily to reach a maximum at 30 days, by which time the rats were severely deficient in selenium and vitamin E. Selenium supplementation in the absence of vitamin E gave rise to a progressive rise in GSH-px(Cum) activity during the 25 days experimental period. The activity of GST(CDNB) (Fig. 3c) rose steadily from day 10 of selenium deprivation to the end of the experimental period, when a more-than-twofold rise in the activity of the enzyme was seen. The time course of this rise suggests that the stimulus for the increased activity was the fall in GSH-px(Cum) that preceded it. Supplementation with selenium in the absence of vitamin E was almost without effect on the GST(CDNB) activity. The GSH-px(H₂O₂) activity (Fig. 3b), which represents the true selenium-dependent GSH-px activity, fell at a slower rate than the GSH-px(Cum) activity, but continued to fall up to 20 days of selenium deprivation, by which time the GSH-px(Cum) activity had begun to rise because of the rise in GST(CDNB) activity. After 25 days of selenium deprivation there was a rise in the GSH-px(H₂O₂) activity caused by the appearance of a new peak of activity at an elution volume of about 230 ml (Fig. 1), compared with the true GSH-px(H₂O₂) activity, which shows a peak elution volume around 190 ml. Supplementation with selenium in the absence of vitamin E gave rise to a steady rise in the GSH-px(H₂O₂) activity during the period of the experiment that was proportionately larger than the rise seen in the GSH-px(Cum) activity (Fig. 2a) in the same period.

The results of the second group of experiments are shown in Fig. 3 and in Tables 1 and 2. Consideration of Fig. 3(a), in which selenium and vitamin E nutrition can be considered as adequate, and which therefore represents the normal pattern, shows resolution of the GST activity of the liver supernatant fraction into five peaks of activity. The identity of GSTs A and C is shown by the fact that these catalyse the conjugation of DCNB, and this was confirmed by SDS/polyacrylamide-gel electrophoresis, which showed that both GSTs are homodimers of subunit Yb. GST-B₂ was shown to be a heterodimer of Ya and Yc subunits and GST-B₁ was a homodimer of Ya subunits. GST-AA was not fully resolved by the method employed here and therefore appears to be composed of subunits Ya and Yc; it has, however, been shown by

Table 1. *Peak-height ratios (GSH-px activities of peaks AA and B₂ relative to peak B₁)*

Ratios were calculated from the peak values of GSH-px(Cum) activity derived from Figs. 3(a)–3(d), and from similar values derived from the curves obtained when the experiment was repeated. The values given are thus mean values derived from the duplicate observations. Abbreviation used: E, vitamin E.

Diet	B ₂ /B ₁	AA/B ₁
+ E + Se	0.51	0.47
+ E – Se	2.20	2.04
– E + Se	1.25	1.07
– E – Se	2.11	2.17

Table 2. *Peak-height ratios (GST activities of peaks A, AA, B₂ and C relative to peak B₁)*

Ratios were calculated from the peak values of the respective GST activities derived from Figs. 3(a)–3(d), and from similar values derived from the curves obtained when the experiment was repeated (results not shown). The values given are thus mean values derived from the duplicate observations. Abbreviation used: E, vitamin E.

Diet	A/B ₁	AA/B ₁	B ₂ /B ₁	C/B ₁
+ E + Se	0.32	0.27	0.85	0.34
+ E – Se	1.04	0.71	1.12	0.75
– E + Se	0.32	0.37	0.81	0.38
– E – Se	0.43	0.75	1.83	0.40

Ketterer *et al.* (1982) to be a homodimer of Yc subunits. The profile of the GSH-px(Cum) activity of the GSTs reveals that the GSH-px(Cum) activity was principally associated with the B₂ and AA GST activities; no definite statement about the GSH-px(Cum) activity of GST-B, can be made because the resolution of the B₁ and B₂ activities was insufficiently defined. Withdrawal of vitamin E from the diet (Fig. 3c) gave rise to little change in relative heights of the peaks of GST and GSH-px(Cum) activity. It is not possible to comment upon the absolute values of the enzyme activities, because the recovery of the enzymes in each experiment was probably different on each occasion because of the extended procedure through which the liver supernatant fraction was put each time the experiment was repeated. The consistent feature of these results, however, was that the relative heights of the peaks of enzyme activity for a given dietary treatment were constant, which suggested that no form of the enzyme was selectively destroyed during the purification procedures.

The effect of withdrawal of selenium was a complex change in the relative peak heights of the

individual GST activities. Inspection of Figs. 3(b) and 3(d) as well as of the results of the duplicate experiment not reported here, suggested that, when the GSH-px(Cum) activities were considered in the GST peaks, the B₂ and AA peaks appeared enhanced relative to the B₁ peak when compared with the selenium-supplemented rats (Fig. 3a). In order to test this possibility, the GSH-px(Cum) activity of the GSTs AA and B₂ relative to B₁ in all the dietary treatments were calculated by using the results in Fig. 3 and the duplicate experiments. The results are given in Table 1. A similar conclusion was arrived at by inspection of the GST(CDNB) profiles of the five transferases and peak-height ratios of GSTs A, AA, C and B₂ relative to GST-B₁ were also calculated. The results are given in Table 2. The results in Table 1 indicate a clear incremental effect of selenium withdrawal on the GSH-px(Cum) activity of the GST-AA and GST-B peaks; surprisingly, withdrawal of vitamin E alone also caused an apparent increment in the GSH-px(Cum) activity of the GST-AA and GST-B₂ peaks, although this effect was not so large as the effect of selenium withdrawal. The results in Table 2 show a clear effect of selenium withdrawal in enhancing the activity of the GSTs AA and B₂. Here, however, vitamin E had no effect. Withdrawal of selenium alone in the presence of adequate amounts of vitamin E also caused an increase in the peak activity of GST-A and GST-C relative to GST-B, whereas when vitamin E was also withdrawn this effect was not seen.

Because of the importance of the unexpected finding that the Yc subunit of GST appeared enhanced in selenium deficiency, the gels were subjected to densitometric measurement to compare the relative amounts of the Ya and Yc subunits in the different dietary conditions. Because the chromatographic methods employed did not fully resolve the GST-AA from GST-B₂, we measured the density of the Ya and Yc bands that were nominally from the B₁, B₂ and AA forms of the enzyme and calculated the Yc/Ya ratio in B₂ and the ratio of the sum of all the Yc and Ya band densities in the three forms of the enzyme. The results are given in Table 3, which indicate that, when selenium was withdrawn from the diet, the amount of the Yc monomer relative to the Ya monomer was doubled.

Discussion

The results presented here demonstrate a profound specific effect of selenium deficiency on the activity of members of the group of enzymes known collectively as the GSTs. The animals used as control subjects were deprived of both vitamin E

Table 3. *Densitometric measurement of Ya and Yc subunits of GST; effect of vitamin E (E) and selenium deprivation*

Ratios were calculated from densitometric measurements made on the Yc and Ya subunits of GSTs separated by the chromatographic and SDS/polyacrylamide-gel-electrophoretic techniques described in the text. The values given are mean values derived from duplicate observations.

Diet	Yc/Ya in B ₂	Yc/Ya in B ₁ , B ₂ and AA together
+ E + Se	0.49	0.23
+ E - Se	0.79	0.59
- E + Se	0.45	0.32
- E - Se	0.93	0.64

and selenium, and were in the pre-necrotic phase of the hepatocellular phenomenon defined by Schwarz & Folz (1957), and experience with similar animals in our laboratory has indicated that, at the time after beginning the diet when the present experiments were carried out, no pathology is discernible under the optical microscope, although some mitochondrial enlargement can be seen under the electron microscope, which is presumed to be associated with the 'respiratory decline' phenomenon.

Consideration of the results in Figs. 1 and 2 shows that the stimulus for the alteration in GST activity is without doubt the fall in the activity of the selenium-dependent GSH-px(H₂O₂). This fell to its minimal level 20 days after beginning the diet, but the activity of GST(CDNB) began to rise at around 10 days of diet. The GSH-px(Cum) activity is thus the sum of the declining selenium-dependent GSH-px activity and the rising GST (peroxidase) activity. The questions raised by this experiment were whether the decline in selenium intake stimulated the activity of all the GST enzymes together, and whether this stimulation was due to an alteration in activity of existing enzymes or whether it was due to an increase in synthesis *de novo* of one or more of the subunits of the GSTs. The apparent rise, after 30 days' diet, in GSH-px(H₂O₂) was not due to a real rise in selenium-dependent GSH-px activity, since Fig. 1 (35 days) shows that this GSH-px activity is due to a protein separated from the position occupied by the Se-dependent enzyme under these chromatographic conditions. The nature of this protein is unknown at present; it was a consistent feature of our experiments with rats that were severely depleted of selenium, and presumably represents a further attempt by the organism to meet the challenge of the oxygen-radical-induced damage that is thought to take place in the absence of GSH-px. In this connection it is noteworthy that Katki & Myers (1980) described a mitochondrial GSH-px

activity that was independent of selenium intake. In the rats given selenium but no vitamin E (Fig. 2), the rise in GSH-px activity measured with both substrates may be a response to the depletion of vitamin E; this is in contrast with the work of others, with different species of mammals (Su & Czallany, 1977; Godwin *et al.*, 1975; Whanger *et al.*, 1977), a fish (Covey *et al.*, 1981) and the Pekin duckling (Xu & Diplock, 1983).

The distribution pattern of the GSTs for rats supplemented with selenium and vitamin E, and the effect of withdrawing either or both nutrient, is shown in Fig. 3. Since it was not possible to quantify these results in absolute terms, due to variable losses that might have occurred during the sequential chromatographic stages, another method of comparison was sought. Inspection of the four distribution patterns obtained (both those given in Fig. 3 and those obtained when the experiment was repeated) suggested that there were changes in the relative amounts of the various forms of the enzyme when selenium was withdrawn from the diet. Calculation of the ratios of the peak heights of the GSH-px activities of GSTs AA and B₂ relative to B₁ gave the mean data shown in Table 1. Calculation was also carried out of the peak heights of GST(CDNB) activities of GSTs A, AA, B₂ and C relative to B₁, and these values are given in Table 2. Table 1 shows that withdrawal of selenium from the diet resulted in a four-fold increase in the activity of both the AA and B₂ forms of GST relative to the B₁ form. Similarly, Table 2 demonstrates a two- to three-fold increase, in selenium deficiency, in the activity of the AA and B₂ forms relative to the B₁ form of GST(CDNB).

The results of the densitometric measurements (Table 3) show that the apparent increase in the activities of GST-AA and GST-B₂ in selenium deficiency is due to an increase in the amount of the Yc monomer present, relative to the Ya monomer. Beale *et al.* (1982, 1983) have concluded that the Ya and Yc subunits are the products of expression of separate genes, which may be inter-related. It is therefore proposed that the results given here are highly suggestive that the enhanced activity in selenium deficiency of the B₂ and AA forms of GST, which are largely responsible for most of the GSH-px activity, is due to a specific effect of the absence of selenium on the expression of the gene that gives rise to the Yc subunit of the enzyme. Conclusive proof of this could be obtained by demonstrating the induction of the Yc monomer immunochemically. It is, however, noteworthy that Reiter & Wendel (1983) have proposed an effect of selenium on gene expression that does not depend on GSH-px activity.

The findings reported here raise fundamental

questions about the possibility that dietary selenium levels may influence the activity of a key group of enzymes whose biological function is more commonly thought to be concerned with the conjugation of a range of different xenobiotics, as well as the removal of active xenobiotic metabolites by direct irreversible covalent binding of the B-forms of the enzyme to the metabolite (Ketterer *et al.*, 1978; Litwack *et al.*, 1971). Among such xenobiotics are polycyclic hydrocarbons, many of which are mutagenic and carcinogenic. In some preliminary experiments we have found that high levels of dietary selenium (2–5 p.p.m.) severely suppress the activity of liver GSTs in the rat. Although it is always dangerous to extrapolate between species, the possibility exists that high dosage with selenium in human subjects could suppress similar GST activities, which lead to an increased toxicity of xenobiotics, a result that is opposite to the objective of prevention of carcinogenesis by selenium supplementation. Investigation of this possibility is urgently needed.

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