Bull. Environm. Contam. Toxicol. 26, 77-84 (1981)

Glutathione Peroxidase Response in Tissues of Rats Fed Diets Containing Fish Protein Concentrate Prepared from Shark Flesh of Known Mercury and Selenium Contents

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The poisoning of people eating fish contaminated with mercury from industrial effluents (McALPINE & ARAKI 1958) has led to stringent limitations being placed on the levels of mercury permitted in seafoods (ANON 1979). These limitations often place constraints on the marketing not only of contaminated fish, but also of fish which naturally accumulate mercury from unpolluted waters.

There is however, evidence that the toxicity of the mercury that accumulates in the flesh of fish living in unpolluted waters is nullified by some associated constituent. Peruvian villagers, Korean fishermen and Samoan cannery workers, who eat large quantities of fish show no evidence of methylmercury poisoning, despite high body loads of mercury (TURNER *et al.* 1975; MARSH *et al.* 1975). Marine mammals which feed exclusively on fish can tolerate high levels of methylmercury in the central nervous system. The increased mercury levels are accompanied by an increase in selenium (KOEMAN *et al.* 1973) and it appears that this selenium, which also accumulates in fish flesh (EGAAS & BRAEKKAN 1977), may detoxify mercury.

Studies have been reported using experimental animals and synthetic diets containing selenium and mercury compounds to demonstrate detoxification of mercury by selenium (GANTHER & SUNDE 1974; STOEWSAND *et al.* 1974; SEKI *et al.* 1975). The mechanism of detoxification remains obscure. Most experiments have involved the use of high levels of both elements and relied on the observation of gross symptoms. The measurement of enzyme systems may be useful in detecting effects of mercury at a lower, subclinical level and in elucidating the biochemistry of mercury/selenium interactions. The activity of the selenoenzyme glutathione peroxidase (GSH-Px) in rats is dependent on dietary selenium (HAFEMAN *et al.* 1974) and attempts have been made to use this enzyme as an indicator of mercury/selenium interactions (WAGNER 1975; PROHASKA & GANTHER 1977).

In previous studies the levels of mercury fed to rats in experimental diets were well in excess of those normally found in food and the levels of selenium were well in excess of the nutritional requirement of approximately 0.1 ppm (HOEKSTRA 1974). The research described in this paper was designed to investigate the

0007-4861/81/0026-0077 $01.60

effect of mercury, in the form and amounts which occur naturally in seafood, on the availability of selenium at levels approximating the nutritional requirement. In anticipation of mercury lowering the GSH-Px response a range of selenium concentrations was used, from nutritional deficiency to three times the nutritional requirement.

EXPERIMENTAL

Sixty-four four-week old male Hooded Wistar rats bred from females that had been maintained on Barastoc Mouse Breeder ration (KMM Pty. Ltd., Kensington, Vic.) were allocated randomly to eight groups. The rats were housed in plastic boxes on sawdust and given distilled water *ad lib.* from acid-washed glass bottles.

Each group of rats was fed (20g/rat/day) on a basal (low selenium) mixture (Table 1) that was in turn mixed with a protein source. The protein used was either Torula yeast, fish protein concentrate (FPC) or a mixture of the two (Table 2). Sufficient DL-methionine (0.6%) was added to the basal mixture to raise the 'protein score' of the yeast diet to 100 (MILLER & DONOSO 1963). A known amount of lipid soluble vitamins was given orally each week to all rats. (1.8 IU α-tocopherol, 150 µg Calciferol, 3000 IU Vitamin A/rat/week).

TABLE 1

Formulation of the basal mixture used in diet preparation.

Component	Percentage
Dextrose	37.5
Wheat Starch	44.3
Salt mix[a]	6.3
Vitamin mix[b]	1.3
DL Methionine	0.6
Lard	6.3
Cod Liver Oil	3.7

[a] $CaCO_3$ 56%, $MgCO_3$ 2.5%, $MgSO_4$ 1.6%, NaCl 4.9%, KCl 11.2%, KH_2PO_4 21.2%, KI 0.008%, $MnSO_4$ 0.35%, NaF 0.1%, $AlK(SO_4)_2.12H_2O$ 0.017%, $CuSO_4.5H_2O$ 0.09%.

[b] Thiamin HCl 0.04%, Glucose monohydrate 88.58%, Riboflavin 0.025%, Pyridoxine HCl 0.02%, Ca-DL-Pantothenate 0.2%, Choline Chloride 10%, Niacin 1%, Menadione 0.01%, Folic acid 0.02%, Biotin 0.01%, Vitamin B_{12} Triturate 0.1%, Vitamin A Palmitate 2500 IU/100g mix, Vitamin D_2 200 IU/100g mix.

TABLE 2

Formulation of Experimental Diets[a]

Diet No.	Protein source[b] (g/100g)	Sodium selenite (ppm Se)	Concentration (ppm) Mercury	Selenium
1	TY-20	-	<0.02	<0.01
2	TY-20	0.15	<0.02	0.15
3	TY-20	0.30	<0.02	0.30
4	F-10 TY-10	-	1.0	0.15
5	F-10 TY-10	0.08	1.0	0.23
6	F-10 TY-10	0.24	1.0	0.39
7	F20	-	2.0	0.30
8	F20	0.16	2.0	0.46

[a] The diets were made up with 80% low selenium basal mixture (Table 1).

[b] TY-Torula yeast; F-Fish Protein Concentrate.

The FPC was prepared from school shark flesh (*Galeorhinus australis*) by first passing the dressed shark carcasses through a Bibun Meat Separator then extracting the flesh with isopropanol:water to remove Vitamin E and polyunsaturated fatty acids that might otherwise have a secondary unquantifiable interaction with mercury (WELSH & SOARES 1976). The process of extraction used was a modification of that developed by the Fisheries Research Board of Canada (IDLER 1968). The minced flesh was twice extracted in a hot 70:30 mixture of isopropanol:water, which reduced lipid and volatile bases to acceptable levels while leaving the mercury and selenium with the flesh (THROWER 1979). Excess isopropanol which is tenaciously held by fish protein concentrate (ACKMAN & ODENSE 1968) was replaced by water (STACHOWSKI 1966) by alternately steaming and vacuum drying at 40°C in a tumble drier. It should be noted that the preliminary treatment with polyphosphoric acid which is normally used to soften connective tissue was omitted, since the acid treatment could have released mercury and selenium from the fish protein. The FPC produced was assayed for mercury and selenium.

Each week rats were weighed and bled from the jugular vein after physical immobilization and anaesthetization with ketamine. Blood samples were immediately centrifuged at 3000xG for 10 min and plasma and erythrocytes stored frozen (-20°C). At the conclusion of the experiment (56 days) rats were sacrificed by bleeding out from the heart; the brain, liver, kidney and the gastrocnemius muscle were then removed weighed, and frozen (-20°C).

Ornithine transcarbamylase (OTC) activity was measured in the plasma each week to detect the onset of any liver damage that might be caused by selenium deficiency (CERIOTTI 1973). GSH-Px activity was measured in the tissues and erythrocytes by a coupled enzyme method based on that of PAGLIA & VALENTINE (1967) using 0.2 mM hydrogen peroxide as substrate and a reaction temperature of 25°C (ANDREWARTHA *et al.* 1979). Protein was estimated by the method of LOWRY *et al.* (1951).

The diets were formulated to provide two levels of mercury from FPC while varying the selenium level with sodium selenite (Table 2). Diets 1 to 3 contained no FPC and so provided a response curve of GSH-Px to added selenite in the absence of mercury. Diet 4 contained 10% FPC which resulted in a mercury level of 1.0 ppm and a selenium level (0.15 ppm) slightly above the nutritional requirement. Sodium selenite was added to this mixture (diets 5 and 6) to investigate the GSH-Px response in the presence of 1.0 ppm mercury.

Diet 7 contained 20% FPC and no added selenite which resulted in a mercury level of 2.0 ppm and a selenium level of 0.3 ppm, well in excess of the nutritional requirement. In order to measure the GSH-Px response to added selenite in the presence of 2.0 ppm mercury, sodium selenite was added to the 20% FPC diet (Diet 8).

RESULTS AND DISCUSSION

There was no evidence of any toxic effects from the FPC used in the experimental diets. Plasma levels of OTC remained normal giving no evidence of liver damage. No differences in growth rate or weight of organs were detected. There were however significant differences in GSH-Px activity.

Erythrocyte GSH-Px activity reached a steady level for each diet within 6 to 8 weeks; this is consistent with the 56 day life span of rat erythrocytes (KANEKO *et al.* 1961). No GSH-Px activity was detected in any of the plasma samples.

Erythrocytes, liver, brain and kidney showed a response of GSH-Px activity to the experimental diets (Table 3). The level of GSH-Px activity in skeletal muscle was constant (44.4 ± 6.1 μ moles NADPH oxidized/min/g protein) and will not be discussed further.

The GSH-Px response to Torula yeast diets supplemented with sodium selenite (diets 1 to 3) is shown in Figure 1. Activity in the liver and brain showed a steady increase with selenium concentration up to 0.3 ppm but the response in the kidney and erythrocytes had tapered off at this selenium concentration. The GSH-Px response was thus more sensitive at the lower (0.15 ppm) selenium concentrations which approximate the nutritional requirement of the rat.

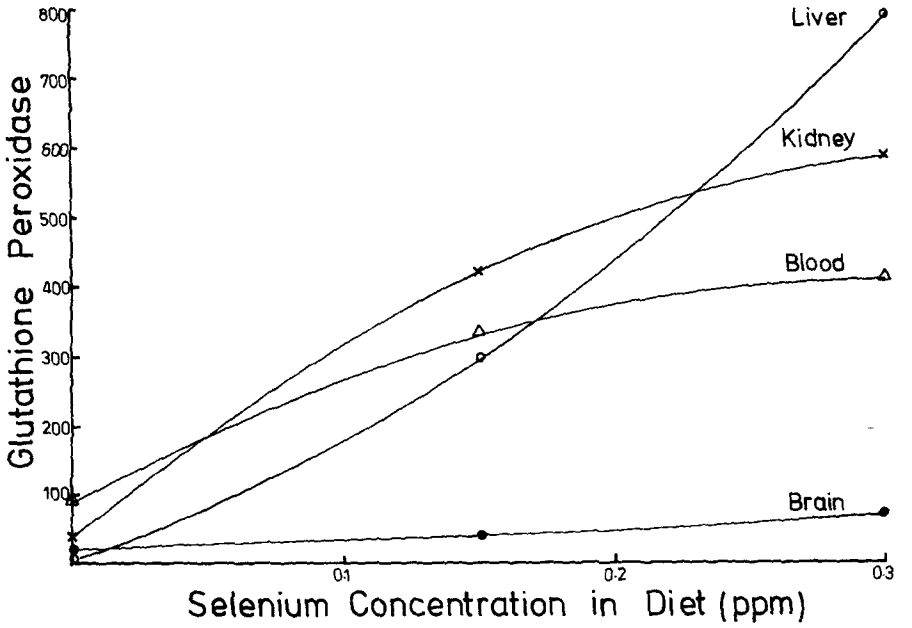

Fig. 1. Glutathione peroxidase activity in tissues of rats fed diets containing selenite (Diets 1 to 3). Units as in Table 3.

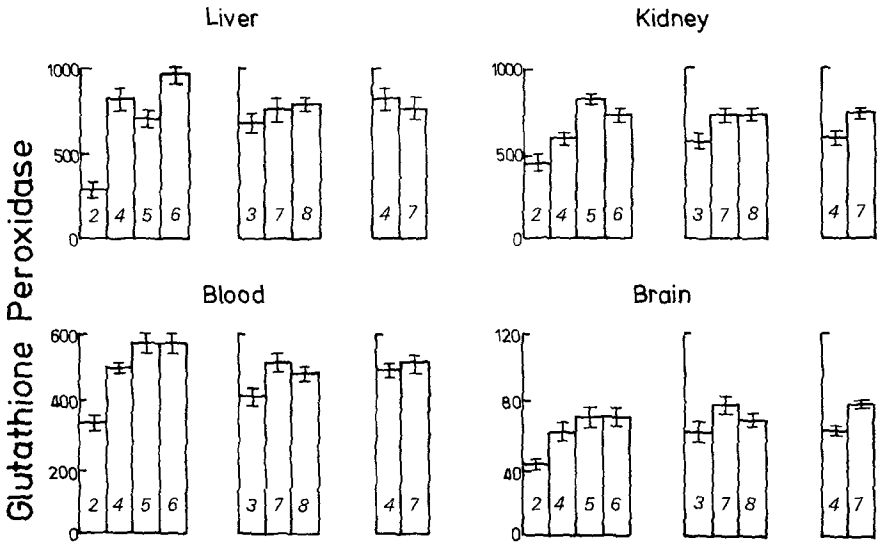

Fig. 2. Glutathione peroxidase activity in tissues of rats fed experimental diets. Units as in Table 3. Numbers in italics indicate diet number, error bars indicate SEM

TABLE 3

Glutathione Peroxidase Activity in Tissues of Rats

Diet No.	Concentration (ppm) Mercury	Selenium	Glutathione Peroxidase Activity[1] Liver[2]	Kidney[2]	Brain[2]	Erythrocytes[3]
2	<0.01	0.15	293^{a}	431^{a}	42^{a}	334^{a}
4	1.0	0.15	815^{b}	571^{b}	62^{b}	486^{b}
5	1.0	0.23	690^{b}	784^{c}	69^{bc}	561^{c}
6	1.0	0.39	950^{b}	710^{c}	68^{bc}	559^{c}
3	<0.01	0.30	676^{b}	573^{b}	60^{b}	401^{ab}
7	2.0	0.30	763^{b}	730^{c}	76^{c}	575^{bc}
8	2.0	0.46	776^{b}	728^{c}	67^{bc}	471^{bc}

[1] Different letters denote significantly different groups ($p<0.001$).
[2] GSH-Px - μ moles NADPH oxidized/min/g haemoglobin.
[3] GSH-Px - μ moles NADPH oxidized/min/g protein.

The GSH-Px response to the FPC diets was higher, in every case, than to the Torula yeast diets containing an equivalent amount of selenium in the form of added selenite. This trend was present in all tissues despite a tenfold difference in the magnitude of the enzyme response between tissues, indicating an increase in the overall selenium status of rats fed FPC (Figure 2). The response, in kidney and brain, to 20% FPC (diet 7) was higher than to 10% FPC (diet 4). There are three possible explanations for the enhancement of GSH-Px activity by FPC. The selenium in FPC may be present in a form that is more available than selenite for synthesis of the enzyme. Some synergistic mechanism may operate between selenium and the excess sulphur amino acids present in FPC to enhance enzyme synthesis. A synergism has been demonstrated between cystine and selenite in studies of mercury toxicity (STILLINGS *et al.* 1974). The methylmercury present in FPC may directly enhance GSH-Px activity. WAGNER (1975) found a consistent though non-significant enhancement of GSH-Px activity in rat liver when methylmercury was added to casein-based diets containing selenite. In no case in the present studies did the presence of 1.0 or 2.0 ppm mercury decrease the enzyme response below that induced by a level of selenium meeting the nutritional requirement.

All the values obtained for FPC diets were either above or in the plateau region of the selenite response curve. The effect, if any, of mercury in the FPC on the response to selenite added to the FPC based diet could not be evaluated because in every case the value for GSH-Px activity exceeded the limits of the selenite response curve. Significant increases in enzyme activity in kidney and erythrocytes were detected when the selenium concentration of the FPC diet was raised to 0.23 ppm with selenite. Increasing the selenium concentration of the diet to higher levels (diets 6 and 8) produced no further

increase in GSH-Px activity possibly due to the insensitivity of the enzyme response at higher levels of selenium (Figure 1).

The GSH-Px response is obviously most sensitive when the dietary selenium level is below 0.15 ppm. In future experiments it should be possible to considerably lower the proportion of FPC, and hence the levels of mercury and selenium, in the diets. This could make possible experiments in which the diet is only marginally altered by the inclusion of FPC. It would still be necessary however to balance the dietary sulphur amino acid content.

GSH-Px activity thus provides a powerful tool for studying mercury/selenium interactions in ingested seafoods in the forms and amounts in which these elements occur in the human diet.

ACKNOWLEDGEMENTS

We acknowledge the assistance of K.O. Godwin, CSIRO Division of Human Nutrition with the formulation of diets, R.K. Joiner and D.J. Casimir, CSIRO Division of Food Research for preparation of the FPC, B. Whittle of the "Attwood" Veterinary Research Laboratory, for care and handling of the rats, and K.A. Francesconi of W.A. Marine Research Laboratories for selenium analyses.

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