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SELENIUM AND DRUG METABOLISM—II

INDEPENDENCE OF GLUTATHIONE PEROXIDASE AND REVERSIBILITY OF HEPATIC ENZYME MODULATIONS IN DEFICIENT MICE

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Abstract—Male mice were fed a diet containing less than 0.01 ppm selenium (Se^-) for 6 months. A control group received the same diet containing 0.5 ppm selenium (Se^+). In the livers of the Se^- animals a drastic decrease in glutathione peroxidase (GSH-Px) activity was observed. It reached undetectable levels after 17 days of the Se^- diet. At that time, GSH-transferase activity began to increase significantly, followed by changes in many other enzyme activities. After the 60th day, these enzyme modulations had reached a plateau with the following percentage changes compared to controls: GSH-transferases: 320% (1,2-dichloro-4-nitrobenzene), 218% (1-chloro-2,4-dinitrobenzene); glutathione reductase: 160%; ethoxycoumarin deethylase: 330%; cytochrome P-450-hydroperoxidase: 230%; heme oxygenase: 240%; UDP-glucuronyltransferase: 200%; GSH-thioltransferase: 64%; sulphotransferase: 62%; NADPH-cytochrome-P-450-reductase: 65%; flavin-containing mono-oxygenase: 57%. No significant changes were observed for GSH-transferase activity assayed with ethacrynic acid or for microsomal H_2O_2 formation and aniline hydroxylase activity. In single-pulse repletion experiments by injection of 250 μg selenium/kg body wt, different individual time constants for the recovery process of the enzymatic perturbations were observed. The half-times for the recovery ranged from 5.7 hr for the microsomal NADPH-cytochrome-P-450 reductase to over 29 hr for GSH-Px up to 44 hr for part of the GSH-transferase activity. 250 μg selenium/kg body wt were needed to restore 50% of GSH-Px activity in the long-term Se^- mice compared to Se^+ controls. All other enzymatic changes in the Se^- mice needed a dose of 7 μg selenium/kg body wt for 50% restorage.

The results demonstrate that processes other than those related to GSH-Px take place in a later phase of selenium deficiency in mouse liver with a chronologically common beginning. The different repletion and depletion kinetics as well as the different need of these processes for the trace element are discussed with respect to the existence of two separate selenium pools.

Evidence is accumulating for selenium-dependent processes other than those mediated by the seleno-enzyme glutathione peroxidase (GSH-Px) (reviewed in ref. [1]). In the preceding paper of this series, we reported the profound influences of prolonged selenium deficiency on drug metabolism in mouse liver [2]. In that study we were unable to differentiate a general metabolic impairment due to the loss of glutathione peroxidase activity from a specific involvement of selenium in a regulatory process as the cause of the observed enzyme modulations. Therefore we undertook a longitudinal study using the time course of selenium depletion and repletion as a tool to investigate the relationship of these modulations to changes in glutathione peroxidase activity and their need of the trace element in order to restore normal metabolism.

MATERIALS AND METHODS

Weaning male albino mice (strain NMRI Han) were purchased from the Zentralinstitut für Versuchtiere (Hannover, F.R.G.) and housed under standard laboratory conditions in groups of 8–10 animals in Macrolon cages with free access to food and water. The low selenium diet was based on a torula yeast based formula, which is adequate in vitamin E and sulphur-containing amino acids [3]. The selenium content of this diet was determined fluorometrically [4] and was found to be below 0.01 ppm selenium. Control animals were fed the same diet supplemented with 0.5 ppm selenium as sodium selenite. For all experiments, dietary groups of 3–6 animals were used. With the exception of the mice used in the depletion study, all animals had been fed a selenium-deficient or control diet for at least 4 months. The preparation of subcellular fractions was performed essentially as described in ref. [5]. GSSG reductase [6] and GSH-thioltransferase [7] were determined at 37°. The latter enzyme was assayed with *S*-sulphocysteine, which was synthesized according to ref. [8]. All other assay procedures were described earlier [2]. Repletion experiments were started with intraperitoneal (i.p.) injection of a single dose of sodium selenite to selenium-deficient mice, dissolved in saline.

NADPH, NADH, GSH, GSSG-reductase, catalase and cytochrome *c* were from Boehringer (Mannheim, F.R.G.). 7-Ethoxycoumarine, umbelliferone, UDP-glucuronic acid, *N,N*-dimethylaniline, ethacrynic acid, *p*-nitrophenol, heme, rotenone, bovine serum albumin, D,L-α-tocopherol acetate and superoxide dismutase were from Serva (Heidelberg, F.R.G.). 1,2-Dichloro-4-nitrobenzene, 1-chloro-2-4-

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dinitrobenzene and tetramethylphenylene diamine were from Fluka (Buchs, Switzerland).

RESULTS

Weanling male mice with an average weight of 8.3 g were fed a selenium-deficient diet, while the control group received the same diet supplemented with 0.5 ppm selenium. Body weight, GSH-Px activity as well as several enzyme activities related to hepatic drug metabolism were monitored over 6 months in both dietary groups. The time course of metabolic changes due to the restricted intake of selenium below 10 ppb is shown in Fig. 1 on a logarithmic time scale. In the animal group fed the selenium-deficient diet, selenium-dependent GSH-Px activity decreased exponentially (Fig. 1A, lower curve). Half of the initial activity was reached after 74 hr. A time of 14 days to drop to zero was extrapolated. The body weight of both dietary groups increased linearly within the first few weeks and was doubled by day 7 of the experiment. During the first 10 days, the only difference observed between the two dietary groups was GSH-Px activity. However, when GSH-Px had decreased below 5% of the control level, minor but significant increases in the activities of GSH-*S*-transferases towards 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene as well as in cytochrome P-450-mediated hydroperoxidase activity occurred. The activities of these two enzymes increased to roughly 120% of the respective control values ($P \leq 0.05$), and remained at this intermediary level until GSH-Px activity was virtually undetectable (cf. Table 1). Later on, complex alterations in hepatic drug metabolism were initiated between the 17th and 21st days of selenium de-

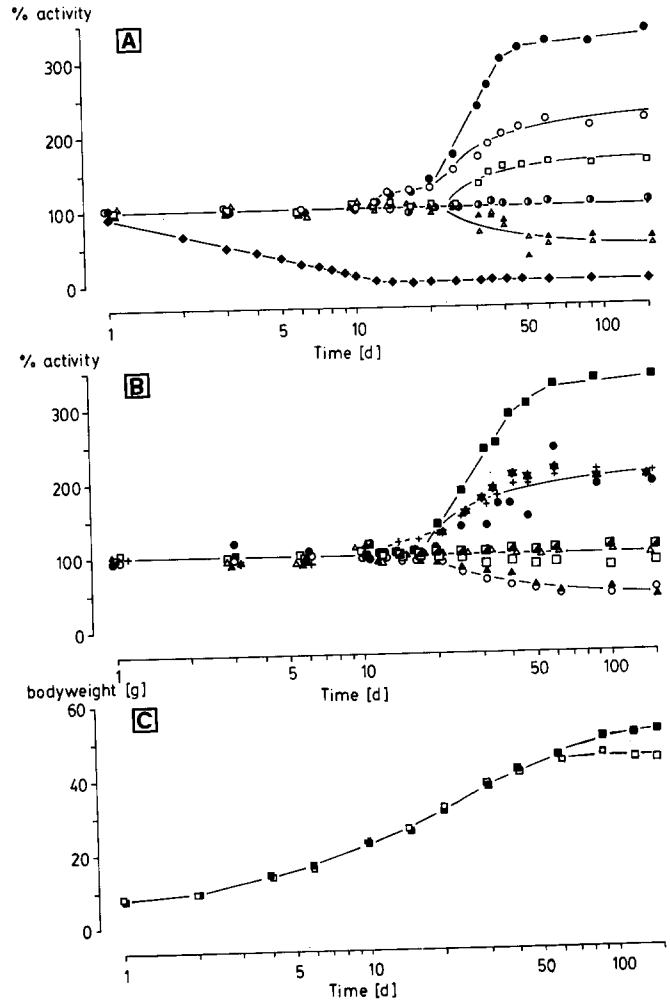

Fig. 1. Alterations in hepatic enzyme activities and body weight of male mice during dietary selenium depletion. (A) Cytosolic enzymes: GSH-*S*-transferases: (●) 1,2-dicholoro-4-nitrobenzene; (○) 1-chloro-2,4-dinitrobenzene; (◑) ethacrynic acid; (□) GSSG-reductase; (△) GSH-thioltransferase; (▲) sulphotransferase; (◆) GSH-peroxidase. (B) Microsomes: cytochrome-P-450-mono-oxygenase: (■) ethoxycoumarin-*O*-de-ethylase; (□) aminopyrin-*N*-demethylase; (◪) aniline hydroxylase; (+) hydroperoxidase; (▲) NADPH-cytochrome P-450-reductase; (△) NADH-cytochrome-b_5-reductase; (●) heme oxygenase; (○) flavin-containing monooxygenase; (★) UDP-glucuronyltransferase. (C) Body weight: (□) selenium-deficient; (■) control. Enzyme parameters are expressed as % activity of control (for absolute values see Table 1).

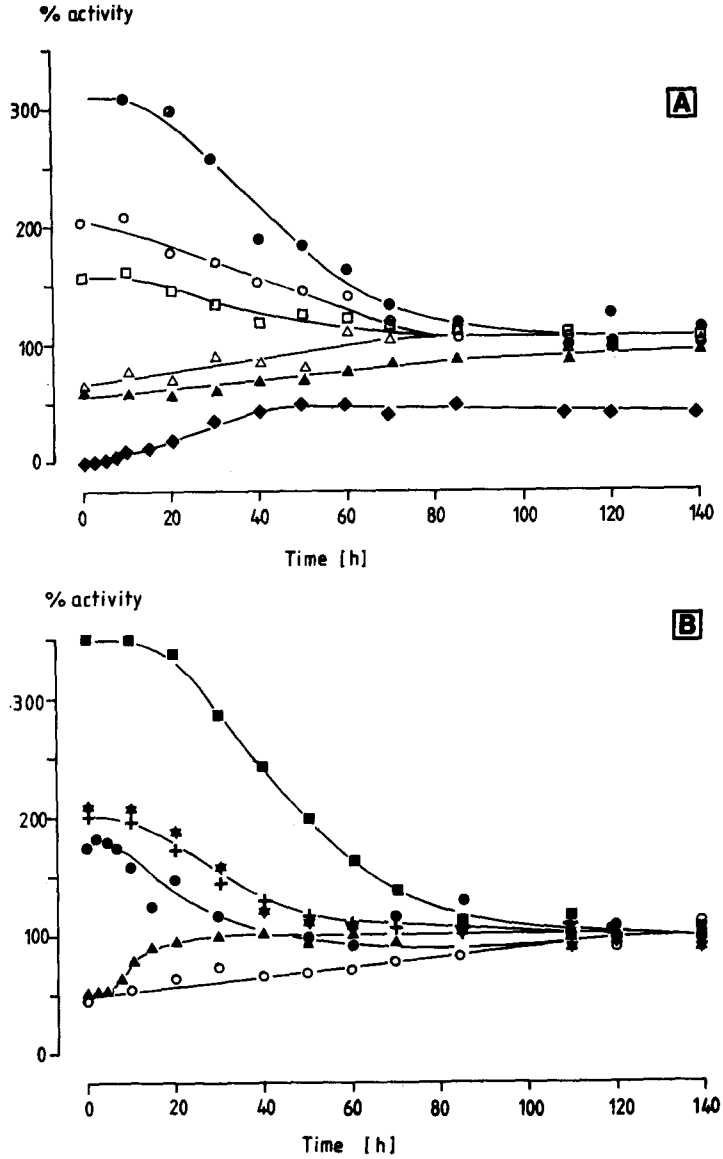

Fig. 2. Recovery of hepatic drug metabolism in selenium-deficient mice repleted with selenium. Se^- mice were injected with a single dose of 250 μg selenium/kg body wt as selenite and enzymatic changes were determined as a function of time. All specific enzyme activities are expressed as % activity of controls (for absolute values, see Table 1). (A) Cytosolic enzymes: GSH-*S*-transferases: (●) 1,2-dichloro-4-nitrobenzene; (○) 1-chloro-2-4-dinitrobenzene; (△) GSH-thioltransferase; (□) GSSG-reductase; (◆) GSH-peroxidase; (▲) sulphotransferase. (B) Microsomes: (■) ethoxycoumarin-*O*-de-ethylase; (+) hydroperoxidase; (▲) NADPH-cytochrome-P-450-reductase; (●) heme oxygenase; (○) flavin-containing mono-oxygenase; (★) UDP-glucuronyltransferase.

pletion. Following an apparently common latency phase, alterations in many different mouse liver enzymes were observed at the end of the third week, leading to enhanced or lowered specific activities. These alterations reached their final plateau levels within a further 30–40 days on the selenium-deficient diet. By this time, the rate of ethoxycoumarin-O-dealkylation had increased to 300% over the control while glucuronidation, cytochrome P-450-dependent hydroperoxidase as well as heme oxygenase roughly doubled in activity. In contrast, NADPH-cytochrome-P-450-reductase and flavin-containing mono-oxygenase activities decreased by 30–50%, while NADH-cytochrome-b_5-reductase and several other cytochrome P-450-dependent reactions exhibited only small changes of varying statistical significance (Table 1). Cytosolic conjugation reactions showed similar prominent changes. GSH-*S*-transferase activities towards 1,2-dichloro-4-nitrobenzene and 1-chloro-2,4-dinitrobenzene increased two to three-fold over the control, while GSH-transferase activity towards ethacrynic acid remained constant. The sulphatation rate was depressed by approximately 40% after this time of selenium deficiency. Beyond enzyme activities related to drug metabolism, the hepatic activity of GSSG-reductase was increased by

Table 1. Mouse liver enzyme parameters during selenium deficiency at three selected times in the course of a longitudinal depletion study over 6 months

Enzyme	Day 10		Day 17		Day 60	
	Se^-	Se^+	Se^-	Se^+	Se^-	Se^+
Cytosolic:						
GSH-peroxidase*	60 ± 28§	753 ± 80	12.7 ± 5.3§	685 ± 107	10.8 ± 3.7†§	816 ± 68
GSH-*S*-transferases						
1,2-Dichloro-4-nitrobenzene*	21.7 ± 3.4	20.3 ± 11.2	25.6 ± 1.6‖	20.9 ± 4.8	63.0 ± 19.0§	19.5 ± 4.9
1-Chloro-2,4-dinitrobenzene†	1856 ± 206	1792 ± 246	1839 ± 71‖	1718 ± 243	3342 ± 162§	1533 ± 56
Ethacrynic acid*	119.8 ± 16.2	123.3 ± 9.7	132.3 ± 1.4	127.4 ± 19.3	123.6 ± 17.2	132.6 ± 7.4
GSSG-reductase*	205.3 ± 32.8	214.3 ± 38.0	199.3 ± 18.5	208.5 ± 23.7	374.5 ± 54.7§	233.8 ± 32.8
GSH-thioltransferase*	13.4 ± 3.8	12.4 ± 3.1	12.1 ± 4.1	13.2 ± 1.9	9.4 ± 3.7§	14.7 ± 2.9
Sulphotransferase‡	0.23 ± 0.04	0.26 ± 0.08	0.25 ± 0.06	0.21 ± 0.04	0.16 ± 0.04§	0.26 ± 0.03
Microsomes:‡						
Ethoxycoumarin-*O*-de-ethylase	1.5 ± 0.4	1.4 ± 0.4	1.5 ± 0.3	1.5 ± 0.3	4.3 ± 0.6§	1.3 ± 0.3
Aminopyrin-*N*-demethylase	7.3 ± 0.6	6.5 ± 1.1	7.9 ± 1.3	8.3 ± 0.8	7.2 ± 0.9¶	7.9 ± 0.6
Aniline hydroxylase	0.47 ± 0.08	0.53 ± 0.03	0.51 ± 0.07	0.49 ± 0.09	0.42 ± 0.07	0.50 ± 0.05
Cytochrome P-450-dependent H_2O_2 formation	4.2 ± 0.8	4.8 ± 1.2	5.4 ± 0.4	5.6 ± 0.8	5.1 ± 0.5	4.8 ± 0.8
Cytochrome P-450-hydroperoxidase	40.3 ± 8.3	39.1 ± 3.8	47.3 ± 6.4‖	38.1 ± 3.3	107.3 ± 12.4§	47.3 ± 9.8
NADPH-P-450-reductase	152.7 ± 21.3	165.3 ± 13.7	148.0 ± 17.4	157.3 ± 30.6	97.4 ± 10.8§	149.7 ± 30.3
NADH-b_5-reductase	673 ± 72	692 ± 42	683 ± 81	657 ± 32	684 ± 42	672 ± 57
Heme oxygenase	0.07 ± 0.02	0.06 ± 0.03	0.07 ± 0.02	0.06 ± 0.01	0.17 ± 0.05§	0.07 ± 0.03
Flavin-containing mono-oxygenase	3.7 ± 0.9	3.9 ± 0.6	4.2 ± 0.4	4.0 ± 0.5	2.5 ± 0.3§	4.4 ± 0.8
UDP-glucuronyltransferase	1.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.2	1.4 ± 0.4	2.6 ± 0.6§	1.3 ± 0.4

* mU/mg.
† units/mg.
‡ nmole/mg × min.
§ $P \leq 0.01$
‖ $P \leq 0.05$
± $P \leq 0.1$
} with respect to controls according to Student's *t*-test.
N = 6.

50% over the control, while GSH-thioltransferase activity dropped to one half of the control activity in selenium deficiency. After 4 months, a statistically significant decrease of 15% in body weight emerged in the Se^- group compared to the supplemented group. No other changes between the groups were observed. In order to relate these effects of selenium depletion, the reversibility of these dietary perturbations needed to be demonstrated. We therefore studied the kinetics and concentration dependence of the recovery process in response to a single dose of i.p.-injected selenite.

As shown in Fig. 2A (lower curve), GSH-Px activity increased linearly with time in response to the injection of 250 μg selenium/kg body wt after an initial time lag of 6 hr. Sixty hours later, the specific activity peaked at a level corresponding to 45% of the control activity, followed by a steady decline. The changes in all the parameters of drug metabolism induced by selenium deficiency were fully reversed and reached control levels within 114 hr in each case. The recovery of enzyme activity to control levels exhibited first-order kinetics. Individual recovery times had time constants from 6 to 48 hr to reach half-maximal effects (Table 2). NADPH-cytochrome-P-450-reductase and heme-oxygenase activities responded the fastest to selenium, i.e. the recovery was completed within 12 to 24 hr. The other microsomal, as well as cytosolic, enzyme activities changed more slowly. Generally, an initial time lag up to 12 hr preceded selenite-induced recoveries regardless of the increase or fall to control levels. Finally, when control mice were used in a similar experiment, a small increase in GSH-Px activity was the only significantly changed enzyme activity (data not shown). Thus we consider the observed enzymatic changes during prolonged selenium deficiency to be specific for selenium.

In order to add to the observed selectivity the parameter of sensitivity, the dose of selenite injected vs the degree of return towards control values, i.e. the percentage of recovery, was investigated. As shown in Fig. 3, a threshold dose of 10–15 μg selenium/kg must be exceeded in order to induce GSH-Px activity. Above this level, enzymatic activity increased linearly up to 250 μg selenium/kg, and above 500 μg/kg, the first signs of saturation became visible. In contrast, drug metabolism enzymes responded in a much more profound way to selenium supplementation. A dose of 7 μg selenium/kg restored 50% of the activities throughout. It is important to notice that almost complete reversal of all long-term changes in hepatic drug metabolism observed in selenium deficiency was reached by a single dose of 10 μg selenium/kg while mouse liver GSH-Px was not detectable. This means, in practical terms, that the effects of selenium deficiency on hepatic drug metabolism can be differentiated from that of GSH-Px activity due to their much greater affinity for the element.

Table 2. Recovery time constants of enzymatic perturbations induced by selenium deficiency in male mouse liver following repletion of selenium by a single dose of 250 μg/kg selenium

Enzyme	$t_{1/2}$(hr)
NADPH-cytochrome-P-450-reductase	5.7
Ethoxycoumarin-*O*-dealkylase	23.2
Hydroperoxidase	21.5
Heme oxygenase	7.5
Flavin-containing mono-oxygenase	42.6
UDP-glucuronyltransferase	14.1
Sulphotransferase*	36.1
GSH-*S*-transferases*	
1,2-Dichloro-4-nitrobenzene	21.6
1-Chloro-2,4-dinitrobenzene	43.8
GSSG-reductase*	34.8
GSH-thioltransferase*	24.6
GSH-peroxidase	28.8

* Cytosolic.

DISCUSSION

This study shows that in male mice the loss of GSH-Px is an early marker of selenium deficiency in

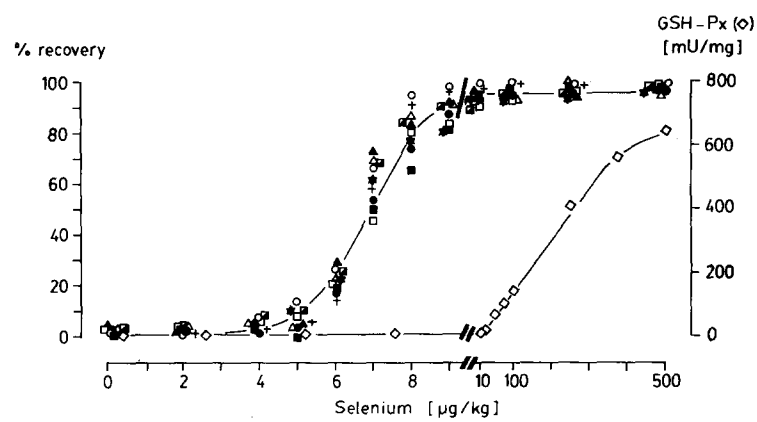

Fig. 3. Dose dependence of enzyme activity changes. Se^- mice were injected with a single dose of selenium as indicated. Changes in enzyme activities after 114 hr are expressed for enzymes of drug metabolism in % control activity restored. Enzymes related to hepatic drug metabolism: (○) ethoxycoumarin de-ethylase; (△) NADPH-cytochrome-P-450-reductase; (+) flavin-containing mono-oxygenase; (■) heme oxygenase. GSH-*S*-transferases: (□) 1,2-dichloro-4-nitrobenzene; (★) 1-chloro-2,4-dinitrobenzene; (●) GSSG-reductase; (▲) GSH-thioltransferase; (◪) sulphotransferase; (◇) GSH-peroxidase.

the liver. The sensitivities of the chemical selenium determination and the enzymatic assay are comparable and indicate that GSH-Px reaches zero at *ca* 80 ng selenium/liver wet wt. On the other hand, changes in drug metabolism represent a phase of more advanced selenium deficiency. Despite a prompt and rapid GSH-Px decrease during the initial selenium depletion period, the animals gained weight normally, and doubled their weight within the first 7 days. This implies that the available amount of selenium in the body is continously diluted. The second phase of depletion resolved distinct phases of aggravation of selenium deficiency.

The initial loss of GSH-Px was followed by complex changes in a number of parameters in drug metabolism. During these two phases, no obvious impairment of vital functions was observed. In a later second phase, the selenium-deficient mice exhibited lower weight gain than the controls. These mild, overall body weight changes resulting from pure selenium deficiency are in contrast to the pathological changes and finally death observed in combined selenium and vitamin E deficiency [9].

The observed changes in drug metabolism and other enzymatic activities suggest that a general phenomenon takes place in a later stage of selenium deficiency. Our experiments on the depletion kinetics indicate that the alterations of enzyme activities observed do not seem to occur independently but rather in a concerted way. This suggests that the primary event may be controlled by a common selenium-sensitive process. Different lines of evidence support the idea that the loss of GSH-Px following dietary selenium deficiency is neither a necessary nor a sufficient cause for the profound changes in other enzyme activities thereafter:

(a) These alterations are neither uniform nor unidirectional. The affected enzymes are so different in structure, function and localization that loss of protection against oxidative challenge could not be evoked as a directly mediated non-specific denominator for their unbalance.
(b) The very short times needed for selenium to restore the activities of heme oxygenase and NADPH-cytochrome-P-450 reductase are well before that of GSH-Px observed in the repletion experiments.
(c) The doses needed for reversal of the selenium deficiency effects are, by more than one order of magnitude, lower for the activities not related to GSH-Px.

These observations suggest that the modulation process is independent of GSH-Px activity, although it cannot be strictly excluded that lacking protection of a central modulator by GSH-Px could have evoked the modulation. Published data including altered rates of vitamin E metabolism [10], phospholipid [11] or glutathione turnover [12] and glucose catabolism [11], as well as modulation of mitochondrial substrate oxidation [13] provide further evidence that various metabolic processes other than drug metabolism are also affected by selenium deficiency. On the other hand, the number as well as the amount of the enzymes responsive to selenium excludes a stoichiometric involvement of selenium in these enzymes themselves. The very small amount of selenium required to restore normal drug metabolism rather suggests that a central regulatory mechanism including a selenium-dependent mediator may be involved. This would result in a mechanism of high amplification with respect to direction, size and versatility of effects. A clue to the possible mechanism is provided by the depletion and repletion kinetics. The depletion process causes uniform changes in all the affected enzyme activities and proceeds with a slow velocity, i.e. half-maximal effects are obtained after 15 days. In contrast, upon abruptly increasing the selenium levels, the return of drug metabolism to normal is a very fast process with individual time constants for the individual enzyme activities. Thus it seems that the balance between synthesis and catabolism of specific enzymes is differently affected in the depletion and repletion phases.

This study demonstrates that different pools of selenium exist in mouse liver. A pool of relatively lower affinity is able to saturate GSH-Px activity to reach 50% of the control enzyme activity with a dose of *ca* 250 μg/kg body wt in single-pulse experiments performed with long-term deficient animals. In contrast, a dose of 7 μg selenium/ kg assigned to the high affinity pool is sufficient to restore 50% of the changes in drug metabolism. In practical terms, a selenium concentration of around 35 ppb selenium in the diet is effective in maintaining normal metabolic conditions. Selenium contents in most commercial diets range around 10–30 ppb selenium i.e. dietary factors can easily limit the extent of selenium depletion. This is probably one of the main reasons for the diversity of effects ascribed to selenium deficiency.

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REFERENCES

1. R. F. Burk, *A. Rev. Nutr.* **3**, 53 (1983).
2. R. Reiter and A. Wendel, *Biochem. Pharmac.* **32**, 3063 (1983).
3. R. A. Lawrence and R. F. Burk, *J. Nutr.* **108**, 211 (1978).
4. J. H. Watkinson, *Analyt. Chem.* **38**, 92 (1966).
5. C. Mitoma, H. S. Potter and H. C. Udenfried, *Archs Biochem. Biophys.* **61**, 431 (1956).
6. C. E. Mize and R. G. Langdon, *J. biol. Chem.* **237**, 1589 (1962).
7. K. Axelsson, S. Eriksson and B. Mannervik, *Biochemistry* **17**, 2978 (1978).
8. I. H. Segel and M. J. Johnson, *Analyt. Biochem.* **5**, 330 (1963).
9. K. Schwarz, *Fedn. Proc,* **24**, 58 (1965).
10. W. C. Fischer and P. D. Whanger, *J. nutr. Sci. Vitamin.* **23**, 273 (1977).
11. W. C. Fischer and P. D. Whanger, *J. Nutr.* **107**, 1493 (1977).
12. K. E. Hill and R. F. Burk, *J. biol. Chem.* **257**, 10668 (1982).
13. H. Sies and K. H. Moss, *Eur. J. Biochem.* **84**, 377 (1978).