Glutathione peroxidase activities during selenium depletion of adult female rats and during selenium repletion of their offspring

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The main purpose of the present investigation was to produce young rats with severe selenium deficiency, but with no clinical signs of this deficiency, and to examine their liver and red blood cell (RBC) glutathione peroxidase activities during selenium repletion. To achieve this goal, female breeders were fed a selenium-deficient diet beginning 2 weeks before mating. The liver glutathione peroxidase activity of the dams was significantly lower than the activity of comparable nonpregnant females after 5 and 10 weeks of selenium depletion. This difference arose exclusively during the period of pregnancy. In contrast, the RBC glutathione peroxidase activity was significantly increased during this period. Only traces of liver enzyme activity were found in the offspring, and the RBC enzyme activity was only 2% of that of the selenium-repleted controls. Body weight was retarded in the male offspring. However, no severe signs of clinical selenium deficiency were observed. The glutathione peroxidase activity in the liver and RBCs of the offspring was determined after 0, 2, 4, 7, 14, and approximately 40 days of selenium repletion. The liver enzyme activity increased faster in females than in males, while the opposite was found for the RBCs. After 14 days of selenium repletion, the glutathione peroxidase activity of the liver was essentially restored, and the RBC enzyme activity was about half that of the control values. This type of rat may prove useful in studies in which young selenium-deficient rats are preferable, as well as in studies of selenium functions that might not be directly related to the role of selenium in glutathione peroxidase.

Keywords: Glutathione peroxidase; selenium; rat; female breeders; offspring

Introduction

The selenoenzyme glutathione peroxidase (GSH-Px: EC 1.11.1.9) may have an important function in protecting membranes from oxidative damage by reducing hydrogen peroxide and some organic peroxides to water and alcohols, respectively.[1] The highest enzyme activity and percentage of selenium bound to GSH-Px is found in liver and red blood cells (RBCs).[2] The hepatic GSH-Px response to dietary selenium content is much faster than that of RBCs.[3] Thus, these tissues are of particular interest for the assessment of selenium status in experimental selenium depletion and repletion. The decrease in GSH-Px activities on feeding a selenium-deficient diet to male rats is well-documented,[3-5] while few studies have been concerned with the female rat.[6] Thus, studies of the recovery of GSH-Px activity after selenium repletion (rats fed a selenium-deficient diet since weaning) have only been performed on older rats of the male sex.[3-5] Hence, this study was initiated to examine the build-up of GSH-Px activities during selenium repletion of young selenium-deficient rats of both sexes. Rats born of selenium-depleted dams were a prerequisite. The aim was to obtain young rats with severe selenium deficiency, but with no obvious signs of this clinical deficiency, such as marked body weight retardation and poor hair coat, which have been observed in second-generation selenium-deficient rats.[7-9]

Three main reasons underlie these experiments. First, feeding a selenium-deficient diet to young rats for 4 to 6 weeks decreases the liver GSH-Px activity to less than 5% of supplemented controls.[3,5] Never-

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theless, there are still significant amounts of selenium left in the RBCs, as indicated in experiments showing that after 20 weeks of selenium deficiency, the activity of RBC GSH-Px was still about 20% of the supplemented control value.[3] Second, novel selenium functions may be undetectable until the RBC pool of selenium is so low that only traces of the element may be redistributed to other tissues. Third, in many toxicologic and biochemical studies, young or young adult rats would be preferable to much older rats, provided that their selenium status is sufficiently low.

The GSH-Px activities during pregnancy and lactation were also determined, as compared with age-matched, nonpregnant female rats, and taken as an indirect estimate of selenium lost to the fetus and offspring, respectively.

Methods and Materials

Animals and diets

Wistar rats from our own breeding colony (originating from Bantin & Kingman Ltd., England) were used. They were housed in a room with controlled temperature (20 to 22°C) and were exposed to a 12-hour light/dark cycle. Rats were fed a Torula yeast-based selenium-deficient purified diet (*Table 1*) with a selenium content of 0.008 mg/kg, as determined by neutron activation analysis (analysis performed by the Swedish Environmental Research Institute, IVL, Stockholm, Sweden). Distilled water with added NaCl (50 mg/l) was used as drinking water and selenium supplementation was with 0.2 mg Se/l drinking water. Selenium was added as Na_2SeO_3. Diet and drinking water were fed ad libitum.

Female rats were fed a nonpurified standard diet (Astra Ewos R3, Astra Ewos, Södertälje, Sweden) with a selenium content of 0.22 mg/kg until the age of 10 weeks. GSH-Px activities of the liver and RBCs were then determined in six females to obtain initial (0) values for the depletion study. Other female rats were selenium-depleted from the same age. After 2 weeks of selenium depletion, some females were placed in groups of three with a proven male breeder for 3 days. The females were weighed 20 days after this grouping (about 4 days before partus). A weight gain of 20% or more was found indicative of pregnancy, and such rats were placed individually in cages. Liver and RBC GSH-Px activities were determined in groups of dams and age-matched nonmated females during the course of selenium depletion. A third group of 10-week-old female rats was supplemented with selenium for 5 weeks and served as a reference group to the depletion study and controls (C) to unmated rats fed the selenium-deficient diet for 5 weeks.

The offspring of selenium-depleted dams were used for determination of the GSH-Px activities in the liver and RBCs during selenium repletion. At the age of 5 weeks, some males and females were supplemented with selenium for 2, 4, 7, 14, or 40 days, while other groups continued on the selenium-deficient regimen.

Table 1 Diet composition

Ingredient	Percent of diet
Torula yeast[a]	30
Sucrose	43.8
Corn starch	15
Corn oil (tocopherol stripped)[a]	6.4
Mineral mixture[a,b]	3.5
Vitamin mixture[c,d]	1.0
dl-methionine[d]	0.3

[a] Purchased from Teklad Test Diets, Madison, WI, USA.
[b] William-Briggs modified mineral mixture, consisting of (g/kg) the following: $CaCO_3$, 207.1; $CaHPO_4$, dibasic, 322.9; $MgSO_4$, anhydrous, 65.7; KCl, 208.6; Na_2HPO_4, 186; $CuSO_4$, anhydrous, 0.37; ferric citrate (16.7% Fe), 4.3; $MnSO_4 \times H_2O$, 4.4; KIO_3, 0.03; and $ZnCO_3$, 0.60.
[c] One percent vitamin mixture gave a final diet addition of (mg or IU) vitamin/100 g diet of 0.04 mg thiamin HCl, 0.25 mg riboflavin, 0.2 mg pyridoxine HCl, 2.0 mg calcium pantothenate, 10.0 mg niacin, 0.1 mg biotin, 0.20 mg folic acid, 0.01 mg vitamin B_{12}, 100 mg choline chloride, 1,400 IU retinyl palmitate, 320 IU ergocalciferol, 5.0 mg tocopheryl acetate, and 0.1 mg menadione.
[d] Purchased from Sigma Chemical Co., St. Louis, MO, USA.

The group supplemented with selenium for 40 days was regarded as fully supplemented.

Tissue preparation

Rats were lightly anesthetized with ether and 5 ml of blood was taken by heart puncture; plasma was removed by centrifugation. A hemolysate was prepared from the sedimented RBCs according to the method of Günzler et al.[10] The transformed hemoglobin containing 10 mg hemoglobin/ml was stored at 4°C and assayed the next day. Immediately after the blood had been removed, the rat was decapitated, and the liver was perfused in situ with ice-cold 0.15 M KCl until it was uniformly pale. The liver was then blotted and minced. A three-volume homogenate was prepared in ice-cold 0.15 M KCl by six passes of a motor-driven Teflon pestle in a glass homogenizing vessel. After centrifugation at 9,000 × *g* for 20 minutes at 4°C, the supernatant was further centrifuged at 105,000 × *g* for 60 minutes at 4°C to yield the postmicrosomal fraction. The protein content of this fraction was determined according to Lowry et al.,[11] using bovine serum albumin as the standard.

Enzyme analysis

The activity of GSH-Px was determined by a coupled assay as described by Günzler et al.[10] Samples were assayed with both 0.25 mM hydrogen peroxide and 1.25 mM tert-butyl hydroperoxide as the substrate. These substrate concentrations were confirmed to be optimal. The GSH-Px activity is expressed in units (U). One unit is 1 μmol NADPH oxidized/(min·g protein or hemoglobin) of postmicrosomal liver supernatant or RBCs, respectively. As there were no substrate-dependent differences in RBC GSH-Px activities, only the results with organic hydroperoxide have been presented. Only the selenium-dependent

GSH-Px activity, measured with H_2O_2, is presented for liver samples.

Chemicals and statistical analyses

The 70% tert-butyl hydroperoxide was from Aldrich (Steinheim, FRG). All other chemicals were of high purity and were purchased from Sigma Chemical Co. The data obtained were tested statistically using the Student's *t* test and are presented as mean values ± SE.

Results

Feeding the selenium-deficient diet had no effect on the success of fertilization, the time of pregnancy, or the number of offspring. Parturition was 24 ± 0.3 days after the first opportunity of copulation, and the number of offspring was 12 ± 0.6 (mean values ± SE of nine dams).

Adult female rats fed the standard diet (0) or the Torula diet with selenium supplementation for 5 weeks (group C) had almost identical GSH-Px activities in the liver (*Figure 1*) and RBCs (*Figure 2*). This indicates that the bioavailability of selenium was comparable for rats fed the standard diet (0.22 ppm selenium) and those fed the selenium-supplemented Torula-based diet. Furthermore, the liver GSH-Px activity of the selenium-supplemented control group (C) could also be regarded as a reasonable estimate of enzyme activities in the present female rats at an age of between 10 and 20 weeks (*Figure 1*). This assumption is based on a reported similarity of liver GSH-Px activity in rats of these ages.[12] The GSH-Px activities of dams and nonmated female rats indicated a pregnancy-dependent (*Figures 1* and *2*) as well as a lactation-dependent (*Figure 2*) effect during selenium depletion.

Growth was retarded in male rats born of selenium-depleted dams, whether they were selenium-repleted for 2 weeks or not (*Table 2*). No such effect was noted for the female offspring. In fact, no sex-dependent difference in body weight was noted between male and female siblings born selenium-deficient, nor between

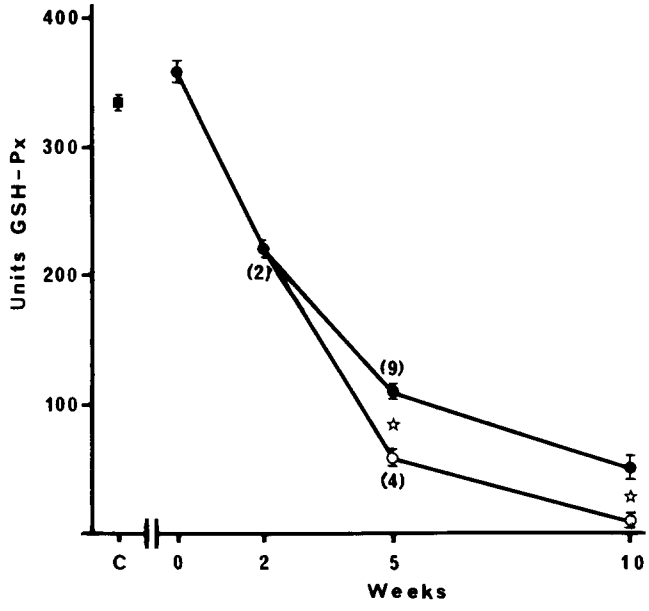

Figure 1 Effect of selenium depletion on GSH-Px activity in liver cytosol from adult female rats. Selenium depletion started with 10-week-old rats, and GSH-Px activity was determined in nonmated females (●) and dams (○) after 2, 5, and 10 weeks of selenium depletion, i.e., at mating, at parturition, and at weaning, respectively. C denotes nonmated females fed the selenium-deficient diet and drinking water with 0.2 mg selenium/l for 5 weeks (■). GSH-Px activity of all selenium-depleted groups was significantly less than those in the C and 0-week groups ($P < 0.001$). ☆ Indicates a significant difference between dams and nonmated females ($P < 0.01$). One unit (U) GSH-Px = 1 μmol NADPH oxidized/min · g protein or hemoglobin) of the 105,000 × *g* liver supernatant or RBCs, respectively. Values are mean ± SE of six (*Figures 1* and *2*) or four (*Figures 3* and *4*) rats if not otherwise stated in parentheses. If not indicated, the SE bar is smaller than the symbol.

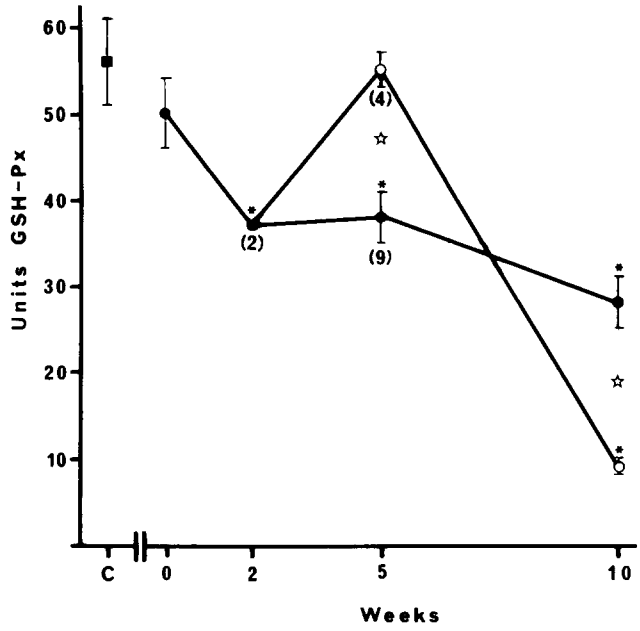

Figure 2 Effect of selenium depletion on GSH-Px activity in RBCs from adult female rats. Rats and treatments are the same as in *Figure 1*. * Indicates significantly less GSH-Px activity than in the C and 0-week groups ($P < 0.05$). ☆ Indicates a significant difference between dams and nonmated females ($P < 0.001$). See *Figure 1* for further details.

Table 2 Effect of breeding and selenium regimen[a] on the body weight of 7-week-old rats

	Body weight (g)[b]			
	Males		Females	
Diet	Control	Born Se^-	Control	Born Se^-
Se^+	204 ± 4	168 ± 6[c]	167 ± 5	162 ± 7
Se^-	200 ± 4	158 ± 3[c]	165 ± 3	170 ± 12

[a] "Control" rats were born and nursed by dams fed a standard diet. They were fed a selenium-supplemented (Se^+) or -deficient (Se^-) diet regimen from 5 weeks of age. "Born Se^-" rats were borne and nursed by selenium-depleted dams. They were continuously fed the selenium-deficient diet (Se^-) or were selenium-repleted (Se^+) via drinking water from 5 weeks of age.
[b] Values are means ± SE of six control rats or of four rats born Se^-.
[c] Significantly different from corresponding control, $P < 0.001$.

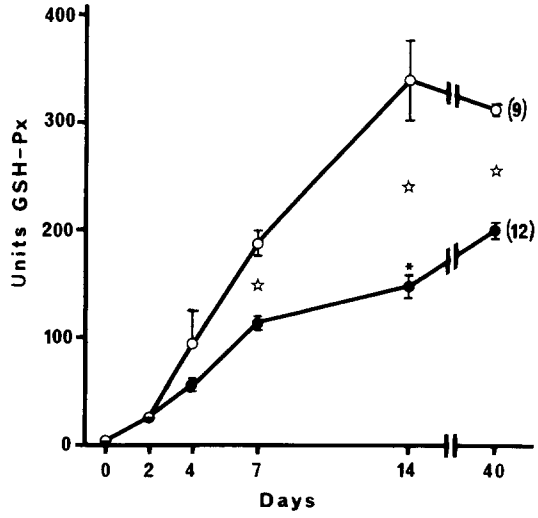

Figure 3 GSH-Px activity in liver cytosol during selenium repletion (0.2 mg selenium/l drinking water) of male (●) and female (○) rats born of selenium-depleted dams. GSH-Px activity for selenium-repleted rats was significantly greater ($P < 0.05$) than those in selenium-deficient rats at day 2 and all following days. ☆ Indicates a significant ($P < 0.01$) sex-dependent difference. * Indicates that GSH-Px activity at day 14 is significantly less ($P < 0.01$) than at day 40. See *Figure 1* for further details.

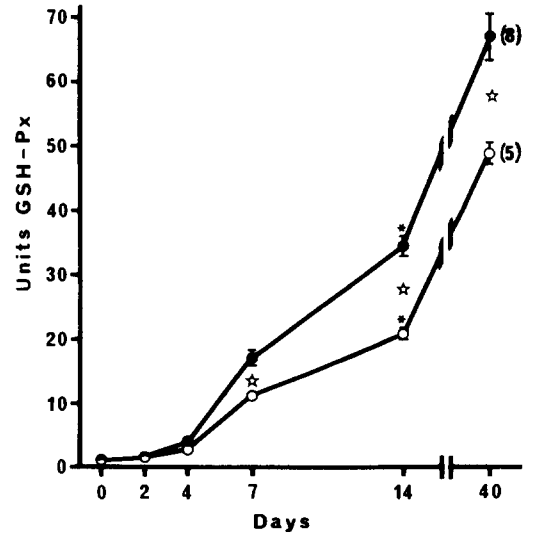

Figure 4 GSH-Px activity in RBCs during selenium repletion (0.2 mg selenium/l drinking water) of male (●) and female (○) rats born of selenium-depleted dams. GSH-Px activity was significantly greater ($P < 0.05$) for selenium-repleted rats than those in selenium-deficient male rats at day 4 and in female rats at day 7 and all following days. ☆ Indicates a significant ($P < 0.02$) sex-dependent difference. * Indicates that GSH-Px activity at day 14 is significantly less ($P < 0.001$) than at day 40. See *Figure 1* for further details.

these groups and the female controls (*Table 2*). In addition to a retarded body weight, unrepleted rats showed signs of a more sparse and out-turned hair coat at 11 weeks of age. However, these effects of selenium deficiency were small compared with described effects on second-generation selenium-deficient rats with their lack of hair growth, eye cataracts, and severely retarded growth.[7,9]

Selenium repletion of the offspring of selenium-deficient dams was followed as increased GSH-Px activity in the liver (*Figure 3*) and RBCs (*Figure 4*) after 2, 4, 7, 14, and 40 days of selenium repletion. Rats repleted with selenium for 40 days were regarded to be fully supplemented and are referred to as controls. The liver GSH-Px activity increased faster in females than in males (*Figure 3*), while the opposite was found regarding the RBC GSH-Px activity (*Figure 4*).

Discussion

The liver GSH-Px activity of dams was significantly lower than the activity of comparable nonpregnant females after 5 and 10 weeks of selenium depletion, i.e., at the time of partus and after weaning, respectively. This difference arose exclusively during the period of pregnancy, while the rate of decline appeared almost identical for dams and nonpregnant females between 5 and 10 weeks of selenium depletion (*Figure 1*). Thus, a previously reported low value of rat liver selenium content and GSH-Px activity in selenium-depleted dams on day 18 of lactation compared with nonlactating females[6] was probably derived from differences during the period of pregnancy rather than due to the effects of lactation.

The RBC GSH-Px activity of nonmated females declined slowly during selenium depletion. This group still retained about 53% of the values for the 0 and C groups after 10 weeks of selenium depletion (*Figure 2*). In contrast, the RBC GSH-Px activity of dams was significantly increased at partus ($P < 0.001$), then dropped to a level significantly below that of nonpregnant rats at 10 weeks of selenium depletion ($P < 0.01$). Increasing activity of RBC GSH-Px until the time of partus has also been observed in humans.[13] The pregnancy-related increase of GSH-Px activity in human[13] and rat (*Figure 2*) RBCs might indicate a pregnancy-dependent hormonal effect. Hormones have, in fact, been shown to influence the activity of GSH-Px.[14] The presently observed changes in liver and RBC GSH-Px activities of selenium-depleted and newly delivered dams might indicate a redistribution of selenium from dams to the offspring. The GSH-Px activities of the liver (*Figure 1*) and RBCs (*Figure 2*) are indicative of a strongly reduced selenium supply in dams during lactation.

Only traces of GSH-Px activity were detected in the liver of unsupplemented male and female rats born of selenium-depleted dams. At day 2 of selenium repletion, the enzyme activity was significantly increased, to the same level for both sexes (*Figure 3*). After more than 2 days of selenium repletion, the female liver GSH-Px activity increased faster than in males, and a statistically significant sex difference was found from day 7 and on the following days of repletion. The greatest sex difference was found after 14 days of Se repletion, when the female liver GSH-Px activity had

reached the control level, while the male activity was still below ($P < 0.01$) its control value (*Figure 3*).

The RBC GSH-Px activity of unrepleted male and female rats was about 2% of the corresponding control values (*Figure 4*). Such a low value for RBC GSH-Px activity was not reached even after 1 year of selenium depletion when starting with weaning male rats with normal selenium status.[3] The first significant ($P < 0.05$) increase of the RBC GSH-Px activity in male and female rats was found after 4 and 7 days of selenium repletion, respectively. In striking contrast to the liver GSH-Px activity, the male RBC GSH-Px activity was higher than in the female rats at all times during selenium repletion (*Figure 4*). This sex difference became significant ($P < 0.02$) from day 7 of selenium repletion and thereafter, i.e., from the time when liver GSH-Px activity was found to be significantly higher in the female rats than in the male rats. At day 14 of selenium repletion, the RBC GSH-Px activity of both sexes was significantly below the corresponding control value ($P < 0.001$). With a RBC lifespan of about 60 days for rats,[15] one should expect that not more than about 25% of the control GSH-Px value could be reached in 14 days of selenium repletion, while, in fact, 52% and 42% of the control values were found in males and females, respectively. This might indicate that the normalization of RBC GSH-Px activity during selenium repletion is not exclusively dependent on newly formed RBCs or that erythropoiesis increases by selenium supplementation.

The breeding regimen with moderately selenium-depleted dams was effective in producing severely selenium-deficient *young* rats. No severe signs of clinical selenium deficiency were observed in these rats, not even when continuously fed the selenium-deficient diet regimen until the age of 11 weeks. The characteristics of liver and RBC GSH-Px activities on selenium repletion (*Figures 3* and *4*) indicate that young rats with significant or even optimal GSH-Px activities are easily produced in 14 days of selenium repletion and, thus, may serve as controls to their severely selenium-deficient siblings. Several biologic effects of selenium may be unrelated to the role of selenium in GSH-Px. This is the case when a single or short-term administration of selenium to selenium-deficient rats restores a deficiency-dependent effect without significantly increasing the GSH-Px activity of the tissue in question.[16] The young, severely selenium-deficient rat presently described may be useful to trace the mechanistic background to such effects. This kind of rat will also be useful in further experiments concerning selenium-dependent effects in the metabolism of ketone bodies[17] and in some detoxication functions.[18,19]

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