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DIFFERENTIAL EFFECT OF CADMIUM ON GSH-PEROXIDASE ACTIVITY IN THE LEYDIG AND THE SERTOLI CELLS OF RAT TESTIS

SUPPRESSION BY SELENIUM AND THE POSSIBLE RELATIONSHIP TO HEME CONCENTRATION

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Abstract—In the testes of rats treated with cadmium acetate (7 or 20 μmoles/kg, 24 hr, s.c.), the activity of glutathione (GSH)-peroxidase was increased. At the same time, the activity of glutathione disulfide (GSSG)-reductase and the cellular GSH concentration were decreased significantly. The basal activity of peroxidase in the Leydig and the Sertoli cell populations was comparable. However, the magnitude of increases in the activities markedly differed in the two cell populations, with that of the Sertoli cells increasing to nearly 450% of the control value in response to treatment with 20 μmoles/kg Cd^{2+}. In the Leydig cells, the enzyme activity in response to the same treatment increased to only about 170% of the control value. Cd^{2+} treatment increased the concentration of heme in the microsomal and the smooth and rough endoplasmic reticulum fractions of the whole testis, as well as in the microsomal fractions of the Leydig and the Sertoli cells. As with the peroxidase activity, the two cell populations vastly differed in their susceptibilities to Cd^{2+} treatment, with the Sertoli cells being more severely affected by the metal. In the Sertoli cells the microsomal heme concentration was increased by approximately 11-fold, whereas only a 2-fold increase in the Leydig cells was noted. The increase in GSH-peroxidase activity was not due to the peroxidase activity of GSH-*S*-transferases, insofar as an increase in transferase activity was not observed in the Leydig and the Sertoli cells. Treatment of rats with sodium selenite (10 μmoles/kg, s.c.) 30 min before Cd^{2+} treatment (20 μmoles/kg) fully suppressed the above-described spectrum of effects of Cd^{2+} in the testis. Also, sodium selenite at a lower dose of 5 μmoles/kg prevented an increase in GSH-peroxidase activity. It is hypothesized that increased GSH-peroxidase activity in the Leydig and the Sertoli cells constitutes an adaptive response to increased cellular levels of heme and to the free radicals generated by the heme molecule. Selenium prevents the increase in GSH-peroxidase activity by circumventing the increase in cellular heme concentration. The protection is believed to be related, at least in part, to increased production of cellular GSH.

It is commonly known that testis constitutes the target organ for Cd^{2+} toxicity [1]. Similarly, the ability of selenium complexes and thiol compounds to protect against Cd^{2+}-mediated hemorrhagic lesions in this organ is well established [2–4]. Although the mechanism by which these agents protect against tissue damage is not clearly understood, selenium, when given as sodium selenite, has been shown to increase the cellular concentration of glutathione (GSH) in the liver by increasing the *de novo* synthesis of enzymes of GSH-biosynthetic pathway, including that of glutathione disulfide (GSSG)-reductase [5]. This increase is dose-related and, when selenium is given at 20 μmoles/kg, a doubling of activities of the enzymes of GSH-biosynthetic pathway is noted [5]. It has been suggested that this activity of selenium contributes to its protection against nephrotoxicity of mercury [6]. Prohaska *et al.* [7] have shown that testicular toxicity of Cd^{2+} is accompanied by an elevation of the activity of GSH-peroxidase; peroxidase utilizes GSH as substrate to inactivate hydrogen peroxide and oxygen-free radicals.

Our previous findings have shown that in the testis of Cd^{2+}-treated rats there is a preferential increase in heme concentration in the Sertoli cell population, when compared with the Leydig cells [8]. Using labeled heme precursors, it was established that the source of heme is the hemoglobin of the erythrocytes. Moreover, the heme molecule was shown to become incorporated into and/or tightly bound to the endoplasmic reticulum membranes [9]. In this connection, a recent study has shown that Cd^{2+}-induced hemorrhage in the testis is accompanied by an increase in lipid peroxidation in the organ [10]. We have shown in the past that lipid peroxidation is promoted by the iron released in the course of degradation of endogenous heme [11–13]. This process is associated with the destruction of cellular membranes and their components, particularly under conditions where cellular GSH levels are decreased [11–13].

The present study was undertaken to examine the effects of Cd^{2+} and selenium on GSH metabolism in different populations of cells in rat testis, and to explore the possibility that increase in GSH-per-

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oxidase activity is a phenomenon linked to increased cellular heme concentrations.

EXPERIMENTAL PROCEDURES

Chemicals, animals and tissue preparation. Cadmium acetate, [Cd^{2+}, $Cd(CH_3Co_2)_2 \cdot 2H_2O$], was obtained from the Fisher Chemical Co., Chicago, IL. Glutathione, GSSG, NADPH, GSSG-reductase (yeast), and *o*-phthalaldehyde were purchased from the Sigma Chemical Co., St. Louis, MO. 1,2-Dichloro 4-nitrobenzene (DCNB) and 1-chloro-2,4-dinitrobenzene (CDNB) were products of the Merck Chemical Co. Sodium selenite (Na_2SeO_3) was obtained from J. T. Baker, Phillipsburg, NJ.

Male Sprague–Dawley rats (180–220 g) were purchased from Harlan Industries, Madison, WI. The animals were maintained on a 12-hr light and dark cycle and were given food and water *ad lib*. All injections were made subcutaneously between 8:00 and 9:00 a.m. The control group received saline. The regimen of treatments is described in the legends to the tables and figure.

The animals were decapitated and the testes were removed. The organs were homogenized in 2 vol. of Tris–HCl (0.01 M, pH 7.4) containing 0.25 M sucrose, and the microsomal and cytosol fractions were prepared as described elsewhere [9]. The 105,000 *g* supernatant fraction was used for the measurement of GSSG-reductase and GSH-peroxidase activities. The content of heme was measured using the microsomal fractions (105,000 *g*, 1 hr). The smooth and the rough endoplasmic reticulum fractions (SER and RER, respectively) were prepared as follows. The organs from two rats were pooled, and the fractionation was accomplished in the presence of CsCl as described by Dallner [14]. The Leydig and the Sertoli cell preparations were obtained using the following procedures. The Leydig cells were isolated by a modification of the procedure of Dufau *et al.* [15]. The decapsulated testes were minced and placed in Leibowitz's medium (approximately 5 ml/g tissue) containing 1 mg/ml bovine serum albumin (BSA) and 0.25 mg/ml collagenase. The preparation was incubated for 10 min at 37° with vigorous shaking. The resulting suspension was filtered through a 100 μm nylon mesh. The filtrate was centrifuged for 10 min at 600 *g*, the cell pellet was resuspended in 20 vol. of Tris–HCl (0.01 M, pH 7.4) containing 0.25 M sucrose, and the centrifugation was repeated. The final cell pellet was used for the preparation of subcellular fractions.

Enriched suspensions of the Sertoli cells were prepared by an adaptation of the methods of Steinberger *et al.* [16] and Dorrington and Fritz [17]. Decapsulated testes were cut into small pieces and incubated with gentle intermittent shaking in Ca^{2+}-free Hanks' medium (approximately 25 ml/g tissue) containing 0.25 mg/ml trypsin and 1 mg/ml BSA for 15 min at 37°. The separated seminiferous tubules were collected on 100 μm nylon mesh and washed repeatedly with the above medium until no evidence of contamination of the tubules with the Leydig cells was observed upon microscopic examination. Subsequently, the tubules were incubated with vigorous shaking in Leibowitz's medium (approximately 5 ml/g tissue) containing 0.25 mg/ml collagenase, 1 mg/ml BSA, and 0.1 mg/ml trypsin inhibitor, for 1 hr at 37°. The resulting suspension was filtered through 100 μm nylon mesh and rinsed with medium, and the retained cells were collected. After settling, the supernatant fraction was removed and the pellet was resuspended in the above-described buffer. The Sertoli cell preparations contained approximately 10% elongated spermatids and fragments from the spermatids. The Leydig cell preparations contained less than 5% contamination with spermatic and cell debris. The suspensions of the Leydig and the Sertoli cells were briefly sonicated, homogenized manually in a glass homogenizer, and used for the preparation of the microsomal and the cytosol fractions.

Assay procedures. Tissue GSH concentrations were measured by a modification of the method described by Cohn and Lyle [18]. The testes were homogenized in 5 vol. of an extraction mixture consisting of 10% trichloroacetic acid/1 mM Na_2EDTA/0.01 N HCl (1/1/1, by vol.). Protein was removed by centrifugation at 5000 *g* for 10 min. One milliliter of 0.5 M Na_2HPO_4 and 0.1 ml of *o*-phthaladehyde in methanol (1 mg/ml) were added to a 50-μl sample of the supernatant fraction. GSH concentration was detected fluorometrically using authentic GSH as the standard. The excitation and the emission wavelengths were 328 and 428 nm respectively.

The activities of GSH-peroxidase and the GSSG-reductase were determined by measuring the disappearance of NADPH at 25° at 340 nm. The GSH-peroxidase assay was conducted by a modified coupling method of Paglia and Valentine [19] as described by Lawrence *et al.* [20]. An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per min. The assay procedure used for the determination of GSSG-reductase activity was based on the method described by Massey and Williams [21]. The assay medium (1.0 ml) contained the enzyme source (60–100 μg protein), Na_2EDTA (3 mM), BSA (2 mg), NADPH (0.1 mM), GSSG (3 mM), and potassium phosphate buffer (50 mM, pH 7.6). The reaction was started by the addition of GSSG. The blank assay mixture did not contain GSSG. An enzyme unit was defined as 1 nmole NADPH oxidized per mg protein per min. GSH-*S*-transferase activities towards CDNB and DCNB were assayed by the procedure of Habig *et al.* [22]. Both substrates were dissolved in ethanol. The concentration of ethanol in the assay mixture was 5%.

Heme concentration was estimated by the pyridine hemochromogen method of Paul *et al.* [23] using the reduced-oxidized difference spectrum between 557 and 575 nm and an extinction coefficient of 32.4 $mM^{-1}\,cm^{-1}$. Protein was measured by the method of Lowry *et al.* [24]. All spectral studies were carried out using an Aminco–Chance DW-2 spectrophotometer. The results are expressed as the mean ± SD for four to six experiments. The data were analyzed using Student's *t*-test, and a P value of ≤ 0.05 denotes significance.

RESULTS

The dose-related effect of Cd^{2+} on the concentration of GSH and the activities of GSSG-

Table 1. Dose-related effect of cadmium treatment on the concentration of GSH and activities of GSSG-reductase and GSH-peroxidase in rat testis

Cd^{2+} Treatment (μmoles/kg)	GSH (mM)	GSSG-reductase (nmoles/mg/min)	GSH-peroxidase (nmoles/mg/min)
0	2.87 ± 0.10	18.1 ± 0.5	28.5 ± 3.1
7	2.35 ± 0.23*	18.6 ± 1.9	38.8 ± 1.2*
20	1.98 ± 0.20*	13.9 ± 0.5*	51.9 ± 1.7*

Sprague–Dawley rats (180–220 g) were treated (s.c.) with the indicated doses of cadmium acetate and killed 24 hr later. The control rats received saline. Testes were removed and decapsulated, and the homogenate and the cytosol fractions were prepared. GSH concentration was determined in tissue homogenate. The cytosol was used for the measurement of enzyme activities. The procedures used are described in detail in Experimental Procedures. The results are expressed as mean ± SD of six determinations. One rat was used for each determination.

* $P \leq 0.05$ when compared with the control animals.

reductase and GSH-peroxidase in rat testes are shown in Table 1. As shown, the GSH level was reduced by nearly 30% in rats treated with 20 μmoles/kg Cd^{2+}; when treated with 7 μmoles/kg, the decrease was less pronounced. Similarly, the activity of GSSG-reductase was reduced substantially with the higher dose of Cd^{2+} but was not affected significantly by the lower dose. The activity of GSH-peroxidase exhibited a dose-dependent increase in response to Cd^{2+} treatment and, at the 20 μmoles/kg level, the activity was increased substantially. The presently observed increase in GSH-peroxidase activity is consistent with the previously reported observation by Prohaska *et al.* [7].

The possibility of a differential response of GSH-peroxidase activity in the Leydig and the Sertoli cells to Cd^{2+} was investigated. As shown in Fig. 1, the rates of peroxidase activity in the control preparation of the two cell types were of similar magnitude. However, the magnitude of the response elicited by Cd^{2+} in the two cell types markedly differed. The Sertoli cells appeared more susceptible to Cd^{2+} treatment; in the Sertoli cell preparation obtained from rats treated with 20 μmoles/kg Cd^{2+}, the peroxidase activity measured nearly 400–450% of that of the control. In the Leydig cells, on the other hand, the activity measured only about 170% of that of the control cells.

We have shown in the past [5] that selenium increases cellular GSH levels by increasing the activities of key enzymes in GSH-biosynthesis pathway [25], i.e. GSSG-reductase and γ-glutamylcysteine synthetase. Accordingly, the possibility of protection by selenium of the Cd^{2+}-mediated increase in GSH-peroxidase activity and decreases in GSSG-reductase activity and GSH concentration was examined. The animals received selenium (10 μmoles/kg, s.c.) 30 min before Cd^{2+} treatment (20 μmoles/kg, s.c.). The results of this study are depicted in Table 2. As shown, this regimen of selenium treatment fully blocked the spectrum of the effects of Cd^{2+} on GSH metabolism. In rats treated with selenium alone, significant effects on the measured parameters were not detected. The treatment of rats with 5 μmoles/kg selenium (i.e. 1:4 molar ratio selenium to Cd^{2+}) was fully effective in preventing an increase in GSH-peroxidase activity. The values obtained for the per-

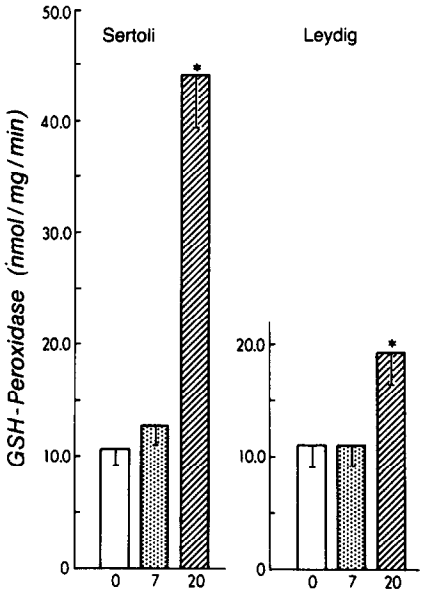

Fig. 1. Dose-related effect of cadmium acetate on GSH-peroxide activity in the Leydig and the Sertoli cell preparations of rat testis. Sprague–Dawley rats (180–220 g) were treated (s.c.) with the indicated doses of cadmium acetate and killed 24 hr later. The Leydig and the Sertoli cell preparations were obtained as described in Experimental Procedures. The cytosol fraction was prepared and used for the measurement of GSH-peroxidase activity as described in the text. The data shown are mean ± SD of four determinations. The pooled testes of two rats were used for each determinate. Key: (□) control; (□) 7 μmoles/kg Cd^{2+} and (□) 20 μmoles/kg Cd^{2+}.

* $P \leq 0.05$ when compared with the control value.

oxidase activity in the control and the selenium p Cd^{2+}-treated rats were 27.4 ± 3.1 and 29.. ± 3.5 nmoles/mg/min respectively.

The mechanism of Cd^{2+}-mediated increase in GSH-peroxidase activity and the protective action of selenium were investigated in relation to the cellular concentration of heme. The effects of Cd^{2+} treatment on heme content in various preparations of testis are shown in Table 3. As noted, treatment of rats with Cd^{2+} (20 μmoles/kg) markedly increased heme concentration in all preparations of the testis, i.e. the

Table 2. Inhibition by sodium selenite of Cd^{2+}-mediated alterations in GSSG-reductase and GSH-peroxidase activities and in the concentration of GSH in rat testis

Treatment (μmoles/kg)		GSH (mM)	GSSG-reductase (nmoles/mg/min)	GSH-peroxidase (nmoles/mg/min)
Cd^{2+}	Selenium			
0	0	2.69 ± 0.30	17.8 ± 1.1	24.7 ± 3.1
20	0	1.91 ± 0.30*	11.0 ± 0.6*	53.1 ± 2.7*
0	10	3.02 ± 0.09	17.7 ± 0.4	32.4 ± 1.2
20	10	2.80 ± 0.11	18.8 ± 0.5	33.1 ± 3.6

Groups of four Sprague–Dawley rats (180–220 g) were treated (s.c.) with the indicated doses of sodium selenite, cadmium acetate or both, and were killed 24 hr after the last injection. When both Cd^{2+} and selenium were administered, selenium was injected 30 min before Cd^{2+}. The control rats received saline. Tissue fractions were prepared as described in the legend to Table 1. The details of experimental procedures are described in the text. Results are expressed as mean ± SD.

* $P \leq 0.05$ when compared with the control.

Table 3. Suppression by sodium selenite of cadmium-mediated increase in heme concentration in the endoplasmic reticulum fractions and the Leydig and the Sertoli cells in rat testis

Treatment (μmoles/kg)		Heme (pmoles/mg)				
Cd^{2+}	Selenium	Microsomes	RER	SER	Sertoli cells	Leydig cells
0	0	60 ± 10	26 ± 1	42 ± 2	7.2 ± 0.8	28.1 ± 3.1
20	0	345 ± 40*	49 ± 9*	144 ± 33*	82.8 ± 7.6*	62.0 ± 4.0*
0	10	70 ± 17	33 ± 3	33 ± 8	10.2 ± 2.0	29.0 ± 2.2
20	10	75 ± 17	21 ± 1	52 ± 7	10.9 ± 2.7	29.1 ± 3.1

Sprague–Dawley rats (180–220 mg) were treated (s.c.) with the indicated doses of cadmium acetate and sodium selenite. Selenium was injected 30 min before Cd^{2+}. The control animals received saline. Twenty-four hours after the last injection, rats were killed, and the Sertoli and the Leydig cell preparations were obtained. The microsomal fraction and the RER and SER fractions were prepared and were utilized for the determination of heme concentration [23]. The methods used are described in Experimental Proccdures. The results are expressed as mean ± SD for four determinations. The pooled testes of two rats were used for each determination.

*$P \leq 0.05$ when compared with the control.

microsomal fraction, RER, SER and the isolated Sertoli and Leydig cells. However, the relative increases in different preparations varied to a notable extent. Compared to the control value, the concentration of heme in the Sertoli cells was increased greatly; the magnitude of increase was nearly 11-fold. In the Leydig cells, heme concentration measured only about twice that of the control level. Differences were also detected in the ER fractions; the heme concentration in the SER fraction was more pronouncedly affected. A greater than 3- to 3.5-fold increase in the SER fraction contrasted the less than 2-fold increase in heme concentration in the RER fraction. As with the peroxidase, the treatment of rats with 10 μmoles/kg selenium 30 min before administration of Cd^{2+} fully prevented the increase in heme concentration in all testicular fractions. Again, selenium alone did not exert a significant effect on heme concentration in the examined tissue preparations. Based on our previous studies [8, 9], it appears that the presently observed increases in heme concentration in the Sertoli and the Leydig cells do not reflect either an increased rate of heme biosynthesis or a decreased rate of heme degradation. In these studies, increases in heme concentrations were not accompanied by changes in activities of δ-aminolevulinic acid synthetase or heme oxygenase; these enzymes are rate-limiting in the synthesis and the degradation of heme respectively. Using labeled heme precursors, however, the increases could be traced to the blood hemoglobin heme.

The following experiments were carried out to investigate whether indeed the activity of GSH-peroxidase was increased in the Sertoli and the Leydig cell populations and that the increased activity was not reflective of an increased activity of GSH-*S*-transferases. In rat liver, GSH-peroxidase activity (i.e. GSSG formation) of transferases has been reported [7]. Accordingly, transferase activity towards DCNB and CDNB, two general substrates for transferases, in cell populations isolated from Cd^{2+}-treated and control rat testis was examined. It appears that transferase activity in rat testis mainly resides in the Sertoli cells. As shown in Table 4, the activity towards both substrates in the control Sertoli cell preparation exceeded that of the Leydig cells by nearly 2-fold; the same pattern was noted in cells obtained from Cd^{2+}-treated rats. Moreover, there was no evidence of an increased transferase activity

Table 4. Effect of cadmium on GSH-*S*-transferase activity in the Leydig and the Sertoli cell preparations of rat testis

Treatment	Enzyme source	Substrate	Activity (nmoles/mg/min)
Control	Sertoli cell preparation	CDNB	1134.0 ± 110.0
		DCNB	18.9 ± 3.0
	Leydig cell preparation	CDNB	546.0 ± 49.0
		DCNB	6.0 ± 0.8
Cd^{2+}		CDNB	1142.0 ± 169.0
	Sertoli cells	DCNB	16.0 ± 1.9
	Leydig cells	CDNB	523.0 ± 26.0
		DCNB	6.6 ± 0.6

Sprague–Dawley rats were treated (s.c.) with 20 μmoles/kg cadmium acetate and killed 24 hr later. The control group received saline. The Sertoli and the Leydig cell preparations were obtained as described in the text and used for the preparation of the cytosol fraction. Enzyme activities were detected at 25° in the presence of 50 mM GSH. Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; and DCNB, 1,2-dichloro-4-nitrobenzene.

in preparations obtained from the Cd^{2+}-treated rats.

DISCUSSION

The data presented in this report describe the differential action of Cd^{2+} to increase GSH-peroxidase activity in two cell populations of rat testis. Clearly, the Sertoli cell population was the target for Cd^{2+}-mediated increase in peroxidase activity. The concentration of heme in this cell population was also preferentially increased. GSH-peroxidase utilizes GSH in the course of inactivating hydrogen peroxide and oxygen-free radicals [26]. Conversely, the heme molecule, or Fe-protoporphyrin IX, causes degradation of components of biological membranes by promoting the formation of oxygen-free radical species [12]. It follows that the individual components of the heme molecule, i.e. iron and porphyrin, are also known promoters of lipid peroxidation and free radical formation [11, 27–32]. As shown in this study, the increase in activity of the peroxidase in the two cell populations closely paralleled the increase in heme concentration (Fig. 1 and Table 3). In the Sertoli cells, the nearly 11-fold increase in heme concentration was accompanied by a more than 4-fold enhancement of the peroxidase activity. In contrast, in the Leydig cells impressive increases in neither parameter were detected. Accordingly, it would appear highly plausible that the magnitude of increase in heme concentration, and the ensuing lipid peroxidative activity, may define the magnitude of response evoked, i.e. increase in GSH-peroxidase activity.

Moreover, as shown here and elsewhere [9], the increase in heme concentration was associated mainly with the SER membranes, where a large concentration of heme was detected. Our previous studies using ^{14}C-labeled glycine, which is a specific precursor for hemoglobin heme synthesis, have suggested that the increase in heme concentration in testicular tissue is due to the binding and/or incorporation of hemoglobin heme to cellular membranes, rather than an increase in the biosynthesis of heme in the tissue [8, 9]. This suggestion is consistent with the findings by Aoki and Hoffer [33] who have shown that endothelial injury is one of the earliest events in testicular damage by Cd^{2+}. A distinct feature of the Sertoli cells is the abundance of SER [34]. In the past, we have demonstrated the catalytic activity of endogenous iron, i.e. iron derived from the degradation of heme, to promote peroxidative destruction of constituents of the ER membranes [11–13]; consistent with this phenomenon is the reported increased lipid peroxidative activity in the testis of rats treated with Cd^{2+} [10]. Accordingly, it would appear unlikely that concurrent increases in GSH-peroxidase activity and cellular heme concentration were unrelated and reflected a coincidental occurrence; rather, it is highly plausible that there was a causal relationship between these occurrences.

It is noteworthy that the activities of the peroxidase in both cell types under control conditions were similar; also the activity of GSH-*S*-transferases in the control Sertoli cell preparation was nearly twice that of the control Leydig cells. These findings may be interpreted as suggesting that the Sertoli cells are physiologically better equipped to protect against potential free radical-mediated damage to cellular constituents. The expressed idea is consistent with the physiological role of the Sertoli cells. These cells, which form part of the testis–blood barrier and have a rich blood supply, in post-pubertal animals lose their ability to divide [34]. In the tubules, they reach from the basal membrane to the tubule lumen and are connected to the neighboring Sertoli cells by tight junctions [35]. Hence, all substances, nutritional, hormonal or toxic, must pass through the Sertoli cell cytoplasm to be available for the germ cells [35]. Accordingly, upon exposure of animals to Cd^{2+}, it would appear likely that the Sertoli cells may acquire higher cytoplasmic concentrations of the metal and would be affected more severely.

The precise mechanism by which Cd^{2+} promotes vascular damage and breakdown of erythrocytes is not fully understood. However, there is evidence suggesting that the metal binds to the capillary endothelium of the testis and initiates cellular events which ultimately cause disruption of the blood–testis barrier [36]. Recent findings provide direct evidence suggesting that hemolysis follows the depletion of erythrocytes' GSH by chemical agents [37]. Moreover, it has been shown that hemolytic conditions follow the exposure of erythrocytes to oxidative conditions and free radical peroxides [38–40]. Maintenance of intracellular GSH concentration is essential to preservation of the integrity of the blood cell membrane, as well as the tertiary structure of hemoglobin [40]. Therefore, it is plausible that the presently observed ability of the metal ion to perturb the cellular levels of free thiols and decrease GSH concentration may be intimately related to the breakdown of erythrocytes. Decrease in GSH concentration and the onset of oxidative stress, in turn, could be caused in part by a decreased formation of the tripeptide, as well as through the direct binding of Cd^{2+} to GSH.

As mentioned above, selenium on the other hand, causes an increase in activities of the enzymes of the GSH-biosynthesis pathway [5]. Accordingly, it

would seem likely that this property of selenium is closely related to the protective action of the element against Cd^{2+} toxicity (Table 2). This concept is strongly supported by the finding that selenium at a 1:4 or 1:2 molar ratio to Cd^{2+} was quite effective against cytotoxicity of the metal. The disparate ratio of selenium to Cd^{2+} negates the possibility that the direct interaction of selenium with Cd^{2+} was solely responsible for the protective action of selenium. The present findings are consistent with our previous observation that selenium is very effective in preventing renal toxicity of mercury when given at a 1:2 molar ratio of selenium/mercury [6]. This suggestion, however, is not intended to dismiss other possible mechanisms of selenium action. For example, Prohaska *et al.* [7] have reported on the intracellular redistribution of Cd^{2+} from low molecular proteins to higher molecular weight proteins by selenium. This possibility could provide, in part, for protection against Cd^{2+} cytotoxicity. Moreover, in the case of other metal ions, such as mercury, selenium has been shown to accumulate in the tissue at an atomic ratio of one [41]. Also, induction by selenium of metallothionein-type Cd^{2+}-binding proteins in the testis [42] may play a role in protection against Cd^{2+} toxicity.

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