In vitro effects of selenodiglutathione on canine mammary cells

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Previous studies have shown that selenodiglutathione (SDG) is toxic to canine mammary tumor cell line 13 (CMT-13), reduces the growth of CMT-11, and does not modify the growth of primary non-neoplastic canine mammary (NCM) cells. In the present studies, approximately 6 times more SDG was required to inhibit by 50% the growth of CMT-11 cells compared with CMT-13 cells. Furthermore, these studies reveal that differences in sensitivity among these mammary cells cannot be explained by cellular retention of selenium from SDG. Data obtained using SDG (3.2 μM) double labelled with ^{75}Se and ^{35}S suggest that differences in the rate of selenium detoxification exist among the three cell cultures examined. The influence of SDG (3.2 μM) on intercellular glutathione depended on the cell examined, increasing in CMT-11, decreasing in CMT-13, and unchanged in NCM. Addition of GSH (100 μM) partially protected CMT-13 and CMT-11 cells against SDG toxicity. Preincubation of CMT-13 or CMT-11 cells with GSH for 48 hr before the addition of SDG completely prevented the growth inhibition caused by this selenocompound. These studies suggest glutathione is critical to the prevention of selenodiglutathione toxicity and accompanying growth depression.

Keywords: selenodiglutathione; neoplastic cell growth; selenium metabolism

Introduction

Cell type, plating density, form, and concentration of selenium are all factors known to modify the response of cells in culture to selenium toxicity.[1-5] Compared to selenite, selenodiglutathione (SDG) is considerably more effective in inhibiting the binding of carcinogen adducts to DNA and in inhibiting the growth of tumor cells in vitro.[1,2,6] Nevertheless, viability and growth studies reveal marked differences in the sensitivity of cells to SDG toxicity. Two neoplastic canine mammary tumor cell lines (CMT-11 and CMT-13) have been shown to be more sensitive to SDG than primary cultures of non-neoplastic canine mammary cells (NCM).[1] In published studies, the order of growth inhibition caused by 9.6 μM SDG was CMT-13 > CMT-11 ≫ NCM.[1,4] Continued examination of the response of these cells may shed insight into the reason why some cells vary in their sensitivity to selenium toxicity.

Glutathione reacts enzymatically and non-enzymatically with selenite to form SDG.[7] Additional glutathione leads to SDG's reduction to less toxic end products.[8] Thus, variation in selenium toxicity among cells may relate to their ability to regulate intracellular glutathione concentrations. Previous studies have shown that intracellular glutathione concentrations are greater in NCM cells, before or following selenite treatment, than occur in either CMT-13 or CMT-11 cells.[1,4] The influence of SDG on the content of glutathione in these cells is unknown.

Vernie et al.[9] have shown that SDG is a potent inhibitor of protein biosynthesis in a cell-free system. Subsequent studies revealed the depression in amino acid incorporation into protein resulting from SDG treatment was due to an inactivation of elongation factor 2.[10] Interestingly, supplementation of the cell-free system with glutathione was found to prevent the marked depression in amino acid incorporation caused by SDG treatment.[10] Thus, exogenous glutathione may modify the response of cells to the toxic effects of SDG.

These present experiments were designed to deter-

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mine if variation in selenium retention or glutathione status accounted for the observed differences in SDG toxicity among CMT-13, CMT-11, and NCM cells. An additional study was conducted to determine if exogenous glutathione modified the ability of CMT-13 cells to withstand the toxicity of SDG.

Materials and methods

Preparation of selenodiglutathione

SDG was prepared by the reaction of sodium selenite with GSH in a 1:4 ratio by the method of Ganther.[7] The products were separated over Dowex 50W (X4) and passed through a column of Sephadex G-10 with twice distilled water as described by Vernie et al.[11] Radioactively labelled SDG was formed by using ^{75}Se-selenite (Amersham Corp., Arlington Heights, IL, USA) and ^{35}S-glutathione (New England Nuclear, Boston, MA, USA).

Tissue culture

The characteristics of the two canine mammary tumor cell lines (CMT-13, CMT-11) and a non-neoplastic primary culture (NCM) of canine mammary cells have been described previously.[1] Culture conditions were generally as previously described.[1] Basically, cells were maintained in chemically defined RPMI medium supplemented with 10% fetal bovine serum and maintained in an environment of 95% air, 5% CO_2 at a relative humidity of 95%. At the time of harvesting, cells were washed twice with phosphate buffered saline (PBS), harvested by scraping, and counted. Cell counts were determined using an electronic counter (Coulter Electronics, Hialeah, FL, USA). The cells were then centrifuged at 600*g* for 10 min and the resulting pellet used for the various analyses as described below. Specific details of analyses and deviation of the plating procedures are given below.

Growth studies

The effect of graded concentrations of SDG on the growth of CMT-13 and CMT-11 cells was examined in experiment 1. CMT-13 and CMT-11 cells were plated at a density of 0.9 and 1.8 $\times$ 10^5 cells, respectively, into 25 cm^2 flask. After 24 hr, SDG was added to the incubation medium at concentrations of selenium ranging from 0–9.6 μM. Attached cells were harvested 24 and 48 hours following the addition of SDG. Four flasks per concentration of selenium were examined at each time point for each cell line. In a separate study, experiment 2, CMT-13 and NCM cells were grown in the presence or absence of SDG in chemically defined medium without added serum as described by Kidwell.[12]

Retention of selenium and sulfur from SDG

In experiment 3, cultures plated at 5 $\times$ 10^6 cells per flask (150 cm^2 flask) and allowed to attach for 24 hr were exposed to doubly labelled SDG (0.1 μCi ^{75}Se and 0.2 μCi ^{35}S/μg SDG) at a final concentration of 3.2 μM. After an additional 24 hr incubation in the presence of SDG, five flasks from each cell line were harvested and the retained radioactivity determined. Gamma radioactivity from ^{75}Se was counted directly using a multi-channel analyzer (Spectrum 88, The Nucleus, Oak Ridge, TN, USA). Beta radioactivity from ^{35}S was detected using liquid scintillation counting (Beckman LS 9000 Scintillation Counter, Irvine, CA, USA). Retention is defined as the fmol of Se or S present in the washed cells after a 24 hr incubation.

Glutathione

Experiment 4 examined the influence of supplemental SDG on the intracellular content of glutathione. In this study, cells GSH and GSSG were determined by the method of Teitze[13] in cells initially plated at 5 $\times$ 10^6 cells and grown in the presence or absence of SDG (3.2 μM) for 24 hr. All samples were assayed in triplicate.

Supplemental glutathione and SDG growth inhibition

Experiment 5 was designed to measure the influence of supplemental glutathione on the toxicity of SDG in cultures of CMT-13 and CMT-11 cells. CMT-13 and CMT-11 cells were plated on day 0 at 1 $\times$ 10^5 cells in 25 cm^2 flask. SDG (3.2 μM) and/or glutathione (100 μM) were added to the incubation medium 24 and 72 hr after plating. Cells were then harvested and counted after an additional 48 and 96 hr incubation. The influence of a 48 hr preincubation with glutathione (100 μM) on the SDG toxicity was also examined.

Statistical analysis

Significant differences ($P < 0.05$) between treatments were determined using an analysis of variance and applying a test of comparison of means (LSD).[14]

Results

Growth alterations

Growth of CMT-13 cells was inhibited significantly when SDG was added to the culture medium at concentrations of 0.32 μM or more. (*Figure 1*). Increasing the concentration of SDG resulted in a progressive decrease in the growth of these cells. Interestingly, concentrations of 6.4 μM or more were required to reduce the growth of CMT-11 cells within the same time period (*Figure 1*). The quantity of SDG required to reduce the growth of CMT-13 and CMT-11 cells by 50% was estimated to be 1.3 and 8.6 μM, respectively. Morphological changes, including widespread vacuolization and cell rounding, were obvious within 24 hr in both tumor cell lines following exposure to 9.6 μM SDG. There was no indication that SDG resulted in cell death in CMT-11, as it did with CMT-13 cells.

SDG supplementation also inhibited the growth of CMT-13 and NCM cells grown in chemically defined

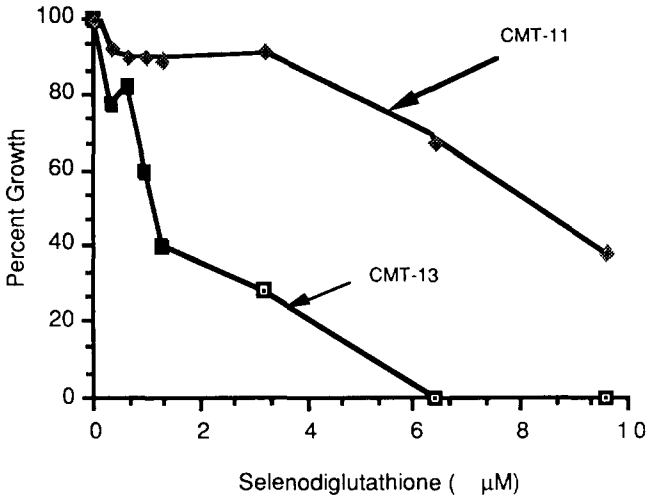

Figure 1 CMT-13 and CMT-11 were plated on day 0 at 0.9 and 1.8 × 10^5 cells per 25 cm^2 flask. Selenodiglutathione was added 24 hr after plating. Points are means of 4 flasks per treatment per cell line. The pooled standard error of the mean was ± 0.02.

medium without serum (*Table 1*). Similar to experiment 1, addition of 1.28 μM resulted in a 48% depression in growth of CMT-13 cells. Addition of 6.4 μM SDG resulted in cell death of CMT-13 cells within 48 hr. Although 12.8 μM inhibited the growth of non-neoplastic cells by about 100%, there was no indication of detachment or changes in the morphological characteristics of these cells. SDG at 25.6 μM produced a more pronounced growth inhibition than was observed in cells exposed to 12.8 μM. However, even this concentration did not result in total death of the culture. In a separate study, the addition of SDG (3.2 μM) to NCM cells (1.1 × 10^6) grown for 2 days in complete media did not significantly alter growth compared to controls (4.9 and 4.4 and 10^6 cells, respectively).

Retention of SDG

Selenium retention was significantly greater in CMT-13 and NCM cells exposed to 3.2 μM SDG than occurred in CMT-11 cells (*Table 2*). NCM cells retained about 3 times more selenium than CMT-13 cells. CMT-13 cells retained approximately 4 times more selenium than CMT-11 cells. The percentage of the ^{75}Se retained from the supplemental SDG was 9.0, 2.3, and 25% for CMT-13, CMT-11, and NCM cells, respectively. In a similar study, the retention of selenium following incubation with 6.4 μM SDG was 2.3, 0.6, and 6.4 fmol selenium per cell for CMT-13, CMT-11, and NCM cells, respectively. NCM and CMT-13 cells also retained considerably more sulfur from SDG than did CMT-11 cells. The percent retention of ^{35}S from supplemental SDG was approximately 24, 6, and 60% in CMT-13, CMT-11, and NCM cells, respectively (*Table 2*). The molar ratio of sulfur to selenium retained in NCM cells was significantly greater than occurred in either tumor cell line. The lowest ratio of sulfur to selenium was observed in CMT-13 cells.

Table 1 Impact of selenodiglutathione on the growth of neoplastic CMT-13 and non-neoplastic mammary cells in chemically defined medium

SDG Added (μM)	Cell Count (× 10^{-5}) CMT-13	NCM
0.0	6.8 ± 0.6[a]	5.8 ± 0.1[a]
1.28	3.6 ± 0.1[b]	—
6.4	0[c]	—
12.8	—	2.0 ± 0.1[b]
25.6	—	1.5 ± 0.3[c]

Note. SDG was added 24 hr after plating. CMT-13 and NCM cultures were seeded at 1 × 10^5 and 2 × 10^5 cells in chemically defined medium, respectively. Cultures were counted 48 hr after the addition of SDG. Values are means ± SEM for 4 flasks per treatment. Vertical means not sharing a common superscript differ ($P < 0.05$).

Effect of SDG on intracellular glutathione

Consistent with previously published results, NCM cells were found to contain higher concentrations of glutathione than either neoplastic cell line (*Table 3*). Addition of SDG to the culture medium did not modify the content of glutathione in NCM cells. However, the content of reduced glutathione increased 2 fold in CMT-11 cells following SDG treatment. SDG resulted in a significant reduction in intracellular glutathione in CMT-13 cells. The ratio of reduced to oxidized glutathione was unaffected in NCM, increased in CMT-11 and decreased with CMT-13 cells following SDG treatment (*Table 3*).

Effect of glutathione on SDG growth inhibition

Consistent with experiment 1, SDG (3.2 μM) markedly inhibited the growth of CMT-13 and CMT-11 cells (*Table 4*). Delaying the time of SDG treatment and thereby increasing the culture density decreased the depression in growth induced by this selenocompound in both CMT-13 and CMT-11 cells. The addition of glutathione (100 μM) to the medium did not consistently alter the growth of either cell line in the absence of SDG. However, GSH supplementation markedly reduced the toxicity of SDG in both CMT-13 and CMT-11 cells as indicated by significantly greater growth rates compared with cells exposed only to

Table 2 Retention of selenium and sulfur from selenodiglutathione by neoplastic and non-neoplastic mammary cells

Cell	Retention (fmol/cell) Selenium	Sulfur	Ratio (S/Se)
CMT-13	0.86 ± 0.19[b]	3.79 ± 0.35[b]	4.3[a]
CMT-11	0.22 ± 0.06[a]	1.04 ± 0.17[a]	4.7[b]
NCM	2.40 ± 0.34[c]	12.17 ± 1.01[c]	5.1[c]

Note. Values are means ± SEM for five flasks per cell culture. SDG was added at 3.2 μM for 24 hr before cell harvesting. Means not sharing a common superscript differ ($P < 0.05$).

Table 3 Effect of selenodiglutathione upon reduced and oxidized glutathione

Cell	SDG	Glutathione		Ratio
		Reduced	Oxidized	
		nmol/10^6 Cells	nmol/10^6 cells	(R/.O)
CMT-13	–	9.9 ± 0.2[b]	0.15 ± 0.03	66
	+	4.5 ± 0.1[a]	0.15 ± 0.02	30
CMT-11	–	2.3 ± 0.2[a]	0.11 ± 0.00	21
	+	5.2 ± 0.4[b]	0.14 ± 0.01	37
NCM	–	31.5 ± 4.8[a]	0.56 ± 0.02	56
	+	26.0 ± 3.8[a]	0.49 ± 0.03	53

Note. Values are means ± SEM for 3 pools of 4 flasks for each cell culture. SDG was added for 24 hr at 3.2 μM. Means within a culture not sharing a common superscript differ ($P < 0.05$).

SDG. Preincubation of CMT-13 and CMT-11 cells with GSH (100 μM) for 48 hr prior to treatment with SDG completely prevented the growth inhibition caused by SDG (*Table 4*).

Discussion

The present studies verify that all cells are not equally sensitive to the toxic effects of selenium supplementation. Over a five-fold variation in the magnitude of growth inhibition resulting from exposure to 1.28 μM SDG was detected among the three cell cultures used in the present investigations. Comparison of data obtained in experiments 1 and 2 reveal that the presence of fetal bovine serum does not alter appreciably the toxicity of SDG. Since the retention of selenium following SDG treatment was 3 and 12 times greater in NCM than CMT-11 cells, respectively, than occurred in CMT-13 cells, it appears that the absolute quantity of selenium retained is not a prerequisite for growth inhibition. Part of the differences in selenium retention may relate to intracellular protein. NCM, CMT-13, and CMT-11 have been shown previously to contain approximately 3.5, 2.0, and 1.0 mg protein per 10^7 cells.[1] Using these protein values, the content of selenium retained from SDG remains significantly higher in NCM than either tumor cell line. Thus, these data suggest that form of selenium retained rather than absolute concentration is critical to cell survival. This point is reinforced by studies by Morrison et al.[15] showing the induction of a 58 kd selenoprotein following selenite treatment correlated closely with the degree of inhibition of DNA biosynthesis caused by selenite.

Selenodiglutathione is known to be more effective than selenite in inhibiting the binding of carcinogens to DNA and in retarding the growth of neoplastic cells.[1,5] Likewise, Vernie et al.[9,11] have shown selenodiglutathione is a more potent inhibitor of protein biosynthesis than selenite. The present studies suggest cell density has minimal impact on SDG toxicity. Previous studies from our laboratory have shown that selenite toxicity increases as the culture density increases.[5] Subsequent studies have shown that the enhanced toxicity of selenite as the culture density increases is associated with an increased cellular retention of selenium.[16] The influence of culture density on the uptake and retention of selenium from SDG requires further examination.

Previous studies from our laboratory have shown that CMT-13 cells have a reduced ability to form dimethylselenide, an end product of selenium metabolism, than do CMT-11 or NCM cells.[4] The lower ratio of selenium to sulfur in NCM cells exposed to SDG (*Table 2*) provides additional evidence that these cells have a slightly greater rate of selenium reduction than neoplastic cells. Support for different rates of selenium clearance also comes from studies showing that the retention of selenium is greater in neoplastic than non-neoplastic tissues.[17]

Glutathione is recognized as an important intracellular detoxification agent.[18,19] Its involvement in normal selenium detoxification makes it a prime candidate for explaining the observed variation in sensitivity to the toxic effects of selenocompounds.[20] In the present studies, NCM cells were found to contain consider-

Table 4 Effect of supplemental glutathione on selenodiglutathione induced growth inhibition

Treatment	Day of Treatment	Cell Numbers (× 10^5)			
		CMT-13		CMT-11	
		Day 3	Day 5	Day 3	Day 5
Control	—	8.3 ± 0.4[a]	35.1 ± 0.3[a,b]	7.1 ± 0.1[b]	30.8 ± 0.9[b]
GSH	1	8.5 ± 0.3[a]	38.7 ± 0.7[a]	8.0 ± 0.2[a]	33.8 ± 0.7[a,b]
SDG	1	2.6 ± 0.3[c]	0.0[e]	3.4 ± 0.2[d]	3.0 ± 0.1[f]
GSH + SDG	1 + 1	6.1 ± 0.2[b]	24.2 ± 0.7[c]	6.0 ± 0.1[c]	11.0 ± 0.3[e]
GSH	3	—	34.9 ± 0.8[b]	—	31.5 ± 0.8[b]
SDG	3	—	13.5 ± 0.3[d]	—	17.7 ± 0.9[d]
GSH + SDG	3 + 3	—	22.2 ± 0.8[c]	—	25.9 ± 0.9[c]
GSH + SDG	1 + 3	—	36.2 ± 2.4[a,b]	—	35.1 ± 1.8[a]

Note. Cells were plated on day 0 at 1 × 10^5 cells per 25 cm^2 flask. GSH was added at a final concentration of 100 μM and SDG was added at final concentration of 3.2 μM Se.
[a-e] Values represent means of cell numbers for 4 flasks per treatment. Vertical means not sharing a common superscript differ ($P < 0.05$).

ably higher intracellular concentrations of glutathione than either neoplastic cell line. This higher glutathione content may have contributed to the ability of these cells to resist the toxic effects of selenodiglutathione. Likewise, the ability of CMT-11 cells to increase glutathione in response to SDG exposure may have contributed to their lower sensitivity to SDG than CMT-13 cells. The inability of CMT-13 cells to maintain intracellular glutathione may have increased their susceptibility to supplemental SDG. These results are consistent with data from Le Boeuf and Hoekstra[21,22] which also suggest that perturbations in GSG, GSSG, and their mixed disulfides can alter cell function.

The present studies show that exogenous GSH (100 μM) can significantly reduce the toxic effects of SDG. Dethmers and Meister[23] have shown that human lymphoid cells are capable of transporting GSH into the cell at the rate of 0.5 nmol/10^7 cells per 15 min. Likewise, recent studies from our laboratory have shown that supplying glutathione to the incubation medium of CMT-13 cells results in an approximate 25% increase in intracellular glutathione concentrations.[5] The present studies show that exogenous glutathione can markedly inhibit SDG induced growth depression in both CMT-13 and CMT-11 cells. The ability of exogenous glutathione to protect cells is in general agreement with its ability to prevent a depression in protein biosynthesis induced by SDG[9]

In summary, these studies document that all cells are not equally sensitive to the toxic effects of selenodiglutathione. Differences in sensitivity are not related to the absolute quantity of selenium retained in the cell. Variation in toxicity is likely explained by variation in the ability of the cell to metabolize selenium to non-toxic forms.

References

1 Fico, M.E., Poirier, K.A., Watrach, M., Watrach, A., and Milner, J.A. (1986). Differential effects of selenium on normal and neoplastic mammary cells. *Cancer Res.* **46,** 3394–3399

2 Poirier, K.A. and Milner, J.A. (1983). Factors influencing the antitumorigenic properties of selenium in mice. *J. Nutr.* **113,** 2147–2154

3 Morrison, D.G., Berdan, R.C., Pauly, D.F., Turner, D.S., Oborn, C.J., and Medina, D. (1988). Selenium Distribution in Mammary Epithelial Cells Reveals its Possible Mechanism of Inhibition of Cell Growth. *Anticancer Res.* **8,** 51–64

4 Kuchan, M.J., Fico-Santoro, M.A., and Milner, J.A. (1990). Consequences of selenite supplementation on the growth and metabolism of cultures of canine mammary cells. *J. Biochem. Nutr.* **1,** 478–483

5 Kuchan, M.J. and Milner, J.A. (1990). Influence of intracellular glutathione on selenite-mediated growth inhibition of canine mammary tumor cells. (submitted for publication)

6 Milner, J.A., Pigott, M.A., and Dipple, A. (1985). Selective effects of sodium selenite on 7,12-Dimethylbenz(a)anthracene-DNA Binding in Fetal Mouse Cell Cultures. *Cancer Res.* **45,** 6347–6354

7 Ganther, H.E. (1966). Selenotrisulfides. Formation by the reaction of thiols with selenious acid. *Biochem.* **7,** 2898–2905

8 Ganther, H.E. (1971). Reduction of the selenotrisulfide derivative of glutathione reductase. *Biochem.* **10,** 4089–4098

9 Vernie, L.N., Collard, J.G., Eker, A.P., de Wildt, A., and Wilders, I.T. (1979). Studies on the inhibition of protein synthesis by selenodiglutathione. *Biochem. J.* **180,** 213–218

10 Vernie, L.N., Bont, W.S., Ginjaar, H.B., and Emmelot, P. (1975). Elongation factor 2 as the target of the reaction product between sodium selenite and glutathione (GSSeSG) in the inhibition of amino acid incorporation. *Biochim. Biophys. Acta* **414,** 283–292

11 Vernie, L.N., Bont, W.S., and Emmelot, P. (1974). Inhibition of in vitro amino acid incorporation by sodium selenite. *Biochem.* **13,** 337–341

12 Kidwell, W.R., Salomon, D.S., Liotta, L.A., and Bano, M. (1982). Effect of growth factors on mammary epithelial cell growth and basement membrane synthesis. In *Growth of Cells in Hormonally Defined Medium,* (G. Sato and D. Sirbasku, eds.), pp. 807–818, Coldspring Harbor Laboratory, Coldspring Harbor, NY

13 Teitze, F. (1969). Enzymatic method for the quantitative determination of nanogram amounts of total and oxidized glutathione. *Anal. Biochem.* **27,** 502–522

14 Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Methods,* pp. 234–235, The Iowa State University Press, Ames

15 Morrison, D.G., Dishart, M.K., and Medina, D. (1988). Intracellular 58-kd selenoprotein levels correlate with inhibition of DNA synthesis in mammary epithelial cells. *Carcinogenesis* **9,** 1801–1810

16 Kuchan, M. (1991). Involvement of Cellular Sulfhydrals in the Selenite Mediated Growth Inhibition of Canine Mammary Tumor Cells in Culture. Doctoral dissertation. University of Illinois, Champaign

17 Cavalieri, R.R., Scott, R.C., and Sairene, E. (1966). Selenite as a tumor localizing agent in man. *J. Nucl. Med.* **7,** 197–208

18 Kosower, N.S. and Kosower, E.M. (1978). The glutathione status of cells. *Int. Rev. Cytol.* **54,** 109–160

19 Meister, A. (1983). Selective modification of glutathione metabolism. *Science* (Washington, D.C.) **200,** 472–477

20 Ganther, H.E. and Hsieh, H.S. (1974). Mechanisms for the conversion of selenite to selenides in mammalian tissues. In *Trace Element Metabolism in Animals* (W.G. Hoekstra, J.W. Suttie, H.E. Ganther, and W. Mertz, eds.), pp. 339–353, University Press, Baltimore

21 LeBoeuf, R.A. and Hoekstra, W.G. (1983). Adaptive changes in hepatic glutathione metabolism in response to excess dietary selenium. *J. Nutr.* **113,** 845–854

22 LeBoeuf, R.A., Laishes, B.A., and Hoekstra, W.G. (1985). Effect of selenium on cell proliferation in rat liver and mammalian cells as indicated by cytokinetic and biochemical analysis. *Cancer Res.* **45,** 5496–5504

23 Dethmers, J.K. and Meister, A. (1981). Glutathione export by human lymphoid cells: Depletion of glutathione by inhibition of its synthesis decreases export and increases sensitivity to irradiation. *Proc. Natl. Acad. Sci. USA* **78,** 7492–7296