

Short Review

## The Antioxidant System

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**Abstract.** The glutathione (GSH) antioxidant system is the principal protective mechanism of the cell and is a crucial factor in the development of the immune response by the immune cells. Experimental data demonstrate that a cysteine-rich whey protein concentrate represents an effective cysteine delivery system for GSH replenishment during the immune response. Animal experiments showed that the concentrates of whey protein also exhibit anticancer activity. They do this via the GSH pathway, the induction of p53 protein in transformed cells and inhibition of neoangiogenesis.

The recent interest in complementary medicine has brought about the increasing use and sometimes the misuse of terms such as "free radicals" and "antioxidants". A free radical can be defined as any species that contains one or more unpaired electrons, an unpaired electron being one that is alone in an orbital. Most biological molecules contain only paired electrons. A radical might donate its unpaired electron to another molecule or it may take an electron from another molecule in order to pair. A feature of the reactions of free radicals is the development of a chain reaction with damage to adjacent biological structures. The majority of free radicals originate in the final stage of cell respiration, in which electrons flow from organic substrate to oxygen, yielding energy. When mitochondria are functioning, an electron passing through the respiratory chain may leak directly onto the oxygen molecule resulting in the formation of a superoxide radical:  $O_2 + e^- \rightarrow O_2^-$ . Figure 1 illustrates the sequence of biochemical events following the appearance of  $O_2^-$ . The term "antioxidant" was initially used to define the cell's own protective mechanisms. It is noteworthy that the two major barriers against released free radicals are located near the mitochondria in proximity to the source of oxidant

formation. Glutathione (L-gamma-glutamyl-L-cysteinylglycine) can spontaneously, or with the help of peroxidase, easily deliver the H necessary for the reduction of the radicals. Figure 2 is an attempt to schematically illustrate the redox system represented by GSH in the cells and by the albumin cysteine in the plasma. In both cases, the active element is the SH (thiol) group of cysteine which when performing its antioxidant activity is oxidized to cystine or cysteine disulfide (Figure 3). It is the ratio cysteine/cystine that defines the redox state which is the major determinant of the optimal function of the cell (Figure 3). Cysteine is the limiting factor of GSH synthesis in the cell and of albumin in the plasma where it is located in position 34. It is noteworthy that nutritional intervention is often ineffective in raising the albumin content of the plasma whereas NAC, an analog of cysteine, can raise blood levels of albumin(1). The antioxidant activity on cholesterol suggest that reduced plasma albumin is far more than a mere expression of nutritional status.

**Immune system.** If we imagine that the cell in Figure 2 is a lymphocyte, we can appreciate why studies *in vitro* have demonstrated that the oxygen-requiring antigen-driven clonal expansion and antibody synthesis in the immune cells depends on their capacity to reconstitute GSH in order to neutralize the increased production of oxygen-derived radicals, hence facilitating a sustained immune response (2,3). This principle was verified by *in vivo* experiments where animals fed cysteine-rich whey protein concentrate showed enhanced immune response to T-dependent antigen (4-6) and Figure 4. This effect is abolished by administration of buthionine sulfoximine which inhibits the synthesis of GSH, hence demonstrating the role of GSH in the effect of this protein on the immune system (7).

**Dietary whey protein concentrates (WPC) and cancer.** The discovered immunoenhancing activity of WPC inspired the first study of WPC feeding on the development of experimental colon carcinoma in mice (8). The positive results of this study were confirmed in rats (9) (Figure 5) and extended to other types of malignancies such as mammary tumors in female rats (10).

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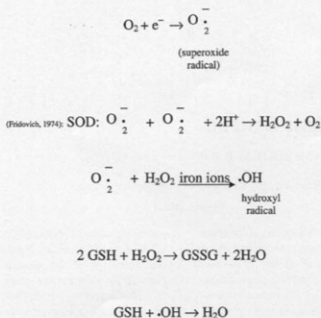
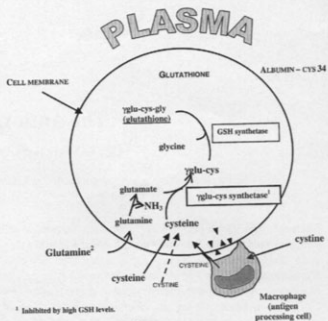


Figure 1. Oxygen-derived radicals and buffer systems.

**Mechanisms.** The effect of a cysteine pro-drug such as NAC on tumor development and carcinogenesis (Figure 6) strongly suggest that WPC acts as a cysteine delivery system in inhibiting tumor growth. In discussing the effects of WPC on tumors, it is important to distinguish between the anti-tumor effect and the anti-carcinogenesis effect. Our hypothesis is that WPC may be important in both; it does this *via* its effect on increasing GSH concentration in relevant tissues probably by providing high levels of substrates for GSH synthesis that could detoxify potential carcinogens or free radicals in spontaneous carcinogenesis. Table I by Parodi illustrates the relationship between cysteine, GSH and experimental tumors (11). Cysteine could also have an anti-tumor effect on low volumes of tumor *via* stimulation of immunity through the GSH pathway. The causative role of cysteine deficiency in the development of the immunological dysfunctions in cancer patients is supported by the observation that an additional source of cysteine can restore natural killer cell activity (13). It is interesting to note that cancer patients show an accelerated shift to more oxidized conditions (12). These data suggest that during the progression of cancers, the antioxidant buffer activity may progressively decline and this could well be related to depletion of the thiol (SH) in the redox equation.

Finally, cysteine itself may exert a direct antitumor effect in two different ways unrelated to GSH synthesis. It was recently demonstrated that several sulfur-containing antioxidants such as NAC and OTZ selectively induce p53-dependent apoptosis in transformed but not in normal cells. In contrast,



<sup>1</sup> Inhibited by high GSH levels.

<sup>2</sup> Crosses the cellular membrane faster than glutamate.

Figure 2. Cellular and plasma redox system.

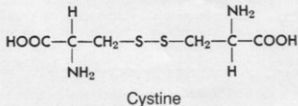
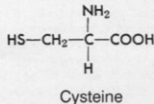


Figure 3. The most important sulfhydryl compounds: the cysteine/cystine redox couple.

antioxidants whose action is limited to scavenging radicals do not seem to have this activity. This activity was found related to a 5 to 10- fold induction of p53 protein and not to GSH formation. Therefore, a natural cysteine donor, such as a whey protein concentrate (WPC), could also inhibit tumors by

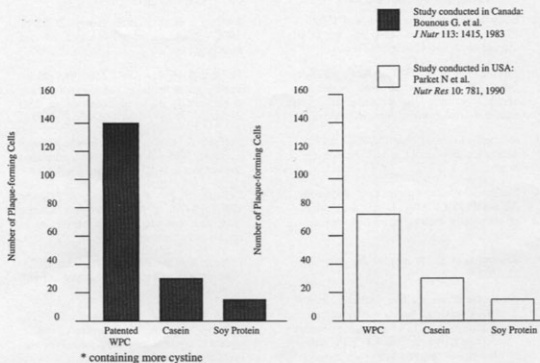
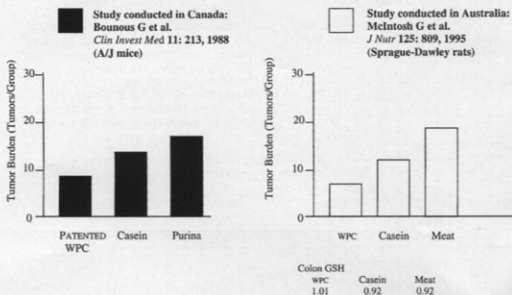


Figure 4. Results of studies demonstrating the immunosustaining role of specially prepared dietary WPCs.



Tumor mass was lower in mice fed patented WPC than in mice fed casein or purina.<sup>3</sup>

No significant difference in tumor mass was noted among the treatment groups.

"... These findings confirmed and extended earlier observations of a Canadian research group [Bounous et al, 1991] that also identified dairy proteins, and whey protein in particular, as being protective against the development of intestinal cancers induced by DMH."

Figure 5. Results of studies demonstrating the role of specially prepared dietary WPCs cancer prevention. Carcinogen was dimethylhydrazine-dihydrochloride (DMH), which induces colon tumors similar to those found in humans (with regard to type of lesions and response to chemotherapy). The diets were fed before and throughout the 24-week DMH-treatment period. No differential effect of diet on body weight was seen.

WHEY PROTEIN CONCENTRATE

1. Bounous G et al. Clin Invest Med 11/213-7, 1988 --The incidence and size of DMH induced colon tumors in mice is lower.
2. Papenburg R et al. Tumor Biol 11/129-36, 1990 -- Similar results as in 1, in addition, dietary treatment is effective also on established malignancy. Improved survival.
3. McIntosh G et al. J Nutr 125/809-16, 1995 -- Almost identical results as in 1 were obtained in rats.
4. Hakkar R et al. Cancer Epid Biomarkers Prevent 9/113-17, 2000 - In rats, the incidence of chemically induced mammary tumors is lower.
5. Bounous G et al. Anticancer Res. 20: 4785-92, 2000:<sup>1</sup>
  - Cancer of the prostate. All had elevated PSA levels with biopsy confirmed cancer of the prostate. 13 of 15 patients showed a progressive decline of PSA values during 3-12 months observation period.
  - Metastasis of renal carcinoma. In a 50 year old lady: following 3 years, significant decrease of metastatic disease in liver, resolution in lung and bone.
  - Bladder cancer. During one year, no recurrence of papillary transitional cell carcinoma.

NAC (N-ACETYL-CYSTEINE)

1. DeFlora S et al. Cancer letters 32/235-41, 1986 -- Inhibition of urethan induced lung tumors in mice.
2. DeFlora S et al. Am J Med 91, 1991 -- Prevention of mutation and cancer by thiols is particularly useful in condition of GSH depletion.
3. DeFlora S et al. J Cell Biochem Suppl 22/33-41, 1995 -- A study of the mechanisms contributing to NAC anticarcinogenesis.
4. DeFlora S et al. Int J of Cancer 17;67(6):842-8, 1996 -- Synergism between NAC and doxorubicin in the prevention of tumors and metastases in mice.
5. Delneste Y et al. Blood 90/1124-32, 1997 -- NAC exhibit potent anti lymphoma activity in mice.
6. D'Agostini F et al. Int J Oncol 13/217-24, 1998 -- In mice, NAC interact with a cytotoxic agent in inhibiting melanoma cell invasion and metastases.
7. Dröge W. Current opinion in Clinical Nutrition and Metabolic care 2/227-33, 1999 -- Plasma albumin level and body cell mass in cancer patients are increased by NAC.
8. Estensen RD et al. Cancer Letters 147/109-114, 1999 -- In patients at risk of colon cancer, NAC produces a decrease of proliferation index in the crypts.
9. Morini M et al. Int J Biol Markers 14/268-271, 1999 -- Inhibition of neo angiogenesis and tumor progression in murine melanomas.

<sup>1</sup> The whey protein concentrate, specifically an isolate defined by protein grade, in non instantized native form, marketed as Immunocal/HMS90, was obtained from Immunotec Research Ltd.

Figure 6. Anticancer effect of cyst(e)ine in natural and pharmaceutical compositions. Cysteine delivery systems.

Table 1. Sulphur amino acids, liver glutathione and tumour data for rats fed various diets<sup>1</sup>.

Diet	Amino acid composition g/100 g			Liver glutathione mmolg (wet tissue)	Tumour incidence	Tumour burden (tumours/group)
	Cysteine	Methionine	Total cysteine + methionine			
Whey	2.3	2.1	4.4	5.21	30	7
Casein	0.3	2.9	3.2	5.62	45	12
Meat	0.5	2.2	2.7	4.16	55	21
Soybean	0.7	1.3	2.0	2.45	60	26

<sup>1</sup>Adapted from McIntosh et al. (1995).

directly increasing cellular thiol levels (14). A second known effect of a cysteine delivery system is related to the inhibitory effect of cysteine on neoangiogenesis and tumor progression (15).

The promising anticancer effect of NAC is hampered by the adverse effect of this drug at pharmacological doses. Long-term use of WPC could represent, therefore, a good option in the long-term treatment of cancer patients.

## References

- 1 Dröge W: Cysteine and glutathione in catabolic conditions and immunological dysfunction. *Current Opinion in Clinical Nutrition and Metabolic Care* 2: 227-233, 1999.
- 2 Noelle RJ and Lawrence DA: Determination of glutathione in lymphocytes and possible association of redox state and proliferative capacity of lymphocytes. *Biochem J* 198: 571-579, 1981.
- 3 Fidelus RK and Tsan MF: Glutathione and lymphocyte activation: A function of aging and auto-immune disease. *Immunology* 61: 503-8, 1987.
- 4 Bounous G, Létourneau L and Kongshavn PAL: Influence of dietary protein type on the immune system of mice. *J Nutr* 113: 1415-1421, 1983.
- 5 Parker NT and Goodrum KJ: A comparison of casein, lactalbumin, and soy protein, effect on the immune response to a T-dependent antigen. *Nutrit Res* 10: 781-792, 1990.
- 6 Wong CW and Watson DL: Immunomodulatory effects of dietary whey proteins in mice. *J Dairy Res* 62: 350-368, 1995.
- 7 Bounous G, Batist G and Gold P: Immunoenhancing property of dietary whey protein in mice: role of glutathione. *Clin Invest Med* 12: 154-161, 1989.
- 8 Bounous G, Papenburg R, Kongshavn PAL, Gold P and Fleischer D: Dietary whey protein inhibits the development of dimethylhydrazine-induced malignancy. *Clin Invest Med* 11: 213-217, 1988.
- 9 McIntosh GH, Register GQ, Le Leu RK and Royle PJ: Dairy proteins protect against dimethylhydrazine-induced intestinal cancers in rats. *J Nutr* 125: 809-816, 1995.
- 10 Hakkak R, Korourian S, Shelnett SR, Lensing S, Ronis M and Badger T: Diets containing whey proteins or soy proteins isolate protect against 7, 12-dimethylbenz(a) anthracene-induced mammary tumors in female rats. *Cancer Epidem, Biomarkers & Prevention* 9: 113-117, 2000.
- 11 Parodi PW: A role for milk proteins in cancer prevention. *Australian J Dairy Technol* 53: 37-47, 1998.
- 12 Hack V, Breitkreutz R, Kinscherf R, Roker H, Batach H, Taul F and Dröge W: The redox state as a correlate of senescence and wasting and as a target for therapeutic intervention. *Blood* 9: 59-67, 1990.
- 13 Dröge W and Breitkreutz R: Glutathione and immune function. *Proc Nutri Soc* 50: 595-600, 2000.
- 14 Liu M, Pelling JG, Ju J, Chu E and Brash DE: Antioxidant action via p53-mediated apoptosis. *Cancer Res* 48: 1723-1729, 1998.
- 15 Morini M, Cai T, Aluigi MG, Noonan DM, Masiello L, De Flora S, D'Agostini F, Albini A and Fassina G: The role of the thiol N-acetylcysteine in the prevention of tumor invasion and angiogenesis. *Int J Biological Markers* 14, No. 4: 268-271, 1999.

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