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*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 illustrate 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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$$O_2 + e^- \rightarrow O_2^{\cdot -}$$

(superoxide radical)

(Fridovich, 1974): SOD: $O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$

$$O_2^{\cdot -} + H_2O_2 \xrightarrow{\text{iron ions}} {\cdot}OH$$

hydroxyl radical

$$2\,GSH + H_2O_2 \rightarrow GSSG + 2H_2O$$

$$GSH + {\cdot}OH \rightarrow H_2O$$

Figure 1. *Oxygen-derived radicals and buffer systems.*

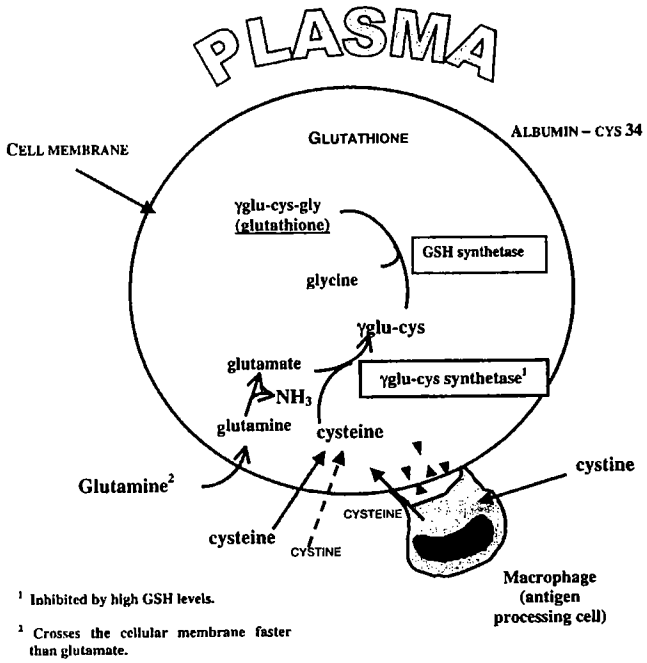
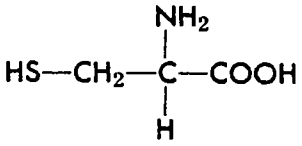
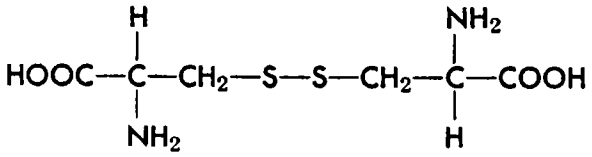



Figure 2. *Cellular and plasma redox system.*

*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.




Cysteine




Cystine

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

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, 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 a whey protein concentrate (WPC), could also inhibit tumors by

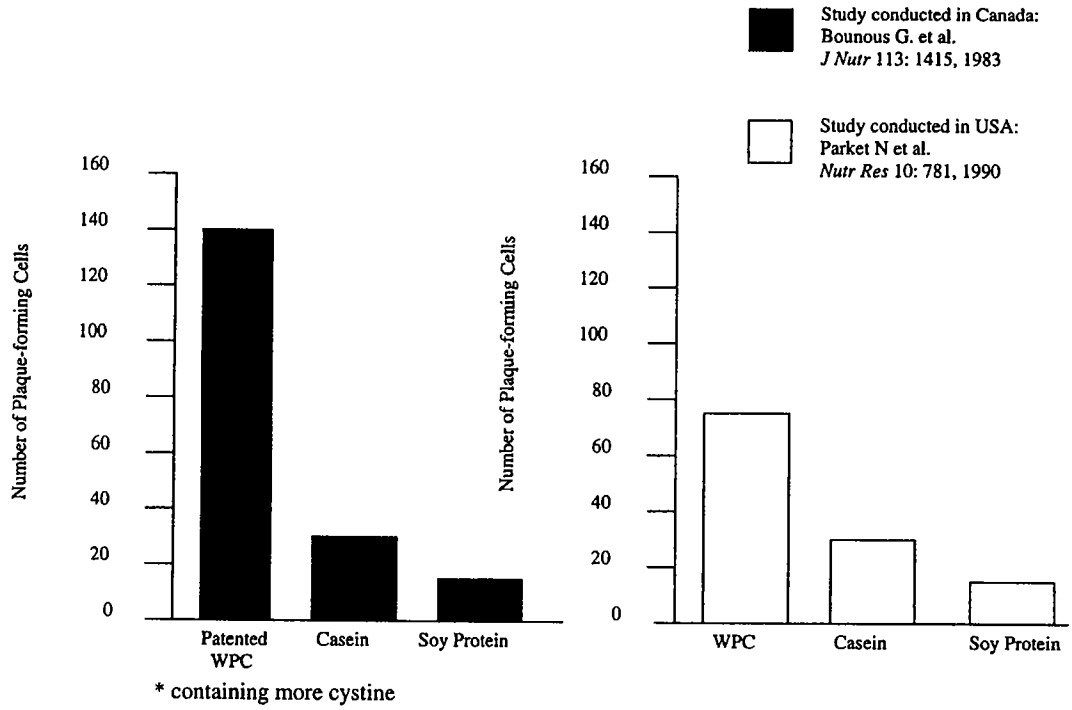
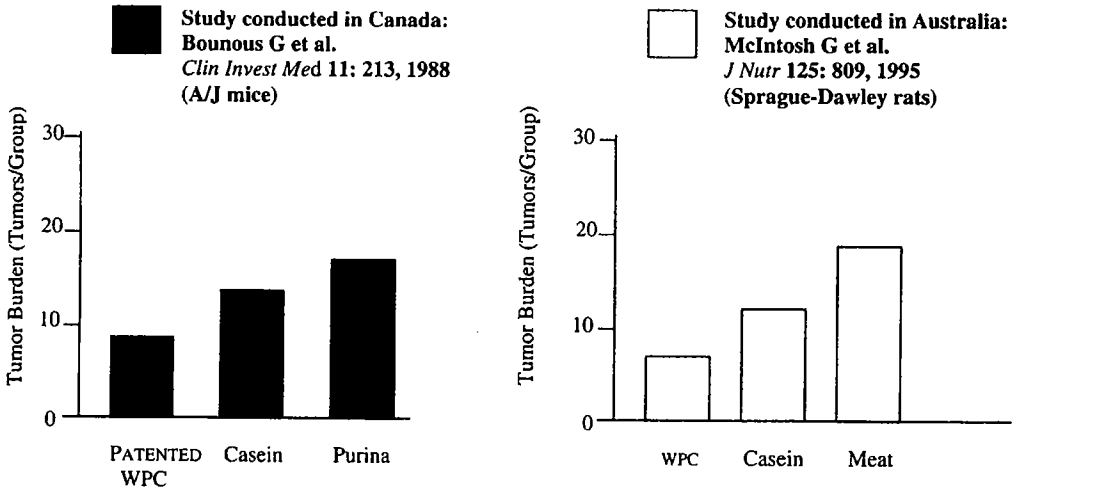



Figure 4. *Results of studies demonstrating the immunosustaining role of specially prepared dietary WPCs.*




Tumor mass was lower in mice fed patented WPC than in mice fed casein or purina.[3]

"... 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."

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

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:[1]
   - 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-ACETYLCYSTEINE)**

1. DeFlora S et al. Cancer letters 32/235-41, 1986 – Inhibition of urethan induced lung tumors in mice.
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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.
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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 crysts.
9. Morini M et al. Int J Biol Markers 14/268-271, 1999 — Inhibition of neo angiogenesis and tumor progression in murine melanomas.

[1] 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 I. *Sulphur amino acids, liver glutathione and tumour data for rats fed various diets*[1].

| Diet | Amino acid composition g/100 g | | | Liver glutathione mmollg (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 |

[1]Adaptedfrom 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.

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