The biological activity of undenatured dietary whey proteins: role of glutathione

Gustavo Bounous
Phil Gold

Departments of Surgery and Medicine, The Montreal General Hospital Research Institute and McGill University, Montreal, Quebec

(Original manuscript submitted 20/7/90; received in revised form 9/1/91; accepted 10/2/91)

Abstract
This study compared the effects of different sources of whey protein concentrate (20g/100 g diet) and of casein on the spleen, liver, and heart glutathione content of C3H/HeJ mice, and on the immune response of their spleen cells to sheep red blod cells. Body weight curves were similar in all dietary groups. Our data indicate that the humoral immune response is highest in mice fed a dietary whey protein concentrate exhibiting the highest solubility (undenatured conformation) and a greater relative concentration of the thermolabile bovine serum albumin and immunoglobulins. In addition, the mice fed this type of whey protein concentrate exhibit higher levels of tissue glutathione. The presence in the serum albumin fraction of glutamylcysteine groups (rare in food protein) and the specific intramolecular bond as related to the undenatured conformation of the molecule are considered to be key factors in the glutathione-promoting activity of the protein mixture.

Résumé
Notre étude compare l'effet des protéines du petit lait (20 g/100 g de diète) de plusieurs sources et de la caséine sur le contenu en glutathion de la rate, du foie, et du coeur de la souris ainsi que sur la réponse immunitaire des cellules spléniques de souris C3H/HeJ provoquée par l'injection d'érythrocytes de moutons. Les courbes de croissance des souris se sont avérées similaires dans tous les groupes. Nos données indiquent une réponse humorale immune plus importante chez les souris nourries avec un concentré de petit lait de grande solubilité (non dénaturé) contenant un concentration élevée d'albumine bovine sérique thermolabile et d'immunoglobulines. De plus les souris nourries avec ce type de concentré protéique ont présenté des niveaux tissulaires élevés de glutathion. La présence dans l'albumine sérique de glutamylcystéine, un peptide rare dans les protéines diététiques et les liaisons spécifiques intramoleculaires réliées à la conformation non-dénaturée de ces molécules, sont donc considérées comme des facteurs positifs permettant l'accumulation de glutathion dans ces circonstances.

Introduction

Our studies have shown that the humoral immune response (number of plaque-forming cells to sheep red blood cells) is significantly higher in mice fed a 20 g whey protein concentrate/100 g diet than in mice fed formula diets of similar nutritional efficiency containing 20 g/100 g diet of any other type of commercially available semipurified food protein, such as casein, soy, wheat, corn, egg white, fish, beef protein, *Spirulina maxima, Scenedesmus* algae protein, or Purina mouse chow [1]. The im-

munoenhancing effect of whey protein concentrate is maintained when the proteins are replaced in formula diet by a pancreatic hydrolysate (oligopeptides with mol. wt. <1000) of undenatured whey protein concentrate [2, 3]. This observation appears to obviate the likelihood of milk protein allergy or some other manifestation of oral immunization. We have further shown that the immunoenhancing activity of dietary whey protein concentrate is related to greater production of splenic glutathione in the whey protein-fed animals during the oxygen-requiring antigen-driven clonal expansion of the lymphocyte [3]. It was then theorized that this might reflect the ability of the lymphocytes of whey protein diet-fed mice to offset potential oxidative damage, thus responding more fully to the antigenic challenge [4, 5]. In fact, the capacity of a cell to recover from an oxidative insult is considered to be represented by its ability to regenerate intracellular stores of glutathione [6]. Our studies also showed that administration of S-(n-butyl) homocysteine sulfoximine, which reduces splenic glutathione in half, significantly reduced the humoral immune response of whey protein-fed mice. This was taken as further evidence for the important role of glutathione in the immunoenhancing effect of dietary whey protein [3]. A recent observation has revealed to us that the described biological activity of whey protein concentrate, already shown to be unrelated to its nutritional quality, is actually dependent on the undenatured conformation of the ingested proteins. This discovery was made accidentally when a batch of whey protein concentrate that was sent to us in 1988 by the usual supplier (Lacprodan from DanMark Protein S.A., Denmark) failed to exhibit the immunoenhancing effect previously described while exhibiting the same nutritional efficiency. Upon analysis, it became apparent that this preparation was less water soluble than the previous samples of undenatured whey protein concentrate exhibiting high biological activity.

The present study was designed to define the effect of changes in the molecular conformation of whey protein concentrate on the immune response and glutathione formation of the host, and to explore the factors and the mechanism of the observed effect of dietary whey protein concentrate on glutathione formation and on humoral immunity.

Table 1. Vitamin and mineral content of formula diets

The vitamin mixture plus the vitamins contained in the basal diet (Mead Johnson, product #80056) provided *in milligrams per 100 g diet*: ascorbic acid, 53.3; niacin, 5.1; riboflavin, 0.38; thiamin, 0.34; folic acid, 0.063; vitamin B-6, 0.26; biotin, 0.031; pantothenic acid, 1.93; choline, 44; *and per 100 g diet*: retinyl palmitate, 1295 IU; ergocalciferol, 260 IU; vitamin E (*dl*-tocopherly acetate), 11.6 IU; vitamin B-12, 0.001 mg; and vitamin K (phylloquinone), 0.06 mg. The mineral content of ions or cations (expressed in milligrams per 100 g diet) and the actual chemical compounds fed were:

Ca, 350 ($CaHPO_4 \cdot 2H_2O$ and $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$); P, 260 ($K_2HPO_2 \cdot 2H_2O$); Fe, 7.9 ($FeSO_4 \cdot 2H_2O$); Mg, 63.2 (MgO); Cu, 0.47 ($CuSO_4 \cdot 5H_2O$); Zn, 3.5 ($ZnSO_4 \cdot 7H_2O$); Mn, 63.2 ($MnSO_4$); Cl, 1108 ($C_5H_{14}CINO$); K, 997 ($K_2HPO_4 \cdot 2H_2O$); Na, 232 (NaCl); Se, 0.01.

Materials and methods

Animals

Male C3H/HeJ mice were obtained from Jackson Laboratories (Bar Harbor, ME.) at seven weeks of age and were maintained five per cage in a temperature-controlled 12 h light-dark cycle room.

Diets

The detailed composition of the common ingredients (vitamins and minerals) in all of the defined formula diets is given in Table 1. Diets were prepared in the following way: 20 g of selected pure protein, 56 g of product 80056 protein-free diet powder containing corn syrup, corn oil, tapioca starch, vitamins, and minerals (Mead-Johnson Co. Inc., IN.), 18 g cornstarch, 2 g wheat bran, 0.05 g Nutramigen vit-iron premix (Bristol-Meyers, Ont.), 2.65 g KCl, 0.84 g NaCl. The only variable in the various purified diets was the type of protein. The formula diets contained 20 g/100 g diet of either of the following types of bovine whey protein concentrate: Product X, especially prepared for us by the "Service de recherche sur les aliments du Ministère de l'agriculture du Québec" in St.-Hyacinthe, Que. (Table 2); Promod (Ross Laboratories, Columbus, OH.); Alacen 855 (New Zealand Dairy); Lacprodan-80 (produced in 1989 by Danmark Protein, Worthington, OH.); Sapro (Saputo, Montréal, Que.); Savorpro-75 (Golden Cheese, CA.); Bioisolate (Lesueur Isolates,

Table 2. A schematic representation of the process to produce Product X

	Raw milk
	↓
	Skimmed at 35°C → cream
	↓
	Skimmed milk pasteurized at 63°C for 30 min
	↓
At **38°C**	Addition of rennet (20 ml/100 kilos), allowing the agitation to resolve at low speed
	↓ → curd
	Whey
	↓
	Filtered with cheese cotton to remove debris (45 min)
	↓
At **40°C**	Ultrafiltration (Romecon UFSI, polysulphone membrane, cut off 50,000 pore diameter 60/1000 of an inch, surface 2–3 m^2) Diafiltration to wash out salts and lactose
	Whey Protein Concentrate
	↓ (Pasteurized at 63°C for 30 min)
At **40°C**	Lyophylization
	↓
	Whey Protein Concentrate Powder

Minneapolis, MN.). An additional group of mice was fed a diet containing 20 g of casein free of whey protein coprecipitate/100 g diet. All purified proteins were vitamin free. The net protein content of the different protein powders was taken into consideration when preparing the various 20 g protein/100 g diets. Diets were continuously available in powder form from stainless steel feeders, 1.5 inches high and especially designed to reduce spillage and spoilage. Mice were placed on the various diets and immunologic studies or spleen glutathione assays commenced 3 weeks later.

Solubility measurements

Heat denaturation unfolds and exposes the poorly soluble hydrophobic amino acid residues of whey proteins to water. The extent of denaturation is normally assessed by loss of solubility at pH 4.6 [7, 8]. In our studies we evaluated solubility by the following method: after dispersion of a 4% protein solution in distilled water at room temperature and pH adjustment at 4.6 acetic acid/sodium acetate buffer, the solution was stirred. Percent solubility was computed as the portion of total protein filtered through a Durieux red III filter paper. Total nitrogen was determined by the micro-kjeldahl method.

Gel electrophoresis

Polyacrylamide gel electrophoresis of the whey protein concentrate samples was carried out with 20% polyacrylamide at pH 8.8 (Laemmli buffer system) after the samples were reduced with 10% 2-mercaptoethanol. An eight-slot gel was run and samples were applied so that each slot received 40 μg of the sample on a dry weight basis. Electrophoresis was performed at 200 volts for 45 min.

Immunization for plaque assays
The diet-fed mice were immunized by an intravenous injection of 5×10^6 washed sheep red blood cells obtained weekly from Institut Armand-Frappier, Laval des Rapides, Quebec.

Plaque forming cell (PFC) assay
The method used for assaying IgM plaque forming cells was as described by Cunningham and Szenberg [9] with minor modifications. Spleen cell suspensions were prepared by gently tamping the spleen through a 50-mesh stainless steel screen, and collecting the cells in balanced salt solution (BSS) supplemented with 10% heat-inactivated calf serum (Grand Island Biological Company, Montreal, Que.). The spleen cells were washed and made up to 15 ml with BSS. Sheep red blood cells were washed twice and made up to a 20% concentration. Guinea pig serum (Grand Island Biological Company, Montreal, Que.) as a source of complement was diluted 1/15 with BSS. All stock solutions were kept on ice water until used. The test consisted of mixing 0.05 ml of spleen cells, 0.15 ml of sheep red blood cells, 0.75 ml of the complement solution in a test tube at 37°C. The whole mixture was immediately withdrawn and put into slide chambers, sealed with warm paraffin wax, and incubated at 37°C for 45–60 min. The number of plaque-forming cells was counted and the total number of plaque-forming cells per spleen estimated by multiplying the number of plaque-forming cells in each sample (0.05 ml spleen cells) by 300. Plaque-forming cells have been expressed per total organ rather than per 10^6 spleen cells, since this appears to reflect more accurately the functional status of the spleen *per se*.

Mice were normally assayed for the plaque-forming cell response to sheep red blood cells on the fifth day after immunization, when the response was shown to peak.

Spleen, heart, and liver glutathione content
Ninety milligrams of mouse tissue were weighed using a Mettler PM-300 balance; samples varied from 90 mg by less than 5 mg (<5%). The samples were homogenized in 5-sulfosalicylic acid (5% w/v). Homogenates were centrifuged for 5 min in a microfuge at 10,000 × g. The assay was carried out using the supernatants on the same day according to the methods of Anderson [10]. Values are expressed as μmol/g/wet tissue.

Statistics
Values were compared among the dietary groups using either Student's t-test, when two groups were being compared, or the analysis of variance (ANOVA) for more than two groups. Each dietary group comprised at least ten mice.

Results

Data in Table 3 indicate that humoral immune response as measured by the plaque assay is highest ($p < 0.004$ by ANOVA) in mice fed Product X, which exhibited the highest level of solubility. The serum albumin content of Product X, at 10% of total whey protein, is almost twice the corresponding values found in any other whey protein concentrate examined ($p < 0.05$ by ANOVA). In addition the level of immunoglobulins is also substantially higher in Product X ($p < 0.05$ by ANOVA). No intergroup difference was noted in body growth, food consumption, or serum protein (data not shown).

On days 2, 3, 4, and 6 after immunization, the spleen glutathione levels in Product X diet-fed mice were 13% ($p < 0.01$), 8% ($p < 0.05$), 21% ($p < 0.01$) and 20% ($p < 0.01$) higher than the corresponding values in Lacprodan-80 diet-fed mice, and they were 12% ($p < 0.01$), 7% ($p < 0.05$), 20% ($p < 0.001$), and 20% ($p < 0.001$) higher than the corresponding values in casein diet-fed mice (Figure 1). No intergroup difference was noted in body growth, food consumption, and serum protein (data not shown). After three weeks of dietary treatment, no significant differences were seen among dietary groups in splenic glutathione levels of unimmunized mice [3.10 ± 0.30 (mean ± standard deviation), 3.09 ± 0.29, 3.07 ± 0.33, μmol/g in Product X diet-fed, Lacprodan-80 diet-fed, or casein diet-fed mice respectively]. These values were maintained up to 5 days later. Hence the values reported in Figure 1 as percentage of values in the corresponding unimmunized mice are considered to be significant variations from control values.

Table 3 shows that glutathione in the liver of unimmunized mice fed Product X diet for 3 weeks is higher than the corresponding values in Promod,

Table 3. Physical-chemical characteristics and biological activity of different types of whey protein concentrate

	Undenatured conformation	Protein composition (% of total whey proteins)				Effect of 3 weeks dietary treatment		
	Solubility index (pH 4.6)	β-LG[1]	Alpha-La[2]	SA[3]	Immunoglobulins	PFC[4] $\times 10^{-3}$	Glutathione (μmol/g)[5] Liver	Heart
Product X	99.5%	57.8±0.9	[illegible]	10±1	[illegible]7	148±16	7.95±0.40	1.15±0.7
Promod	97%	61±3	20±1	4±1	15±3	65±14	6.64±0.41	1.0±0.7
Alacen 855	97.1%	62±8	22±3	4±1	12±6	66±17	6.04±0.36	–
Lacprodan-80	96%	62±4	18±2	4.8±2	15±4	44±15	6.70±0.20	1.01±0.5
Sapro	95%	60±3	21±2	4.8±0.1	14±1	67±16	–	–
Savorpro-75	98%	63±3	20±1	4±1	13±3	31±8	–	–
Bioisolate	90.1%	66±4	15±1	5±1	12±3	65±20	–	–
Casein	–	–	–	–	–	35±9	–	1.0±0.8

Values are expressed as mean ± SD; 20 g protein/100 g diet
[1]β-LG, β-Lactoglobulin
[2]Alpha-La, Alpha-Lactalbumin
[3]S.A., Serum Albumin
[4]Number of plaque-forming cells / spleen 5 days following immunization with 5×10^6 sheep red blood cells
[5]Effect of 3 weeks of dietary treatment in unimmunized mice
– Not done
*Please see text for statistical significance

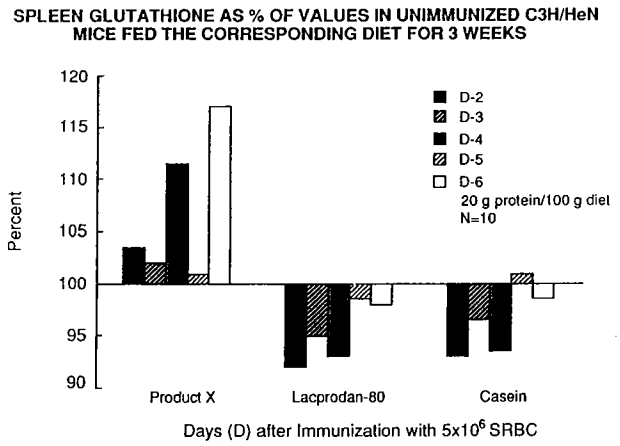

FIG. 1. Spleen glutathione levels expressed as percent of values in unimmunized mice fed the corresponding diets for 3 weeks. Effect of 3 weeks dietary treatment with 20 g/100 g diet of either Product X, or Lacprodan-80, or casein. D-day following immunization with 5×10^6 sheep red blood cells (SRBC). Each value represents the mean of 10 mice. See text for statistical analysis.

Alacen or Lacprodan-80 diet-fed mice ($p < 0.01$ by ANOVA). Glutathione in the heart of unimmunized mice fed Product X diet for 3 weeks is higher than the corresponding values in Promod, or casein diet-fed mice ($p < 0.05$ by ANOVA). No intergroup difference was noted in body growth, food consumption, or serum protein (data not shown).

Discussion

Our data are consistent with previous findings demonstrating the correlation between enhancement of plaque-forming cells response and spleen glutathione levels in immunized mice fed whey protein in comparison to mice fed the casein diet, and the central role of glutathione in the immunoenhancing effect of dietary whey protein [3]. The current findings indicate that the previously described biological activity of dietary whey protein is restricted to the undenatured form of the protein, and it is not related to its nutritional efficiency. The differences in solubility of the protein mixture as measured by filtration may reflect conformational changes biologically more significant than the actual numbers appear to indicate. Indeed specific thermolabile proteins, crucial to the biological activity of the protein mixture, may be involved. In addition, partial unfolding of some molecules, undetected by solubility changes, could initiate further unfolding and / or

Table 4. Protein composition of cow and human milks

	Cow milk			Human milk		
Protein	(g/L)	Mol. wt.	Glu-Cys	(g/L)	Glu-Cys	Reference
Caseins	26	19,000–25,000	0	3.2	0	17
β-lactoglobulin	3.2	18,300		trace		18
β-lactoglobulin**			1**			18
Alfa-lactalbumin	1.2	14,160	0	2.8	0	18
Serum albumin	0.4	66,267	6*	0.6	5*	18–20
IgG	0.6	155,000	not known	0.04	not known	
IgA	0.1	400,000	not known	1.0	1 (known)	21
Lactoferrin	trace 0.07	80,000	4	2.0	4	22

*Disulfide bond

**60% of total β-lactoglobulin is constituted by the B variant (KWAI, NG, HAYES NF, MOXLEY JF, MONARDES HG: Association of genetic variants of casein and milk serum proteins with milk, fat, and protein production by dairy cattle. *J Dairy Science* 67: 835–40, 1984.) A and B variants have the same Glu-Cys group (WHITNEY RM: Proteins of milk. In: WANG NP, ed. Fundamentals of dairy chemistry. 3rd edn. New York: Reynolds Publishing, 1988: 81–169.) In columns 3 and 5 the numbers of Glu-Cys groups per molecule of protein are given.

other biologically significant alterations during the process of digestion in the gastrointestinal tract. The type of protein present in the whey protein concentrate is of obvious importance. Our analysis shows that the relative concentration of the heat-labile bovine serum albumin and immunoglobulin is highest in Product X, which exhibits greater biological and glutathione-promoting activity.

The factors and mechanisms of the observed effect of some undenatured whey proteins on glutathione formation is a matter of interest. Glutathione (L-gamma-glutamyl-L-cysteinylglycine) is dependent upon the supply of cysteine which is derived from dietary protein. At the present time, the optimal strategy for obtaining "normal" glutathione tissue levels in healthy animals is by providing the animal with a diet containing adequate levels of protein. Little or no increase in tissue or organ glutathione has been found when animals on a diet with 20–25% protein are given supplemental sources of sulfur amino acids [11]. Recent studies in rats showed that whereas a growth-retarding low casein diet caused decreased hepatic glutathione concentration, neither excess casein (30 and 45%) nor excess sulfur amino acids could increase glutathione concentrations above the levels found when rats were fed a diet adequate in protein (15%) [12]. Whereas concentrations of glutathione are dependent on the supply of cysteine, administration of cysteine is not an ideal way to increase glutathione concentration because cysteine is rapidly metabolized and furthermore, it is toxic [13, 14]. On the other hand, tissue glutathione concentration may be increased by administration of gamma-glutamylcysteine: glutathione increased in the kidney by about 50%, 40–60 min after s.c. injection in mice, returning to control values 2 h later [15]. The administered gamma-glutamylcysteine is transported intact and serves as substrate of glutathione synthetase [16]. Advances in amino acid sequencing of food proteins allowed us to investigate the occurrence of glutamylcysteine groups in whey protein and the possible relation to glutathione promotion. Indeed, whey protein concentrate from bovine milk contains substantial amounts of glutamylcysteine groups, unlike casein, which does not increase tissue glutathione when fed to mice [1] (Table 4). The glutamylcysteine groups are located primarily in the serum albumin fraction (Table 4). Although the amino acid sequence of bovine milk immunoglobulins is not totally known, the high levels of cysteine and glutamine in bovine immunoglobulin suggest the possi-

bility of glutamylcysteine groups as described in human milk immunoglobulin (Table 4). Glutamylcysteine groups are extremely rare in animal and plant edible proteins. An extensive search of all available data on amino acid sequencing of edible proteins [23–57] reveals that the Glu-Cys group with disulfide link is indeed limited to some of the whey protein as indicated in Table 4, and to the ovomucoid fraction of egg white which contains 2 of these groups in a 30,000 mol. wt. molecule [26]. This molecule represents about 8% of total egg white proteins which are 75% ovalbumin [26]. This literature search included 81 different animal and plant proteins from several species as indicated in the title of the references [23–57]. In the natural state the milk whey proteins have a definite configuration which, when exposed to heat above a certain critical level, is disrupted [58]. In addition to heating, other processing treatments, e.g., pumping, mixing, aeration, vacuum evaporation, and spray drying further promote denaturation [59]. The half-cystine residues, frequent in some of the whey proteins [18] are connected by intramolecular disulfide bonds which contribute to the spatial conformation of the molecule and partly block unfolding of the molecule [60]. It is interesting to note that the cystine residues of all glutamylcysteine groups in β-lactoglobulin and serum albumin are connected by intramolecular disulfide bonds [18]. It is also relevant to note that pancreatic trypsin does not hydrolyse the disulfide cross-linkage characteristic of the native whey proteins [61]. It is our hypothesis that the glutathione-promoting activity of dietary whey protein concentrate is dependent on the glutamylcysteine groups contained in serum albumin fractions, in the β-lactoglobulin and possibly in the immunoglobulin G fraction. The preservation of the disulfide bond (which involves the cysteine) may be crucial to the release, upon digestion, of intact glutamylcysteine peptide for absorption by the intestinal mucosa. Denaturation, on the other hand, by unfolding the protein molecule, exposes the glutamylcysteine sequence to the digestive enzymes with subsequent release of either of the single amino acids or other peptide combinations. This would be consistent with the lesser glutathione-promoting activity of the denatured form of whey protein concentrate which contains the same amount of cysteine. It is conceivable that the single glutamylcysteine group in the small β-lactoglobulin molecule is more vulnerable during the intestinal transit than some of the 6 groups in the folded, larger serum albumin molecule. Whether glutamylcysteine enters the cells as a cysteine-mixed disulfide is not known. If this were the case, it could more readily cross cell membranes [62].

The two phases in the production of whey protein concentrate involve firstly the thermal treatment of milk (pasteurization) prior to and during the manufacture of cheese. The second step involves the concentration of the proteins from whey through various phases of ultrafiltration, and freeze or spray drying or lyophylization to obtain a powder that may contain 80–90% whey proteins. If denaturation of a whey protein during the first phase is irreversible, it is evident that the denatured protein would be lost from whey by precipitating with the caseins in the formation of cheese. During the subsequent treatment of whey with the objective of concentrating the remaining proteins, more protein could be denatured but not lost from the whey protein concentrate. The extent of denaturation produced during the second phase of whey protein concentrate production is normally assessed by the loss of solubility at a pH of 4.6. With a denaturation temperature of 78°C, β-lactoglobulin is the least denaturable of the serum proteins. On the other hand, with a denaturation temperature of 64°C, bovine serum albumin is denatured almost as easily as alpha-lactalbumin. Since its denaturation is not as reversible as that of alpha-lactalbumin, it appears to be the most easily denatured serum protein. It precipitates between 40°C and 50°C as a result of hydrophobicity-directed unfolding [63]. Whereas extensive investigations of this protein isolated from bovine blood serum have been made, the configuration of bovine serum albumin isolated from milk has not been investigated [63]. Immunoglobulins in milk are also very heat labile, especially below a pH of 6 [63].

Our Product X whey protein concentrate was prepared in the most lineant way compatible with accepted standards of safety with regard to bacterial contamination. The extremely high solubility index indicates that the proteins present are essentially undenatured, hence demonstrating the lineancy of the ultrafiltration process. Although the proteins contained in the concentrates from the other sources examined were mostly in undenatured form, as indicated by the relatively high solubility of the concen-

trates, the content of serum albumin and immunoglobulins in these mixtures is below the level apparently necessary to produce a biological activity. These very thermolabile proteins are denatured, hence precipitated and partially lost from whey when high pasteurization temperatures are utilized. Conversely, the relatively high concentrations of the thermosensitive serum albumin and immunoglobulins resulting from the low pasteurization of milk in Product X, may reflect more closely the pattern of raw milk. These data lend support to the hypothesis that the thermolabile Glu-Cys containing proteins such as serum albumin in undenatured conformation are crucial elements for the biological activity of whey protein concentrate.

We have also tested another, more rapid, method of low level milk pasteurization (72°C for 13 s) with demonstrated antibacterial effect comparable to that obtained with the method described in Table 2. The characteristic features of relatively high serum albumin and immunoglobulin levels noted in Product X (Table 3) are more pronounced in the whey concentrate obtained from milk pasteurized at 72°C for 13 s: β-lactoglobulin 52 ± 1%; alpha-lactalbumin 6 ± 1%; serum albumin 14 ± 1%; immunoglobulins 28 ± 2%.

Enhancement of glutathione levels in the tissues may well represent the common denominator underlying the beneficial effect observed by a diet of whey protein, as demonstrated by increased longevity in hamsters [64] and mice [65], increased resistance to carcinogens [66] and pneumococcal infection [67] in mice.

Mammalian cells have evolved numerous mechanisms to either prevent or treat injurious events that might result from normal oxidative byproducts of cellular metabolism. Foremost among these endogenous protective systems is the glutathione redox system. Glutathione is a ubiquitous tripeptide (L-gamma-glutamyl-L-cysteinyl-glycine) present in high concentrations (generally in the millimolar range) in most mammalian cells as reduced glutathione [68, 69]. Glutathione peroxidase eliminates H_2O_2 generated by mitochondrial O_2 consumption employing glutathione as a hydrogen donor to reduce H_2O_2 to water. Therefore glutathione plays a critical role in the defense against oxygen toxicity by breaking the chain of reactions leading from superoxide anion to the very active hydroxyl radical through intermediate H_2O_2. Undoubtedly the efficiency of this reaction is important in modulating the aging process [69].

In addition to detoxification of endogenous toxins, glutathione plays a crucial and unique role in the elimination of exogenous toxins and xenobiotics. Accordingly, the liver, having the greater content of glutathione [69], is the major organ involved in the elimination and detoxification of xenobiotics. Glutathione readily binds transition metals and is an important factor in their elimination [70]. Among the reactions associated with the -SH group of glutathione are those which convert β-ketoaldehydes (which are toxic) to the corresponding alpha-hydroxyacids [71]. Glutathione inactivates electrophilic drugs and carcinogen metabolites by formation of glutathione conjugates [72]. The reactive ultimate carcinogenic forms of chemical carcinogens are electrophiles, and thus good candidates for detoxification by reaction catalyzed by glutathione [68]. This peptide plays a role in protection against tissue damage resulting from exposure to ozone [73] and can also protect against radiation [74]. It has been reported that resistance to x-irradiation varies synchronously with glutathione content. Cellular sensitivity is increased when glutathione level is low [75–77]. A damage-producing product of ionizing radiation is considered to be the hydroxyl radical which reacts rapidly with most organic molecules, including DNA [78]. Thiols react with hydroxyl radicals almost exclusively by donation of a hydrogen atom from the SH bond. A second way in which thiols can protect DNA is through chemical repair of DNA [78].

The free radical theory of aging [79] hypothesizes that the degenerative changes associated with aging might result from toxic effects of the free radicals produced during cellular metabolism. Aging is thus considered to be caused by the byproducts of normal physiological metabolic processes of life. One approach taken to verify the free radical theory of aging has been to determine whether any age-related changes occur in cellular antioxidative protective mechanisms. One such principal mechanism is glutathione, which is a ubiquitous cellular constituent and the most abundant thiol-reducing agent in mammalian tissues. The ubiquitous nature of the aging process makes glutathione an interesting object of aging-related research. It appears that, where-

as data or age-related changes in tissue vitamin E and other antioxidants are, at best, contradictory [79] the tissue glutathione levels are more consistently reported to decline with old age. Thus glutathione contents of liver, kidney, heart [80], and brain [81] were respectively 30%, 34%, 20%, and 30% lower in very old mice than in mature mice. A lower glutathione status has been demonstrated in erythrocytes of aging mice [82] and humans [83]. Glutathione peroxidase activity was also reported lower in the elderly population [84].

More specifically, some characteristic age-related diseases appear to be preceded by or associated with a drop in glutathione content in the organ or systems involved. The immune system loses its effectiveness with age. A major factor in this loss of functions is a progressive age-related failure of T-lymphocytes to respond to proliferating stimuli [85]. The age-related decline in immune responsiveness of mice was found to be associated with a 19% drop in spleen glutathione content [86]. Our experiments indicate that the immunoenhancement by whey protein feeding is dependent upon the glutathione-promoting activity of this diet [3]. Even non age-related conditions of immunodepression have been found to be characterized by decreased levels of glutathione. Systemic glutathione deficiency has been recently observed in symptom-free HIV seropositive individuals [87]. Intracellular glutathione appears to play an important role with the defense of endothelial cells against oxidized low density lipoproteins, hence in the pathogenesis of arteriosclerosis [88].

Osteoarthritis has many characteristics of a free radical-produced disease. The breakdown of the polymer hyaluronic acid, which acts as a joint lubricant, may be caused by oxygen free radicals produced by neutrophils that accumulate in the affected joint. In addition to its action as free radical scavenger, glutathione could be implicated as a source of cysteine [73], because cysteine serves as the active sulfate for the biosynthesis of chondroitin sulfate in the cartilage [89]. In addition, oxygen-derived free radicals stimulate osteoclastic bone reabsorption in rodent bone *in vitro* and *in vivo* [90].

One aspect of aging is the formation of cataracts. This process in part involves lipid peroxidation. Severe glutathione depletion in the lens by administration of buthionine sulfoximine produces cataract formation in young mice [91].

Many hypotheses have been proposed to explain the physiology of Alzheimer's disease. The increased prevalence of Alzheimer's disease with age and the fact that the neuropathological injuries are similar in Alzheimer's disease and in normal cerebral aging suggest that the so-called Alzheimer's disease is in fact accelerated aging. As mentioned earlier, the free radical theory of aging postulates that aging can be induced by the deleterious effect of free radicals. Indeed, the level of lipid peroxidation is higher in the brain of subjects affected by Alzheimer's disease than in the brain of the non-demented elderly [92], although an age-related rise in blood lipid peroxides has been reported with fully adequate vitamin E status [93]. A significant decrease in the level of glutathione peroxidase has been reported in erythrocytes of patients with Alzheimer's disease together with evidence of lipid peroxidation [94].

Finally, a recent multicentre study has demonstrated that glutathione concentrations were significantly lower in the putamen, globus pallidus, substantia nigra, and frontal cortex of Parkinsonian brains. A direct positive correlation between the severity of cellular deficit and the glutathione content could be calculated, whereas the distribution of ascorbate was relatively even with no significant differences between controls and Parkinsonian brains [95].

Our current and previous studies demonstrate that it is feasible to produce a moderate but sustained increase in tissue glutathione levels in young and old mice by the simple method of administering undenatured whey protein concentrate containing most of the bovine serum albumin originally present in the raw milk. The present discovery obviates the toxic effects of other known methods for increasing the intracellular levels of glutathione. Most cell types have no direct system for transport of intact glutathione into cells [74]. The methods involving the use of acetyl-cysteine [13], gamma-glutamyl-cyst(e)ine [15], athiazolidine [96], or glutathione esters [97] offer an interesting possibility for short-term intervention. However, their long-term effectiveness in producing sustained elevation of cellular glutathione has not been shown, nor has the possible toxicity of their long-term use been disproved. Our previous studies [65] indicate that sustained elevation of tissue glutathione levels can be obtained by

the administration of undenatured whey protein concentrate (tested up to three months). This discovery could provide a method for efficiently increasing cellular glutathione levels for any purpose for which elevated glutathione levels are desired such as for drug detoxification, arteriosclerosis, Alzheimer's and Parkinson diseases; cellular protection against oxygen and its metabolites such as peroxides, free radicals or foreign compounds, carcinogens, irradiation, immunodeficiency states, etc.

The main similarities between egg white and milk whey are unique features in the plant and animal kingdom: the highest cysteine content of any food protein (2.4 g %) and the presence of glutamylcysteine (Glu-Cys) groups (glutathione precursor) with disulfide bonds. The Glu-Cys groups of egg white proteins are contained in a minor protein constituent (ovomucoid) and their total amount in g/litre of egg white is comparable to that found in milk whey. It may also be noteworthy that from time immemorial, whey from raw milk and / or undenatured raw egg white have been administered to children and to the sick as prophylactic or therapeutic measures in folk medicine. This is probably one of the very few instances in which uncooked, i.e., undenatured animal products are fed to humans, in modern times. Whey protein concentrate was administered in our experiment as the protein component of a normal mouse diet containing all the other ingredients, such as energy, vitamins, minerals, and trace metals including selenium.

The increments of tissue glutathione described in our experiments were moderate but sustained throughout the feeding period. It is noteworthy that the effects of a small increase in cellular glutathione may be greater than expected. For example, there are many reports of human and murine tumor cell lines selected *in vitro* for resistance to a variety of chemotherapeutic agents. In a number of these cell lines cellular glutathione is increased consistently by 2-fold compared to the drug-sensitive parental cell line, despite the fact that the level of drug resistance is often much greater, e.g., as much as 30-fold [97, 98]. Given the fact that cellular glutathione is very tightly regulated, that a 2-fold increase may be maximal, and that the effect of small increments in glutathione may be amplified by a variety of glutathione-utilizing enzymes (e.g., glutathione peroxidase, glutathione-S-transferase), the reproducible change in glutathione concentration observed in animals fed Product X diet is likely to have biological importance. The chronic nature of this augmentation may contribute significantly to this effect.

In conclusion, our data lend support to the concept that the concentration of serum albumin and possibly the immunoglobulins, as well as the undenatured conformation of the molecules, are crucial factors in determining the biological activity of dietary whey protein concentrate. The relative content of the active and thermolabile proteins must, in fact, be close to the values found in the whey from raw milk as easily determined by gel electrophoresis. The increasing use of high temperature milk pasteurization in recent years appears to have caused a drop in the levels of serum albumin and immunoglobulin and in the biological activity of most currently produced whey protein concentrates.

Acknowledgement

We wish to express our deep gratitude to Mr Bernard Aurouze, "Chef de Service de Recherche sur les Aliments", Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Gouvernement du Québec, who was instrumental in the production of Product X. The authors thank Mrs Louise Gilbert for technical assistance, Mrs Tina Parks-Picard for secretarial assistance in preparing this manuscript, and Miss Marie Aubin for her collaboration in the project. This work was supported by grants from the Dairy Bureau of Canada and the Medical Research Council of Canada.

References

1. BOUNOUS G, KONGSHAVN PAL, GOLD P: The immunoenhancing property of dietary whey protein concentrate. *Clin Invest Med* 11: 271–8, 1988
2. BOUNOUS G, STEVENSON MM, KONGSHAVN PAL: Influence of dietary lactalbumin hydrolysate on the immune system of mice and resistance to salmonellosis. *J of Infect Dis* 144: 281, 1981
3. BOUNOUS G, BATIST G, GOLD P: Immunoenhancing property of dietary whey protein in mice: Role of glutathione. *Clin Invest Med* 12: 154–61, 1989
4. FIDELUS RK, TSAN MF: Enhancement of intracellular glutathione promotes lymphocyte activation by mitogen. *Cell Immunol* 97: 155–63, 1986

5. GOUGEROT-POCIDALO MA, FAY M, ROCHE S: Mechanisms by which oxidative injury inhibits the proliferative response of human lymphocytes to PHA. Effect of the thiol compound 2-mercaptoethanol. *Immunology* 64: 281–8, 1988
6. NOELLE RJ, LAWRENCE DA: Determination of glutathione in lymphocytes and possible association of redox state and proliferative capacity of lymphocytes. *Biochem J* 198: 571–9, 1981
7. DEWIT JN, HONTELEX-BACKX E: Les propriétés functionelles des proteines du lactoserum: consequences des traitements thermiques. *Tech Lait* 952: 19–22, 1981
8. KINSELLA JE: Milk protein: Physicochemical and functional properties. *C.R.C. Critical review in food science and nutrition*. 21: 197–262, 1984
9. CUNNINGHAM AJ, SZENBERG A: Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology* 14: 599–600, 1968
10. ANDERSON ME: Tissue glutathione: In: C.R.C. Handbook of methods for oxygen radical research. Boca Raton, Florida: CRC Press, Inc., 1985: 317–29
11. DENEKE SM, FANBURG BL: Regulation of cellular glutathione. *Am J Physiol* 257: L163–73, 1989
12. BAUMAN PF, SMITH TK, BRAY TM: The effect of dietary protein and sulfur amino acids on hepatic glutathione concentration and glutathione-dependent enzyme activities in the rat. *Can J Physiol Pharmacol* 66: 1048–52, 1988
13. ESTRELA JM, SAEZ GT, SUCH L, VINA J: The effect of cysteine and N-acetyl cysteine on rat liver glutathione (GSH). *Biochem Pharmacol* 32: 3483–5, 1983
14. GLATT H, SABLJIC MP, OESCH F: Mutagenicity of glutathione and cysteine in the Ames test. *Science* 220: 961–3, 1983
15. ANDERSON ME, MEISTER A: Transport and direct utilization of gamma-glutamylcyst(e)ine for glutathione synthesis. *Proc Natl Acad Sci* 80: 707–11, 1983
16. MEISTER A: 5-Oxoprolinuria and other disorders of glutathione biosynthesis. In: STRANBURY JB, WYMGAARDEN JB, FREDRIKSON DS, eds. Metabolic basis of inherited diseases 4th edn. McGraw Hill, 1978: 328–35
17. JENNES R: Interspecies comparison of milk protein. In: FOX PF, ed. Developments in diary chemistry-1. Applied Sciences Publisher, 1982: 87–114
18. EIGEL WN, BUTLER JE, ERNSTROM CA, FARREL HM, HARWALKAR VR, JENNES R, WHITNEY R: Nomenclature of proteins of cow's milk: Fifth Revision. *J Dairy Sci* 67: 1599–631, 1984
19. FASMAN GD: Handbook of biochemistry and molecular biology. 3rd edn. Proteins. Vol. III. Boca Raton, Florida: C.R.C. Press, Inc., 1976: 497
20. FINDLAY JBC, BREW K: The complete amino acid sequence of human alfa-lactalbumin. *Europ J Biochem* 27: 65–86, 1972
21. LIU YV, PUTMAN FW: Primary structure of a human IgA immunoglobulin. *J Biol Chem* 254: 2839–49, 1979
22. METZ-BOUTIQUE MH, JOLLES J, MAZURIER J: Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *Europ J Biochem* 145: 659–76, 1984
23. ROMANOFF AL, ROMANOFF AJ: The avian egg. New York: Wiley Publishers, 1949: 918
24. HENDERSON JY, MOIR AJG, FOTHERGILL LA: Sequences of sixteen phosphoserine peptides from ovalbumins of eight species. *Europ J Biochem* 114: 439–50, 1981
25. NISBET D, SAUNDRY RH, MOIR A, FOTHERGILL LA: The complete amino acid sequence of hen ovalbumin. *Europ J Biochem* 115: 335–45, 1981
26. KATO I, SCHRODE J, KOHR WJ, LASKOWSKI M: Chicken ovomucoid amino acid sequence. *Biochem* 26: 193–201, 1987
27. DELANGE RJ, HUANG TS: Egg white avidin. *J Biol Chem* 246: 698–709, 1971
28. JOLLES J, JAREGUL-ADELL J, BERNIER I: La structure chimique du lysozyme de blanc d'oeuf de poule: étude détaillée. *Biochem Biophys Acta* 78: 668–80, 1963
29. LESZYK J, MORNET D, AUDEMARD E, COLLINS JH: Amino acid sequence of a 15 kilodalton actin-binding fragment of turkey gizzard caldesmon: Similarity with dystrophin, tropomyosin and the tropomyosin-binding region of troponin T. *Biochem and Biophys Res Commun* 160: 210–6, 1989
30. TAKAGI T, KONISHI K, MABUCHI I: Amino acid sequence of starfish oocyte depactin. *J Biol Chem* 263: 3097–102, 1988
31. KOBAYASHI T, TAKAGI T, KONISHI K, COX JA: Amino acid sequence of crayfish troponin I. *J Biol Chem* 264: 1551–7, 1989
32. LESZYK J, DUMASWALA R, POTTER JD, GUSEV NB, VERIN AD, TOBACMAN LS, COLLINS JH: Bovine car-

diac troponin T: Amino acid sequences of the two isoforms. *Biochemistry* 26: 7035–42, 1987

33. RICH CB, FOSTER JA: Characterization of rat heart tropoelastin. *Arch Biochem and Biophys* 268: 551–8, 1989
34. RAJU K, ANWAR RA: A comparative analysis of the amino acid and cDNA sequences of bovine elastin and chick elastin. *Biochem and Cell Biol* 65: 842–5, 1987
35. COLLINS JH, JAKES R, KENDRICK-JONES J, LESZYK J, BAROUCH W, THEIBERT JL, SPIEGEL J, SZENT-GYÖRGYI AG: Amino acid sequence of myosin essential light chain from the scallop: *Aquipecten irradians*. *Biochemistry* 25: 7651–6, 1986
36. MAITA T, KONNO K, MARUTA S, NORISUE H, MATSUDA G: Amino acid sequence of the essential light chain of adductor muscle myosin from Ezo giant scallop, *patinopecten yessoensis*. *J Biochem* 102: 1141–9, 1987
37. MAITA T, TANAKA H, KONNO K, MATSUDA G: Amino acid sequence of the regulatory light chain of squid mantle muscle myosin. *J Biochem* 102: 1151–7, 1987
38. MAITA T, HAYASHIDA M, TANIOKA Y, KOMINE Y, MATSUDA G: The primary structure of the myosin head. *Proc Natl Acad Sci (USA)* 84: 416–20, 1987
39. ONISHI H, MAITA T, MIYANISHI T, WATANABE S, MATSUDA G: Amino acid sequence of the 203-residue fragment of the heavy chain of chicken gizzard myosin containing the SH_1-type cysteine residue. *J Biochem* 100: 1433–47, 1986
40. MAITA T, ONISHI H, YAJIMA E, MATSUDA G: Amino acid sequence of the amino-terminal 24 kDa fragment of the heavy chain of chicken gizzard myosin. *J Biochem* 102: 133–45, 1987
41. WATANABE B: Amino-acid sequence of the hinge region in chicken myosin subfragment-2. *Biol Chem Hoppe-Seyler* 370: 55–61, 1989
42. FASMAN GD: C.R.C. Handbook of biochemistry and molecular biology. 3rd edn. Proteins. Vol. III. Boca Raton, Florida: C.R.C. Press, Inc., 1976: 305
43. CHU M-L, CONWAY D, PAN T-C, BALDWIN C, MANN K, DEUTZMANN TIMPL R: Amino acid sequence of the triple-helical domain of human collagen type VI. *J Biol Chem* 263(35): 18601–6, 1988
44. MENENDEZ-ARIAS L, MONEO I, DOMINGUEZ J, RODRIGUEZ R: Primary structure of the major collagens of yellow mustard seed. *Europ J Biochem* 177: 159, 1988
45. ANDERSON KIRIHARA J, BERNHARD PETRI J, MESSING J: Isolation and sequence of a gene encoding a methionine-rich 10-kDa zein protein from maize. *Gene* 71: 359–70, 1988
46. WALLACE JC, GALILI G, KAWATA EE, CUELLAR RE, SHOTWELL MA, LARKINS BA: Aggregation of lysine-containing zeins into protein bodies in *xenopus* oocytes. *Science* 240: 662–4, 1988
47. WANDELT C, FEIX G: Sequence of a 21 kd zein gene from maize containing an in-frame stop codon. *Nucleic Acids Res* 17(6): 2354, 1989
48. PEDERSEN K, ARGOS P, NARAVANA SVL, LARKINS BA: Sequence analysis and characterization of a maize gene encoding a high-sulfur zein protein of M_r 15,000. *J Biol Chem* 261(14): 6279–84, 1986
49. COLOT V, BARTELS D, THOMPSON R, FLAVELL R: Molecular characterization of an active wheat LMW glutenin gene and its relation to other wheat and barley prolamin genes. *Mol Gen Genet* 216: 81–90, 1989
50. OKITA TW, CHEESBROUGH V, REEVES CD: Evolution and heterogeneity of the alfa-β-type and alfa-type gliadin DNA sequences. *J Biol Chem* 260(13): 8203–13, 1985
51. HIGUCHI W, FUKAZAWA C: A rice glutelin and a soybean glycinin have evolved from a common ancestral gene. *Gene* 55: 245–53, 1987
52. FUKAZAWA C, MOMMA T, HIGUCHI W, UDAKA K: Complete nucleotide sequence of the gene encoding a glycinin A_2B_{1a} subunit precursor of soybean. *Nucleic Acids Res* 15(19): 1987
53. SIMS TL, GOLDBERG RB: The glycin G_{y1} gene from soybean. *Nucleic Acids Res* 17(11): 4386, 1989
54. THANH VH, TUMER NE, NIELSEN NC: The glycinin G_{y2} gene from soybean. *Nucleic Acids Res* 17(11): 4387, 1989
55. BÄUMLEIN H, WOBUS U, PUSTELL J, KAFATOS FC: The legumin gene family: Structure of a B type gene of *Vicia faba* and a possible legumin gene specific regulatory element. *Nucleic Acids Res* 14(6): 1986
56. MARCH JF, PAPPIN DJC, CASEY R: Isolation and characterization of a minor legumin and its constituent polypeptides from *Pisum sativum* (pea). *Biochem J* 250: 911–5, 1988
57. HAYASHI M, MORI H, NISHIMURA M, AKAZAWA T, HARA-NISHIMURA I: Nucleotide sequence of cloned cDNA coding for pumpkin 11-S globulin β subunit. *Eur J Biochem* 172: 627–32, 1988
58. DOUGLAS FW, GREENBERG R, FARRELL HM,

EDMONDSON LF: Effect of ultra-high-temperature pasteurization on milk proteins. *J Agric and Food Chem* 29: 11–5, 1981

59. MORR CV: Functional properties of milk proteins and their use as food ingredients. In: FOX PF, ed. Developments in dairy chemistry-1. London: Appl Sci Publ. 1982: 375–99
60. DEWIT JN: New approach to the functional characterization of whey proteins for use in food products. In: GALESLOOT TE, TINBERGER BJ, eds. Milk proteins 1984. The Netherlands: Pudoc. Wageningen (Publ.). 1985: 183–4
61. LEHNINGER AL: Principles of biochemistry. New York: Worth. Publ. Inc., 1982: 688
62. ISSELS RD, NAGELE A: Promotion of cystine uptake, increase of glutathione biosynthesis, and modulation of glutathione status by S-2-(3-Aminopropylamino) ethyl phosphorothioic acid (WR-2721) in Chinese hamster cells. *Cancer Res* 49: 2082–6, 1989
63. BROWN RJ: Milk coagulation and protein denaturation. In: WONG NP, ed. Fundamentals of dairy chemistry. 3rd edn. New York: Van Nostrand Reynold Co., 1988: 583–607
64. BIRT DF, BAKER PY, HRUZA DS: Nutritional evaluations of three dietary levels of lactalbumin throughout the lifespan of two generations of Syrian hamsters. *J Nutr* 112: 2151–60, 1982
65. BOUNOUS G, GERVAIS F, AMER V, BATIST G, GOLD P: The influence of dietary whey protein on tissue glutathione and the diseases of aging. *Clin Invest Med* 12: 343–9, 1989
66. BOUNOUS G, PAPENBURG R, KONGSHAVN PAL, GOLD P, FLEISZER D: Dietary whey protein inhibits the development of dimethyl-hydrazine induced malignancy. *Clin Invest Med* 11: 213–7, 1988
67. BOUNOUS G, KONGSHAVN PAL: Influence of protein type in nutritionally adequate diets on the development of immunity. In: FRIEDMAN M, ed. Absorption and utilization of amino acids. Vol. II. Boca Raton, Florida: C.R.C. Press, Inc., 1989: 212–33
68. MEISTER A, ANDERSON ME: Glutathione. *Ann Rev Biochem* 52: 711–60, 1983
69. KAPLOWITZ N, AW TY, OOKTENS M: The regulation of hepatic glutathione. *Ann Rev Pharmacol Toxicol* 25: 715–44, 1985
70. MEGAW JM: Glutathione and ocular photobiology. *Current Eye Research* 3: 83–7, 1984
71. ORRENIUS S, THOR H, BELLOMO G, MOLDEUS P: Glutathione and tissue toxicity. MITCHELL PW, ed. 9th Int Cong Pharmacol. London: McMillan Press, 1984
72. FLOEHE L, GUNZLER WA: Glutathione-dependent enzymatic oxidoreduction reactions. In: ARIAS IM, JAKOBY WB, eds. Glutathione: Metabolism and function. New York: Raven Press, 1976: 17–34
73. DELUCIA AJ, MUSTAFA MG, HUSSAIN MZ, CROSS CE: Ozone interaction with rodent lung. III. Oxidation of reduced glutathione and formation of mixed disulfides between protein and non-protein sulfydryls. *J Clin Invest Med* 55: 794–802, 1975
74. DENEKE S, FANBURG BL: Regulation of cellular glutathione. *Am J Physiol* 257: L163–73, 1989
75. VOS O, ROOS-VERHEY SD: Endogenous *vs* exogenous thiols in radioprotection. *Pharmac Ther* 39: 169–77, 1988
76. DESCHAVANNE PJ, MALAISSE EP: Radiation survival of glutathione-deficient human fibroblasts in culture. *Br J Radiol* 54: 361–2, 1981
77. CLARK EP, EPP ER, BIAGLOW JE: Glutathione depletion, radiosensitization and misonidazole potentiation in hypoxic Chinese hamster ovary cells by buthionine sulfoximine. *Radiat Res* 98: 370–80, 1984
78. FAHEY RC: Protection of DNA by thiols. *Pharmacol Ther* 39: 101–8, 1988
79. BLUMBERG JB, MEYDANI SN: Role of dietary antioxidants in aging. In: HUTCHINSON MG, MUNRO HN, eds. Nutrition and aging. New York: Academic Press, 1986: 85–97
80. HAZELTON GA, LANG CA: Glutathione contents of tissues in the aging mouse. *Biochem J* 188: 25–30, 1980
81. LANG CA, RICHIE JP, CHEN TS: Differential glutathione and cysteine levels in the brain of the aging mouse. Abstr. 8327. *Federation of American Societies for Experimental Biology*: 1988
82. ABRAHAM EC, TAYLOR JF, LANG CA: Influence of mouse age and erythrocyte age on glutathione metabolism. *Biochem J* 174: 819–25, 1978
83. WALKER HD, BIRKE G, TIGGS FJ, BENOHR H: Glutathiongehalt und glutathion reduzierende enzyme in erythrocyten verschiedenen. *Alters Klin Wochensch* 52: 179–84, 1974
84. ROBINSON MF, GODFREY PJ, THOMPSON CD, REA HM, VAN RIJ AM: Blood selenium and glutathione peroxidase activity in normal subjects and surgical patients with and without cancer in New Zealand. *Am J Clin Nutr* 32: 1477–85, 1979

85. COFFMAN FD, COHEN S: Aging and defective lymphoid cell activation. *Exp Geront* 24: 437–49, 1989
86. FURUKAWA T, MEYDANI SN, BLUMBERG JB: Reversal of age-associated decline in immune responsiveness by dietary glutathione supplementation in mice. *Mechanisms of Aging and Development* 38: 107–17, 1987
87. BUHL R, HOLROYD KJ, MASTRANGELI A, CANTIN AM, JAFFE HA, WELLS C, SALTINI C, CRYSTAL RG: Systemic glutathione deficiency in symptom-free HIV-seropositive individuals. *Lancet* (Dec. 2nd): 1294–7, 1989
88. KUZUYA M, NAITO M, FUNAKI C, HAYASHI T, ASAI K, KUZUYA F: Protective role of intracellular glutathione against oxidized low density lipoprotein in cultured endothelial cells. *Bioch Biophys Res Commun* 163: 1466–72, 1989
89. BAKER DH, CZARNECKI-MAULDEN GL: Pharmacologic role of cysteine in ameliorating or exacerbating mineral toxicities. *J Nutr* 117: 1003–10, 1987
90. GARRETT R, BOYCE BF, OREFFO ROC, BONEWALD L: Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone *in vitro* and *in vivo*. *J Clin Invest Med* 85: 632–9, 1990
91. CALVIN HI, MEDVEDOVSKY C, WORGUL BV: Near-total glutathione depletion and age-specific cataracts induced by buthionine sulfoximine in mice. *Science* 28: 553–5, 1986
92. CHIA LS, THOMPSON JE, MOSCORELLO MA: X-ray diffraction evidence for myelin disorder in brain from humans with Alzheimer's disease. *Can Bioch Biophys Acta Ser Biomemb* 775: 308–12, 1984
93. SCHÄFER L, THORLING EB: Lipid peroxidation and antioxidant supplementation in old age. *Scand J Clin Lab Invest* 50: 69–75, 1990
94. JEANDEL C, NICHOLAS MB, DUBOIS F, NABET-BELLEVILLE F, PENIN F, CUNY G: Lipid peroxidation and free radical scavengers in Alzheimer's disease. *Gerontology* 35: 275–82, 1989
95. RIEDERER P, SOFIC E, RAUSCH WD, SCHMIDT B, REYNOLDS GP, JELLINGER K, YOUDIM MBH: Transition metals, ferritin, glutathione, and ascorbic acid in Parkinsonian brains. *J Neurochem* 52: 515–20, 1989
96. WILLIAMSON JM, BOETTCHER B, MEISTER A: Intracellular delivery system that protects against toxicity by promoting glutathione synthesis. *Proc Natl Acad Sci (USA)* 79: 6246–9, 1982
97. PURI RN, MEISTER A: Transport of glutathione as alpha-glutamylcysteinylglycyl ester, into liver and kidney. *Proc Natl Acad Sci (USA)* 80: 5258–60, 1983
98. SUZUKAKE K, VISTICA BP, VISTICA DT: Dechlorination of L-phenylalanine mustard by sensitive and resistant tumor cells and its relationship to intracellular glutathione content. *Biochem Pharm* 32: 165–71, 1983
99. HAMILTON TC, WINKLER MA, LOUIE KG, BATIST G: Augmentation of adriamycin, melphalan, and cisplastin cytotoxicity in drug-resistant and sensitive human ovarian cancer cell lines by BSO mediated GSH depletion. *Biochem Pharm* 34: 2583–6, 1985

Key words: undenatured whey proteins, effect on glutathione

Address reprint requests to: Dr G. Bounous, Room 947, U.S.C., The Montreal General Hospital, 1650 Cedar Avenue, Montreal, Quebec H3G 1A4