



1

Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

Preamble

- Glutathione is a tripeptide made of Cysteine, Glutamic Acid or Glutamine and Glycine. It is synthesized in every cell of the body. The synthesis follows an evolutionary path during which the amino acids are joined together by substrate specific enzymes to synthesize Glutathione. After Glutathione is synthesized, approximately 90 percent is then stored in the cytosol, waiting to be used either inside or outside of the cell as protection from challenges to the immune system in its role as the major and most versatile antioxidant. Glutathione is frequently referred to as “The Master Antioxidant.”
- Physiological Glutathione is defined as the Precursor Method of Glutathione synthesis. The Precursor Method includes these certain amino acids. The Method embraces the same path which follows the step-by-step synthesis by the substrate specific enzymes that has been evolutionarily perfected and adapted only for this purpose. To illustrate the magnitude and specificity of their role, we look at the function of the substrate specific enzymes.
- The first enzyme encountered is gamma-glutamyl cysteine synthetase. This enzyme joins L-cysteine and the L-glutamate source together. That is the sole purpose of this enzyme—the anabolism of two amino acids. The result of this anabolism is a di-peptide called gamma-glutamylcysteine. Thereafter another substrate-specific enzyme called Glutathione synthetase anabolizes the di-peptide gamma-glutamylcysteine plus glycine to form the tri-peptide Glutathione.
- Each of the two substrate specific enzymes described above exist only to perform their one function, the joining together (anabolism) of the two amino acids to which they are assigned. Those two enzymes are only cytosolic. They exist only in the intracellular space. If their critical roles are bypassed by forced importation of the whole Glutathione molecule, then at least two known negative physiological consequences can result: reductive stress and vestigiality of the substrate specific enzymes which are thrown into disuse.
- Certain repetitions will follow for emphasis.



2

Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

Immune Formulation 200® Key Background Data on Glutathione

- Immune Formulation 200® is a proprietary patented formulation based on US RE42,645E: "Nutritional and Therapeutic Compositions and Methods to increase Bodily Glutathione." Immune Formulation 200® is *different* from other products available to consumers today which claim that they *are* Glutathione or claiming to deliver *the benefits* of Glutathione. (Att. 1)
- Glutathione is a tripeptide of L-Cysteine, Glycine, and Glutamic Acid/or/Glutamine as Glutamate. These three amino acids are the required precursors for Glutathione synthesis and replenishment. Over millions of years, these specific amino acids have successfully evolved and been favored by natural selection for Glutathione synthesis.
- Glutathione is manufactured physiologically **only** within each bodily cell. Key principle: Immune Formulation 200® provides these crucial constituents for Glutathione to be manufactured only intracellularly, i.e., *within each and every cell of the body*. • Why is this cellular milieu so important? Because the substrate-specific enzymes necessary for physiological Glutathione synthesis to take place are present only within cells.
- **The intracellular synthesis of Glutathione is regulated by an enzymatically-controlled shutdown mechanism, which monitors the production of Glutathione.** When sufficient Glutathione is made, each cell's enzymatically-controlled shutdown mechanism turns the extra amino acids aside for other functions--making proteins--or elimination. Bypassing the enzymatically regulated shutdown mechanism can open the Glutathione synthesis mechanisms to the danger of vestigiality by throwing the substrate-specific enzymes into disuse. This enzymatically controlled shutdown mechanism is necessary for the regulation, maintenance, and monitoring of Glutathione production. It has also been perfected by natural selection over millions of years. The specific constituents of Immune Formulation 200® do not bypass the regulation and monitoring of the specific enzymatically controlled shutdown mechanism.



3

**Unique Features and Key Advantages of
Immune Formulation 200®
July 24, 2016**

**Key Differences of Immune Formulation 200® from
other Glutathione Dietary Supplements**

- Glutathione is a tripeptide manufactured within each cell. Glutathione is composed of three specific amino acids: L-Cysteine, Glycine, and Glutamic Acid/or/L-Glutamine as Glutamate, all of which have been adapted for their roles by Natural Selection.
- Immune Formulation 200®, the patented proprietary formulation, provides all of the specific amino acid constituents that the body needs to manufacture its own Glutathione *physiologically, plus* the "starter" Selenium (in the form of Selenomethionine) required for Glutathione to work after it is made. These constituents have been favored by natural selection for Glutathione synthesis over millions of years. A more comprehensive discussion of this subject is being addressed in a 500-page book, based on the Patent, and commissioned by John Wiley Publishers. The book is entitled: *Glutathione Synthesis: Unraveling the Pleiotropic Paradox and Its Vital Immune Roles*. (Att. 2).
- The most functional amino acid in Glutathione synthesis is the rate-limiting L-Cysteine. However, solo L-Cysteine is highly oxidizable and too unstable to arrive safely at cells for inclusion into the Glutathione synthesis template. L-Cysteine can become indiscriminately active as a free radical and therefore become somewhat toxic itself, i.e., it has a propensity to jettison its hydrogen indiscriminately, and, without its hydrogen, L-Cysteine, with an open valence, can become an unstable free radical. This free radical can create toxicity problems for DNA, for the genome, and for other cellular mechanisms, etc. Notable researchers have written: "Cysteine is often the limiting [primary] amino acid for Glutathione synthesis, but administration of Cysteine is associated with toxicity."¹ In Immune Formulation 200®, the L-Cysteine is safely secured and stabilized in the disulfide bond of L-Cystine.
- The safe delivery of L-Cysteine to cells for Glutathione synthesis had been a

¹ Meister A, "On the Biochemistry of Glutathione" citing Anderson ME, Meister A (1987) *Methods Enzymol* 143, 313-325 in *Glutathione Centennial*, Editors Naoyuki Taniguchi, Taneaki Higashi, Yukiya Sakamoto, Alton Meister © 1989 Academic Press, Inc. p. 13.



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

4

scientific enigma for many distinguished researchers for many years.²

- Immune Formulation 200® uses L-Cystine with its stable disulfide bond to deliver the highly oxidizable L-Cysteine to cells, rather than solo L-Cysteine itself or a synthetic of L-Cysteine such as NAC. Immune Formulation 200® also uses the safe delivery of L-Methionine to assure sufficient availability of the vital rate-limiting L-Cysteine.
- The body is protected from the oxidizability of L-Cysteine by the mechanism of auto-oxidation, which directs its sulfhydryl moiety into the stable L-Cystine. The principle inherent in auto-oxidation was utilized by Patent RE42,645E for the use of the disulfide bond in the stable L-Cystine, as the safe delivery system of L-Cysteine into the cytosol for its introduction into the Glutathione synthesis chain.
- Although L-Cysteine is the rate-limiting amino acid and controllable *when it is in the Glutathione synthesis chain* (template), it can be an unstable free radical, when it is *alone* outside of cells. Such instability limits its probability of arriving safely at cell walls for incorporation into the Glutathione synthesis chain. L-Cysteine's high oxidizability creates precursor delivery problems for Glutathione replenishment. An advantage of Immune Formulation 200® is that it uses predictably physiological and safe delivery systems for the conveyance of L-Cysteine. It does not use a synthetic compound or a molecular substitute for L-Cysteine to gain entrance into the cytosol. Immune Formulation 200® uses the physiological methods that have been monitored and tested for eons by natural selection.
- The physiologically safe delivery systems utilize four pleiotropic routes in order for the vital L-Cysteine to be supplied and entered into the cytosol for Glutathione synthesis. These diverse pleiotropic physiological routes include:
 - Safe delivery via the disulfide bond.
 - Safe delivery by the L-Cysteine coupling mechanism with the disulfide bond

² Anderson ME, Meister A. "Intracellular delivery of Cysteine." *Methods of Enzymology: Sulfur and Sulfur Amino Acids*. (1987) 143;313-325.



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

- Safe delivery of L-Cysteine via the transsulfuration of L-Methionine.
- Recognition of the safe delivery of L-Cysteine itself throughout the body by the body's natural pleiotropic use of the Glutathione molecule itself via autophagy. Not by some non-physiological mechanism forcing its way into the cell.
- L-Cystine carries two molecules of L-Cysteine. Covering all constituents, in addition to safely carrying L-Cysteines in L-Cystine, Immune Formulation 200® contains the two other Glutathione constituents, Glycine and Glutamine. In addition, Immune Formulation 200® carries Selenium in the form of Selenomethionine--the Selenium as a co-factor necessary to activate Glutathione when it has been synthesized and the L-Methionine in Selenomethionine as another source of L-Cysteine via its transsulfuration into L-Cysteine.
- An important role for Selenium. Immune Formulation 200® contains and utilizes the important cofactor Selenium, which acts as a "starter" that is necessary for Glutathione to function after it has been synthesized. Selenium is necessary to be present in order for Glutathione to work.
- An equation can only proceed as far as the lowest constituent concentration allows it to proceed. Thus, Immune Formulation 200® provides sufficient concentrations of all vital amino acid Precursor constituents, so the Glutathione synthesis equation will not be slowed or come to a halt.
- Using the oxidized form of L-Cysteine (L-Cystine, which is two L-Cysteines "locked" in a disulfide bond) allows two L-Cysteines to be carried safely to cells for recycling. As L-Cystine enters the cell wall, it is decoupled into two L-Cysteines via substrate specific enzymes (such as Thioltransferase) so that the important L-Cysteines can be included in the Glutathione synthesis.
- Using the pleiotropy of the disulfide bond via the oxidized form of L-Cysteine (as L-Cystine) allows L-Cysteine to be stabilized and carried safely into the intracellular space. L-Cystine is then decoupled (becoming two Cysteines) in the reducing milieu of the cytosol by substrate-specific enzymes that act upon and reduce (decouple) the disulfide bond. Thus, the L-Cysteines become available to be utilized into Glutathione synthesis.
- The substrate-specific enzymes in the cytosol that act on and reduce



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

(decouple) the disulfide bond of L-Cystine are:

- “Thioltransferase will catalyze reduction of oxidized thiol groups in proteins as well as in low molecular mass compounds.”^{3 4 5}
- Thioredoxin.
- Oxidoreductase⁶
- In addition, the non-enzymatic pleiotropic activity of intracellular Glutathione itself, can donate a Hydrogen to decouple L-Cystine.
- Two diagrams (Att. 3 and 4) of the equation of the Glutathione synthesis chain (template) are attached. One of these (Att. 3) illustrates the two Glutathione synthesis enzymes 1) glutamyl-cysteine synthetase and 2) Glutathione synthetase, That diagram also highlights the vital position of these evolutionarily successful constituents and the vitally important enzymatic shutdown mechanism. That diagram also illustrates the regulatory protection afforded by Natural Selection of the Glutathione chain from reductive stress and vestigiality. The protection is that the intact Glutathione molecule is physiologically restrained from being imported into the cell. Only the correct constituents of Glutathione should pass through the cell membrane to take their place in the Glutathione synthesis chain. This is why whole Glutathione is physiologically prevented from entering cells, bypassing the Glutathione synthesis chain and its regulatory shutdown mechanism. The hazards of vestigiality and reductive stress can occur when attempts are made to import the intact Glutathione molecule
- Use of L-Cystine 1) carries highly oxidizable L-Cysteines docilely (because it is

³ Mannervik B, Widersten M, Board PG “Glutathione-linked Enzymes in Detoxication Reactions” p. 24, in Glutathione Centennial, Editors Naoyuki Taniguchi, Taneaki Higashi, Yukiya Sakamoto, Alton Meister © 1989 Academic Press, Inc.

⁴ Eriksson SA, Mannervik B, “The Reduction of the L-Cysteine-Glutathione mixed disulfide in rat liver. Involvement of an enzyme catalyzing thiol-disulfide interchange” *FEBS Letters* V 7, No. 2, March 1970; 27-29.

⁵ Askelöf P, Axelsson K, Eriksson S, Mannerik B. “Mechanism of Action of Enzymes catalyzing thiol-disulfide interchange. Thioltransferases rather than Transhydrogenases.” *FEBS Letters* V38, No. 3, March 1974, 263-267.

⁶ Jones et al .



7

Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

without an open valence) to cell walls and 2) while so doing, protects the body from highly oxidizable solo L-Cysteine.

- Natural selection has also favored L-Methionine as another safe delivery system for L-Cysteine via transsulfuration, i.e., “the caterpillar becoming a butterfly.” Immune Formulation 200® has provided this added modality to provide L-Cysteine, in the constituent Selenomethionine.
- Immune Formulation 200®'s disulfide bond of L-Cystine has natural properties that augment safe transport of the rate limiting L-Cysteine into the Glutathione synthesis chain. Each L-Cystine is a stable disulfide bond for two L-Cysteines, plus its coupling capacities. per below
- Coupling on the disulfide bond: additional L-Cysteines can also be carried by the disulfide bond with its coupling mechanism, by which a free L-Cysteine can couple, because the attraction of sulfur to sulfur is strong. The coupling mechanism maintains non-polarity by using a quantum-like electron exchange to carry an extra one or two L-Cysteines safely into the cytosol. Sulfur's self-attraction to L-Cystine's disulfide bond keeps additional solo L-Cysteines in a “non-free-radical state.” Consequently, these one or two extra L-Cysteines may also be delivered to cells for introduction into the Glutathione synthesis chain.
- By the above pleiotropic and diverse supply routes sufficient for the replenishment of the vital precursors required by the Glutathione synthesis templates in 300,000,000,000,000⁷ (trillion) human bodily cells is maintained. By the Glutathione synthesis template being expressed in every one of the 300 trillion bodily cells, Natural Selection is declaring the priority that has been placed on Glutathione Immunity and its roles in species' survival. This ubiquitous propagation of the Glutathione synthesis template to all bodily cells is parallel to the ubiquitous spread of Mitochondria. Whether it be a liver cell, heart cell, reproductive cell, hormone cell, brain cell, etc., the Glutathione synthesis template form holds true.
- The Glutathione template is present even in every erythrocyte (red blood cells) which does not even have Mitochondria!

⁷ J. Michael Bshop, M.D., *How to win the Nobel Prize*. Harvard University Press. © 2003, p. 39.



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

8

- After much activity with the U. S. Patent Trademark Office, the Patent Office issued U.S. RE42,645E in which no ratios confined the Patent Holder's ability to create an almost infinite number of formulations of RE42,645E. At the same time, it curtails and restricts any outsider's ability to infringe using a different ratio.

Taste and Stability of Immune Formulation 200®

- Immune Formulation 200® has a neutral to pleasantly sweet taste without adding sugars or fats to the diet. It can be taken alone or in foods or beverages. The constituent amino acid Glycine is responsible for the pleasantly sweet taste, which is a property of its molecular structure. The stable disulfide bond of L-Cystine is responsible for the neutral taste.
- The Taste Test: Immune Formulation 200® has a neutral to sweet taste. Knowing that taste, one can be assured that the disulfide bond is intact. If the disulfide bond were prematurely decoupled, the highly oxidizable L-Cysteine would cause Immune Formulation 200® to have an unpleasantly sour to bitter taste. L-Cysteines held in the stable, neutral L-Cystine state L-Cysteine's "taste" is only released *intracellularly*, and that does not affect the mouth-taste at all. Immune Formulation 200® reflects the naturally sweet taste of the Glycine constituent.
- Immune Formulation 200® constituents have very low molecular weights: the Formulation can withstand high temperatures⁸, a wide range of pH values, and may be stirred, mixed, shaken and strongly agitated without biodegradation. Thus it may be readily added to many foods and beverages.

Amino Acid	Molecular Weight	Denaturation- Decomposition Temperatures
L-Cystine	240.3	260 C or 500 F
L-Glutamine	146.1	185 C or 365 F
Glycine	75.0	290 C or 554 F
L-Methionine	149.2	281 C or 537.8 F

⁸ Handbook of Chemistry and Physics, CRC Press, 85th Edition, 2004-2005.



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

Bioavailability of Immune Formulation 200®

- Immune Formulation 200® becomes bioavailable as soon as it comes into contact with the epithelial cells of the mouth. Epithelial cells, as do other cells, contain the substrate specific enzymes that bring the Precursor constituents into the cytosol for entry into the Glutathione synthesis chain.

Structure/Function Accepted Claims on Record with FDA

- Structure/function Claims are registered with the U. S. Food & Drug Administration
 - Immune Formulation 200® supports the Immune System
 - Immune Formulation 200® increases bodily Glutathione.
 - Immune Formulation 200® activates the NRF2 gene, which reduces oxyradicals and free radicals and reduces oxidative stress.

Trademarks of The ProlImmune Company

- ProlImmune® is a registered trademark of The ProlImmune Company, L.L.C.
- Immune Formulation 200® is a registered trademark of The ProlImmune Company, L.L.C.
- Physiological Glutathione itself, via its pleiotropic synthesis roles and because of its vital immune support roles, could possibly become the 14th Vitamin in the history of Medicine.

Consumer Availability

- Immune Formulation 200® is available in 100-gram containers (since 2009) through The ProlImmune Company LLC and through its World-Wide Distributors.
- Through The ProlImmune Company, LLC., Immune Formulation 200® is available in bulk form as an ingredient for food and beverage manufacturers who wish to have this outstanding dietary supplement in a product.
- ProlImmune Global, Inc., a separate corporation, has been organized and incorporated to make and market Immune Formulation 200® in convenient, easy-to-carry, consumer friendly packets.



Unique Features and Key Advantages of Immune Formulation 200® July 24, 2016

Positive Consumer History

- During the over six years of marketing Immune Formulation 200® to the public, The ProlImmune Company has never received a customer complaint, and there has never been any safety issue reported to ProlImmune® or to the U.S. Food and Drug Administration or to ProlImmune's Insurance Carrier. To the contrary, we have been complimented over and over, and there are many letters of appreciation in our files.
- Immune Formulation 200® has been Certified for Sport® by the distinguished NSF International® (Att. 5). Immune Formulation 200® was put through over 200 tests for banned substances required to qualify for the Certification for Sport and successfully passed these tests (Att. 6).
- Immune Formulation 200® has been found to increase intracellular Glutathione better than N-acetylcysteine (NAC), the pharmaceutical standard. Before the California medical schools invested \$25,000,000.00 (million) in research, they had to be convinced that Immune Formulation 200® was superior to NAC. Extensive university research findings are available, and a Human Clinical Trial is available at ClinicalTrials.gov. See NCT01251315.

Attachments:

1. See also *Natural Products Insider* (a Virgo Publication) news story about the patent issuance RE42,645E. (1 page).
2. Wiley Letter to Dr. Crum August 9, 2013.
3. Diagram Albert Crum, M.D.
4. Diagram from Patent "Legal Remarks" on Record in US Patent Trademark Office (1 page).
5. Certified for Sport® from NSF International.
6. List of NSF Tests Passed to qualify as Certified for Sport®.

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