Oral Proteolytic Enzyme Therapy

Enzymes are needed for every chemical action and reaction in the body. Our organs, tissues, and cells are all run by metabolic enzymes. Enzymes are essentially proteins which require coenzymes and cofactors (vitamins and minerals) to work. They act as catalysts to change the structure of molecules by splitting them or combining them. Enzymes build new proteins, cells, and tissues, and tear down old cells no longer needed. Without enzymes, life would not exist. More than three thousand different enzymes have been identified in the body and each one has a specific function.

Most metabolic enzymes in the body, the enzymes that regulate everything from pancreas and liver function to the immune system, are proteases, or proteolytic enzymes (PEs). Proteolytic is a generalized phrase for enzymes that specifically facilitate the chemical breakdown of proteins by breaking the bonds between the amino acids that make up those proteins. Proteases are involved in a multitude of physiological reactions from simple digestion of food proteins to highly regulated cascades (e.g. the blood clotting cascade, the complementary immune system, and the apoptosis activation cascade). Most people think of enzymes in relation to digestion. While it is true that a great many enzymes are derived or created from what could be termed “protein digesting enzymes”, digestion is only part of the human body’s enzyme story. Studies done *in vitro* and in mice have shown that proteolytic enzymes are not only essential for digestion and sustaining and maintaining optimal health, but also have immunomodulatory and tumoricidal properties.[1, 2, 3, 4, 5, 6, 7, 8] This article will discuss the history, research, and mechanisms behind proteolytic enzymes therapy in the treatment and prevention of various pathologies, inflammation and cancer.

Proteolytic enzymes occur naturally in all organisms and constitute 1-5% of all genetic content. They are different from other enzymes in the body in that they can adapt to changing needs. For example, the same proteolytic enzyme can meet both digestive and metabolic needs in the body. This is the reason that you will see some of the same proteolytic enzymes in both digestive enzyme formulas and systemic metabolic formulas.

Biochemically speaking, just about everything that causes illness is either a protein or is protected by a protein - and is therefore subject to control by proteolytic enzymes. For example:

- DNA stores the code for all the body's proteins and enzymes and is a protein manufacturing plant. Genetic diseases are the result of DNA no longer producing those proteins and enzymes accurately or doing it insufficiently or excessively.
- Bacteria, viruses, yeasts, and fungi are all protected by proteins. Attacking those proteins is key to destroying the invaders.
- Food allergens are almost all proteins.
- Cancer cells are protected by proteins.

Proteolytic enzymes can digest and destroy the protein-based defense shield of each pathogen, allergen, and rogue cell, thereby leading to their ultimate elimination. In addition, established cancers reprogram the production of enzymes in the body to both accelerate their own growth and protect themselves from the immune system. Supplemental proteolytic enzymes can alter that dynamic.

Most PEs, such as chymotrypsin and trypsin, are generated by the pancreas, and so they are known as pancreatic enzymes. When food enters the stomach, pancreatic juices containing enzymes, together with bile from the gall bladder, are released into the duodenum. Pancreatic enzymes help break down fats, proteins and carbohydrates. A normally functioning pancreas can secrete one liter of pancreatic juice into the duodenum daily. This fluid contains not only pancreatic enzymes to help with digestion, but also bicarbonate to neutralize stomach acid as it enters the small intestine. In degenerative illness, like cancer, the pancreas is weakened and becomes deficient in its function of releasing enzymes and bicarbonate.

Additionally, processing and cooking food destroys enzymes that were originally in the food. In fact, any sustained heat of approximately 118°-129°F destroys virtually all enzymes. The result is that much of the cooked food we eat enters our stomach seriously devoid of enzymes. This, together with a deficiency of pancreatic enzymes, results in overall enzyme depletion. Such problems can be solved with different types of oral enzyme therapy. Many people benefit from the oral intake of digestive enzymes that include proteases for protein digestion, amylase for carbohydrate, and lipase for fat

digestion. For situations such as chronic or acute inflammation, vascular disease, or malignant illness, systemic oral proteolytic enzyme therapy is therapeutic.

Pancreatic proteases, or proteolytic enzymes, have long been used in medicine both diagnostically and therapeutically for acute and chronic inflammatory conditions, circulatory disturbances, pancreatic disease, and in cancer metastases. Proteases represent a diverse array of enzymes that act on the peptide bonds within proteins. They can be divided into six discrete protein families that differ with respect to structure and catalytic type. Hence, there are six classifications or groups of proteases in the human body:

- Serine protease
- Threonine protease
- Cysteine protease
- Aspartate protease
- Glutamic acid protease
- Metalloproteases

Most of the proteases discussed in this article are serine proteases. Serine proteases are enzymes that break peptide bonds in those proteins in which the amino acid serine plays a key role at the enzyme's active site. In humans, serine proteases are responsible for coordinating various physiological functions including digestion, immune response, blood coagulation, inflammation, and reproduction. Equally important, serine proteases are widely distributed in nature and found in all kingdoms of cellular life as well as many viral genomes. The ability to break down serine protein bonds in invading viruses carries some obvious advantages when it comes to defending your body. The two proteases commonly used in cancer treatment are trypsin and chymotrypsin, which are grouped into the serine protease family.

The two exceptions that commonly figure in supplemental enzyme formulas are bromelain and papain, which are cysteine proteases. In humans, cysteine proteases are responsible for cell aging and cell death, and certain immune responses. Thus, enzymes that regulate and enhance those reactions provide a perfect complement in any systemic, proteolytic enzyme formula. In addition, cysteine proteases play a role in bringing macrophages back into line when they are misprogrammed and attacking

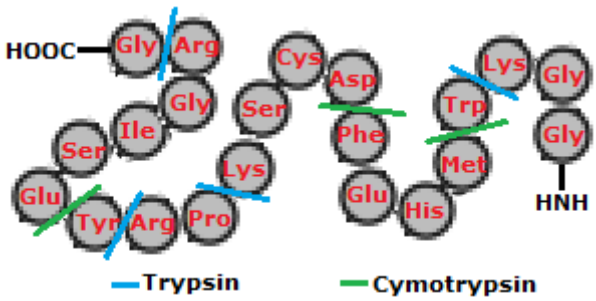
collagen and elastin at sites of inflammation such as arterial walls in atherosclerosis and lung tissue in emphysema.

Trypsin is a protein-digesting enzyme present in pancreatic juices that is secreted into the small intestine during a meal. The pancreas secretes trypsin as an inactive proenzyme called trypsinogen. Once in the intestine, an enzyme called entero-peptidase, which is secreted from intestinal cells, cuts off a small piece of trypsinogen to produce the active trypsin enzyme. Trypsin acts to hydrolyze peptides into their smaller building blocks, namely amino acids. Peptides are the result of the enzyme pepsins breaking down the proteins in the stomach. This enables the uptake of protein in the food because peptides, though smaller than proteins, are too big to be absorbed through the lining of the ileum. Trypsin catalyzes the hydrolysis of peptide bonds at arginine and lysine linkages, and activates other enzymes secreted as proenzymes with pancreatic juice. Therefore, trypsin is essential for normal function of digestive processes that convert food proteins into amino acids for absorption.

Chymotrypsin is a protease like trypsin, whose synthesis occurs primarily in the pancreas. Instead of the active form, however, it is produced as an inactive zymogen called chymotrypsinogen to prevent its protease activity from digesting the pancreas. Upon secretion into the lumen of the small intestine, chymotrypsinogen is converted to its active form by trypsin. This dependence on a different enzyme to activate a protease is a common way for the body to prevent the digestion of organs and other harmful enzymatic side effects. Chymotrypsin hydrolyzes peptide bonds at carboxyl groups and liberates the amino acids L-tyrosine, L-tryptophan, and L-phenylalanine as well as other molecules including several synthetic esters and amides. Not only do these proteases assist in digesting proteins, but they are also absorbed into the bloodstream where they act to reduce inflammation and assist in breaking down diseased and cancerous cells.

Both trypsin and chymotrypsin cut from the c-terminal of the protein. In a protein, each amino acid residue contains two sides: the n-terminal and the c-terminal. The n-terminal refers to the nitrogen-terminal, or the side of the amino acid that has the nitrogen. The c-terminal refers to the carbon-terminal, or the side of the amino acid that has the carbon. So, for example, chymotrypsin will only cut the protein on the side of phenylalanine that has the carbon (not on the side with the nitrogen).

In a single protein there will be several amino acid residues for each enzyme to act on. But each amino acid is only going to select for their specific amino acids and cut the protein in that position:



Trypsin and chymotrypsin each cleave the protein at different points.

Proteolytic Enzymes and Inflammation

Inflammation is a natural response of the body to injury and a secondary detoxification mechanism. However, prolonged inflammation retards the healing process. Proteolytic enzymes support and accelerate the natural inflammatory process without letting it get out of control. They affect the entire inflammatory process while maintaining the endogenous defense and repair mechanisms. Proteolytic enzymes neutralize the biochemicals of inflammation (bradykinins and pro-inflammatory eicosanoids) to levels where the synthesis, repair and regeneration of injured tissues can take place. Reducing inflammation can have immediate impact on improved organ function, cancer prevention and recovery, and degenerative disease prevention. It also helps speed up recovery from sprains, strains, fractures, bruises, contusions, and surgery.

One of the main functions of the proteolytic enzymes is to break down and destroy harmful proteins known as circulating immune complexes (CICs). CICs are protein chains created by the immune system in response to irritation that forms between antibodies produced by the immune system and antigens. CICs are also associated with autoimmune diseases, such as systemic lupus erythematosus, immune complex glomerulonephritis, rheumatoid arthritis and vasculitis. These immune complexes build a “wall” of fibrin around traumatized areas, creating a barrier for healing nutrients. Proteolytic enzymes increase the breakdown of the fibrin that blocks blood and lymph vessels which cause swelling, and dissolve the CICs and antibodies that cause inflammation. Inflammatory responses can be resolved before irreversible damage to internal organs or tissue occurs.

Proteolytic enzymes are superior to conventional pharmacological therapies because, unlike non-steroidal anti-inflammatories (NSAIDs) and cortico-steroid therapies, enzymes do not suppress natural immune responses. NSAIDs cause prostaglandin biosynthesis inhibition and hence, are associated with numerous side effects. For example, the cyclooxygenase (COX)–1 enzyme is responsible for the production of prostaglandins and thromboxane, which are involved in routine physiological functions such as gastrointestinal protection, platelet aggregation and renal blood flow. COX-2 is induced by inflammatory mediators, local injury and cytokines. The prostaglandins produced by COX-2 contribute to pain and fever, but are also important in regulating renal function, tissue repair and reproduction. Hence, both COX-1 and COX-2 enzymes play an important role in homeostasis: prostacyclin (prostaglandin I2) is produced via COX-2 of endothelial cells and has antithrombotic effects, and thromboxane is produced by COX-1 found in platelets and is prothrombotic. Unlike NSAIDs, proteolytic enzymes do not inhibit prostaglandin biosynthesis. By breaking down and destroying CICs, proteolytic enzymes cleanse the blood of these harmful proteins and clear the way for the body's natural repair and healing processes to take over.

History and Research of Proteolytic Enzymes and Cancer

This brief history reveals the shameful politics that have impeded the medical acceptance of proteolytic enzymes used as an ancillary cancer treatment. Though proteolytic enzymes have become globally accepted as anti-inflammatory, anti-edematous and analgesic, their use in cancer as immunomodulatory and tumoricidal has been a battlefield of controversy. Most chemotherapeutic agents currently used in the clinical management of cancer interfere with fundamental cellular mechanisms, which result in cytotoxicity to healthy cells. Oral proteolytic enzymes are not cytotoxic, nor are they expensive. Consequently, pharmaceutical companies cannot generate money marketing them (unlike patentable products, such as cytotoxic chemotherapy).

History reveals their proven benefits in disease and cancer treatment. In the Bible, second Book of Kings, chapter 20, verse 7, a poultice of figs was laid on Hezekiah's boil - and he recovered. Figs contain the proteolytic enzyme ficin which reduces inflammation and promotes healing. Many plants and their fruits contain proteolytic enzymes that have been used historically as traditional medicine by many cultures. For example, pineapples contain bromelain, papayas contain papain, and kiwi contains actinidin, all of which are good food sources of proteolytic enzymes.

In 1906, Dr. John Beard, Scottish embryologist and professor at the University of Edinburgh, proposed that the pancreatic proteolytic enzyme trypsin represents the body's primary defense against cancer and would be useful as a cancer treatment. Beard used injectable trypsin to successfully treat tumors in mice.[9] During the first two decades of the twentieth century, Beard's research[10] attracted some attention in academic circles, and several case reports in the medical literature documented tumor regression and even remission in terminal cancer patients treated with proteolytic enzymes. Beard was also the first to report that trophoblast cells act and behave in a manner identical to cancer cells, acting invasively, and inducing their own blood supply.

The trophoblast "theory" of cancer will not be covered here, but this important information may be accessed from the book by Drs. Nicholas Gonzalez and Linda Isaacs, *The Trophoblast and the Origins of Cancer: One Solution to the Medical Enigma of Our Times*. In this book, Dr. Gonzalez and Dr. Isaacs provide a comprehensive review of Dr. John Beard's 100-year-old theories about cancer, from the perspective of contemporary molecular biology. The authors explain how Dr. Beard most likely discovered stem cells, and very well may have uncovered the root origins of cancer and its effective treatment with proteolytic enzymes.

At that time, Beard assumed the proteolytic enzymes had to be injected to prevent their destruction by hydrochloric acid in the stomach. However, research demonstrates that orally-ingested proteolytic enzymes are acid stable[11], pass intact into the small intestine, and are absorbed through the intestinal mucosa into the bloodstream as part of an enteropancreatic recycling process.[12, 13] The problem Beard and others faced in their research was reproducibility due to the chemical instability of injectable proteolytic enzymes.

After Beard's death in 1923, proteolytic enzyme cancer therapy was largely forgotten in the U.S., but in Europe, a few physicians continued to utilize proteolytic enzymes in metabolic cancer treatment programs. Dr. Max Wolf of Austria followed Beard's work and continued to research how to stabilize enzymes, so they maintained their potency. Wolf found that people who have cancer tend to be deficient in proteolytic enzymes, and that enzyme production diminishes in humans with age. While people have historically supplemented their enzyme levels by consuming fresh, raw foods, modern preservation and heat preparation techniques destroy many of the enzymes in foods. Combined, these conditions can create an enzyme deficiency that results in poor digestion and metabolic dysfunction. Dr. Wolf, together with Dr. Helen Benitez, decided to conduct research to discover whether PEs manufactured by the human body were like those

that occurred in other plants and animals. They identified and isolated several enzymes from natural sources and used these enzymes in cancer treatment.[14] Wolf and Benitez labeled their enzyme preparations Wobenzym ("WOBE" derived from their surnames).[15] These oral enzyme formulations are world renowned and still marketed today. In the years that followed, hundreds of doctors throughout Europe began experimenting with proteolytic enzymes as a therapy for cancer. However, in the U.S., it took years for the NCI to consider conducting a study of proteolytic enzymes as a cancer treatment.

Another notable medical pioneer of the 70s and early 80s to advocate proteolytic enzymes for cancer was William Donald Kelley, D.D.S. Kelley had been a successful orthodontist with a keen interest in nutrition, practicing in Grapevine, Texas. In the early 1960s, while he was in his mid-30s, he became severely ill. His doctors diagnosed him with advanced pancreatic cancer, though this diagnosis was never confirmed with a biopsy. Kelley self-treated his condition using a nutritional program that included an organic, largely vegetarian, raw foods diet, a variety of food supplements, and coffee enemas. He also added high doses of oral pancreatic enzymes to his regimen, not because of any familiarity with Beard's ideas, but to help relieve his severe digestive distress, as often occurs in patients with pancreatic malignancy. Kelley soon became well and started using his protocol to treat others with cancer. In 1969, Kelley published his book *One Answer to Cancer*, which may be freely accessed from the internet.[16]

Kelley's cancer treatment utilized a very comprehensive metabolic program involving specific diets individualized to match the metabolic character of each patient, considering their physiology, basic metabolic rate, and psychoemotional condition. Proteolytic enzymes, vitamins, minerals, and a rigid detoxification protocol were all part of Kelley's cancer program. Kelley's clinic in Mexico treated hundreds of cancer patients, many of whom were terminal. His success became renowned, and for better or worse, he secured his position as a preeminent alternative cancer practitioner. This notoriety inevitably resulted in Kelley becoming a target for mainstream medical and cancer societies that had little interest in nutritional cancer approaches.

The actor Steve McQueen, who had metastatic mesothelioma, heard of Kelley's cancer program and traveled to Mexico to undergo treatment. McQueen had pleural mesothelioma, a cancer associated with asbestos exposure for which conventional medicine has no known cure. McQueen developed a chronic cough and his conventional doctors misdiagnosed him for a year while giving him antibiotics. His condition worsened; when finally, he was correctly diagnosed with metastatic mesothelioma, his oncologists administered radiation and immune therapy.

Unfortunately, conventional treatments are not effective for advanced metastatic mesothelioma. McQueen was terminal and dying when he came to Kelley's clinic. Kelley, the compassionate and gracious man that he was, agreed to treat him, but there was an enormous risk involved in taking on such a high-profile terminal case. After leaving Kelley's clinic, McQueen still lived several months with his terrible mesothelioma. He developed a large abdominal tumor on his liver, and was advised to undergo surgery. McQueen died from complications after the surgery, and even though he had been pronounced terminal by his oncologists, many media sources and conventional cancer societies blamed Kelley for McQueen's death. Kelley's reputation suffered greatly from the bad publicity. In 1971, the American Cancer Society added Kelley's regimen to its long list of "unproven therapies".

Oncologist Dr. Nicholas Gonzalez had a private practice in New York City from 1987 until his death in 2015, treating patients with cancer and other serious degenerative illnesses. Beginning in 1980 as a medical student, Dr. Gonzalez reviewed more than 1,000 medical charts from Donald Kelley. Gonzalez' successful cancer treatment approaches were in part based on the previous research of both Dr. Beard and Dr. Kelley. Dr. Gonzalez' cancer regimen focused on the use of high dose PEs, but also included rigorous dietary protocols, nutritional supplements, and coffee enemas that can be found in the earlier work of Dr. Max Gerson.

The Gonzalez protocol, briefly described, consists of six basic components:

1. Metabolic diet – Gonzalez used 10 basic diets with 94 variations ranging from strict vegetarian to red meat depending on the cancer and the patient.

2. Intensive nutritional support – various vitamins, minerals, trace elements, essential fatty acids and amino acids are prescribed - depending on each patient's nutritional status.

3. Protomorphogen support - these are concentrates, in pill form, of specific bovine organs and glands. The word is derived from the Greek "*proto*" (meaning primary) and "*morphogen*" (meaning form or structure of the initial element).
Protomorphogen products are composed of nuclear proteins and used to supply a better RNA/DNA template so that the body can assemble available nutrients to repair damaged tissue in the glands being treated. What differentiates protomorphogens from glandulars and cytosol extracts is the patented extraction process of the company Standard Process Inc.

4. Digestive aids, such as digestive enzymes, hydrochloric acid, and probiotics.

5. Pancreatic enzyme therapy – high dose proteolytic pancreatic enzymes are taken orally between meals.

6. Detoxification – among the many regimens used is the coffee enema. The purpose is to help the body eliminate the unnatural abundance of toxins and waste products as tumors break down in the body. Coffee enemas have also been found by many patients to alleviate pain.

Dr. Gonzalez authored three books: *What Went Wrong: The Truth Behind the Clinical Trial of the Enzyme Treatment of Cancer*, a William Kelley history entitled *One Man Alone: An Investigation of Nutrition, Cancer, and William Donald Kelley*, and *The Trophoblast and the Origins of Cancer: One Solution to the Medical Enigma of Our Times*.[17]

Due to Dr. Gonzalez' reported successes treating pancreatic cancer in 1993, the NCI invited him to conduct a pilot study of PEs in inoperable adenocarcinoma of the pancreas. According to the American Cancer Society, for all stages of pancreatic cancer combined, the one-year relative survival rate is 20%, and the five-year rate is 7%. The study included 11 patients diagnosed with adenocarcinoma of the pancreas (stage II through stage IV). None of the patients had received chemotherapy or radiation therapy, and none had undergone surgical resection with curative intent. All of the patients had pancreatic tumors that were either unresected or partially resected. Survival from the time of diagnosis was the only study endpoint, and all 11 patients were included in this survival analysis. Of the participants in the trial, 82% lived longer than one year, 45% lived two years, and 4 patients (36%) lived over three years. When the results of the study were released, two patients were alive: one who had survived 3 years, and one who had survived 4 years. The researchers concluded that one- and two-year survival percentages for this group of patients were superior to those observed for other U.S. patients diagnosed with adenocarcinoma of the pancreas.[18] In spite of the study's positive outcome, cancer societies turned a deaf ear and empty pocket to Dr. Gonzalez' work and PE therapy. However, Dr. Gonzalez' research did inspire other researchers to further investigate proteolytic enzymes.[19, 20]

In 2001, a study was designed to evaluate the impact of postoperative treatment with an oral enzyme preparation given complementary to an antineoplastic therapy in patients

with breast cancer.[21] The design of this epidemiological study was a retrospective cohort analysis with parallel groups. The researchers concluded:

> Complementary treatment of breast cancer patients with oral enzymes improves the quality of life by reducing signs and symptoms of the disease and the side effects of adjuvant antineoplastic therapies. This epidemiological retrospective cohort analysis provides evidence that the patients may also gain benefit by a prolongation of the time to event for cancer recurrence, metastasis and survival. Oral enzymes were generally well tolerated.

A 2005 study investigating purified pancreatic enzymes, trypsinogen and trypsin, chymotrypsinogen and chymotrypsin, and amylase showed that the mixture of these enzymatic activities produces potent anti-metastatic and antitumor effects in cellular, animal and human systems. The researchers concluded:

> These findings support the conclusion that proteolysis is the active mechanism of the proenzyme treatment. Future studies will focus on the molecular mechanisms of the proenzyme therapy including the identification of molecular target(s) on the tumor cells. In conclusion, we have discovered that proenzyme therapy, mandated first by John Beard nearly one hundred years ago, shows remarkable selective effects that result in growth inhibition of tumor cells with metastatic potential.[22]

This study showed that this mixture of proteases and amylase produces potent anti-metastatic and antitumor effects. This is in part because the outer coating of a cancer cell contains human chorionic gonadotropin (hCG). The enzyme amylase breaks down the hCG so that the proteases can then break down the protein coat of the cancer cells. Hence, some physicians use amylase as percussive enzyme activity before employing the proteolytic enzymes. Proteases, like carboxypeptidase, trypsin and chymotrypsin, are unable to reach the protein until amylase breaks down the hCG. This is the rationale for using amylase in abundance alone or in combination.

In 2010, another NCI-funded study emerged that compared pancreatic proteolytic enzyme therapy with gemcitabine-based chemotherapy for pancreatic cancer treatment.[23] The outcome was less than positive for PE therapy. The researchers concluded, “Among patients who have pancreatic cancer, those who chose gemcitabine-based chemotherapy survived more than three times as long and had better quality of life than those who chose proteolytic enzyme treatment.” John Chabot of Columbia, who helped

develop the gemcitabine-based chemotherapy regimen used in the study, was chosen as the principle investigator. This was a clear conflict of interest that should have precluded him from serving as principle investigator. In any research study, the chief investigator should be free of any financial attachment to the treatment being studied. Besides this, there were several other methodology flaws that were later described by the Office of Human Research Protections, the NIH agency in charge of investigating mismanagement on government funded studies.[24] Dr. Gonzalez also wrote a rebuttal to the study in the *Journal of Clinical Oncology*.[25] In spite of the study's questionable methodology, this study was, and still is, used as a case against proteolytic enzyme cancer therapy.

Research of proteolytic enzymes as a cancer treatment continues, mostly *in vitro* and in animal studies. Over the last 60 years, we have learned much about effective protocols of oral enzyme combinations and optimum dosage from these pioneering doctors.

Proteolytic Enzymes Anticarcinogenic Mechanisms

Proteolytic enzymes (serine endopeptidases such as trypsin or chymotrypsin and cysteine endoproteinases such as bromelain and papain or combinations of those enzymes) have long been available for diverse medical indications. However, their mechanisms of action, for example, in complementary oncology are not yet fully understood. There are a variety of mechanisms by which they are thought to contribute to efficacy.

PEs have been shown to work against tumors and cancerous terrains through several mechanisms. For over a century, a volume of data has accumulated establishing that the blood protein fibrin facilitates the persistence and progression of cancerous cells.[26, 27, 28, 29] Basically, fibrin deposition on and around cancerous cells during their migration in the blood protects them from elimination by natural killer (NK) or other cytotoxic cells. This layer of fibrin may be up to 15 times thicker than the layer of fibrin that surrounds normal cells. The cancer cells with their fibrin coating can adhere to tissues where they congregate and multiply. This protein coat hides cancer cells like camouflage, including their antigens, from the body's cellular immunity.

One important feature of proteolytic enzymes is their ability to break down the protein fibrin coating around cancer cells.[30] Normally, with a healthy immune system, the tumor and cancer cells are destroyed by natural killer cells, macrophages, lymphocytes, and

Tumor Necrosis Factor (TNF). But these immune cells must first recognize the cancer cell antigens. TNF normally binds to receptors on the cancer cell wall, which then allows it to destroy the cancer cell. But cancer cells are capable of releasing decoy antigens that bind to TNF, so that TNF cannot bind to the real cancer cell surface antigen and exert its necrosis effect. These decoy antigens, together with the fibrin layer, permit malignant cells to escape destruction by immune cells. Thus, tumor cells protect themselves from being recognized by antibodies and from the attack by cells of the immune system by shedding soluble surface antigens. Proteolytic enzymes break up these decoy antigens and free the TNF to bind to the real cancer surface antigens on their cell wall. Proteolytic enzymes also effectively break down and dissolve the fibrin protein-based defense shield of these cancerous cells without harming normal cellular activity. Additionally, proteolytic enzymes break down and neutralize other reactive, toxic proteins in the blood and lymphatic circulation. This allows for improved cellular oxygenation and allows the blood and lymph to flow more freely. However, to achieve this, there needs to be an abundant quantity of proteolytic enzymes in the bloodstream.

In addition to breaking down fibrin and destroying decoy antigens, proteolytic enzymes are also thought to exert immunomodulatory effects by causing increased release of reactive oxygen species by polymorphonuclear leukocytes[31], and by production of tumor necrosis factor-alpha and interleukins IL-6 and IL-1B that cause cytotoxic effects.[32, 33, 34] Yet, another more complicated way in which PEs attack cancer cells involves their ability to inactivate Transforming Growth Factor Beta 1 (TGF-β1). TGF-β1 is a polypeptide member of the transforming growth factor beta superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis. In normal cells, TGF-β stops the cell cycle at the G1 stage to stop proliferation, induce differentiation, or promote apoptosis. However, in many cancer cells, parts of the TGF-β signaling pathway are mutated, and TGF-β no longer controls the cell. Hence, these cancer cells proliferate and cause immunosuppression and angiogenesis, which makes the cancer more invasive.[35] It has been demonstrated that proteolytic enzymes reduce TGF-beta levels in serum by converting the protease inhibitor alpha2 macroglobulin (alpha2M) from the "slow" form into the "fast" form, whereby the "fast" form binds and inactivates TGF-beta irreversibly.[36]

Proteolytic enzymes exert anti-inflammatory responses through a variety of mechanisms. They reduce swelling of mucous membranes, decrease capillary permeability, and dissolve blood clot-forming fibrin deposits and microthrombi. Scientists believe that the tumor microenvironment is in part created by inflammatory cells that

foster proliferation, survival and migration of the tumor.[37] Proteolytic enzymes, because of their anti-inflammatory effect, help improve a corrupted, toxic tumor microenvironment.

Oral proteolytic enzyme therapy is a safe and effective ancillary cancer treatment that works by inducing cancer cell death (apoptosis), by breaking down the fibrin coating around the tumor, by eliminating reactive toxic proteins, and by stimulating several mechanisms of the immune system to fight cancer. To effectively treat cancer, it is necessary to break down the tumor's defense, then to change the internal environment which supports cancer's growth, while strengthening the cellular immunity to detect and destroy any mutated cells. Oral PE therapy helps facilitate all these necessary conditions.

Oral Proteolytic Enzyme Sources

Sources of proteolytic enzymes include animals, plants, insects, bacteria and fungi. However, the most researched and clinically-utilized source in tumor management has been primarily animal-based enzymes. Animal-based proteolytic enzymes come from the pancreas of pigs and sometimes cows. These glands are defatted and dried, yielding a raw pancreatin mixture that includes trypsin, chymotrypsin and other peptidases. Further refinement yields a trypsin-chymotrypsin blend with significantly more trypsin. When animal-based enzymes are enterically coated, they can resist stomach acidity, thus yielding higher serum levels. Some formulas also include the enzyme amylase that assists the proteases in breaking down the protein coat of the cancer cells.

Enzyme formulas are ever improving with clinical experience and continued research. Many physicians now believe a properly designed supplemental proteolytic enzyme formula usually contains the following:

- First, a lot of protease - at least 200,000 HUT per dose. This is far more than found in a digestive formula. 300,000 HUT is even better. For some individuals, plant-based enzymes are ideal since they are both tolerant of stomach acid and work in a much wider range of pH environments.

- Secondly, it should contain a good variety of enzymes which utilize different biological pathways and whose benefits complement, rather than duplicate, each

other. Wobenzym N (formula below) is an example of a formula with a variety of enzymes.

- Third, better formulas often contain some non-proteolytic enzyme ingredients, both because they increase the efficacy of the proteolytic enzymes in the formula and because they provide their own complementary benefits as well. They may contain amylase, lipase, curcumin (turmeric) or ginger.

- Lastly, the trypsin to chymotrypsin ratio (t/c ratio) should ideally be greater than 5 to 1.

Calculating Enzyme Quantity

When comparing enzyme formulas, it is necessary to compare activity levels, not milligrams. When choosing an enzyme formula, the weight (usually listed in mg) means nothing when comparing the activity units of the enzymes. Quality enzyme products will use the internationally recognized and accepted standard for measurement by FCC (Food Chemicals Codex) Units. These are usually expressed in different activity units for each type of enzyme. Here is a table of several enzymes with approximate conversion factors:

- Protease – HUT (Hemoglobin Units, Tyrosine basis); USP (United States Pharmacopeia) 1 HUT approximately = 6.5 USP
- Amylase – SKB (named after the creators of the test Sandstedt, Kneen, and Blish) or DU (used in the brewing industry). SKB and DU match up one to one. However, 1 DU approximately = 48 USP
- Lipase – FIP. Formerly measured in LU (Lipase Units), but now more commonly measured in FIP units (i.e., according to the test methods of the Fédération Internationale Pharmaceutique). On a unit to unit basis, 1FIP approximately = 2.5 LU/FCCLU; No conversion available to USP
- Cellulase – CU (Cellulase unit)
- Invertase – IAU (Invertase Activity unit)
- Lactase – LacU (Lactase unit)
- Maltase – DP (degrees Diastatic power)

- Nattokinase – FU (Fibrinolytic Units) refers to nattokinase's ability to break down the blood clotting enzyme, fibrin.
- Serrapeptase – SPC (Serrapeptase Units)
- Papain – MCU (Milk Clotting Units). Based on how fast the enzyme digests milk protein. Sometimes listed as PU (Papain Units), which are equivalent to 0.1 MCU; may be listed as FIP
- Bromelain – GDU (Gelatin Digesting Units). Based on how fast the enzyme digests gelatin. 1 MCU is equivalent to 0.67 GDU.

One of most popular proteolytic enzyme formulations in the world is Wobenzym N manufactured by Mucos Pharma in Germany.[38] It is a mixture of animal- and plant-derived enzymes. Wobenzym N formulation (3 enteric-coated tablets) contains:

- Pancreatin 56,000 USP units protease (pancreas) *Sus scrofa* 300 mg
- Papain 492 FIP unit *Carica papaya* 180 mg
- Bromelain 675 FIP unit *Ananus comosus* 135 mg
- Trypsin 2160 FIP unit (pancreas) *Sus scrofa* 72 mg
- Chymotrypsin 900 FIP unit (pancreas) *Bos taurus* 3 mg
- Rutoside trihydrate (Rutin) *Sophora japonica* 150 mg

(Notice that the trypsin to chymotrypsin ratio of this product is 24:1.)

Proteolytic Enzyme Absorption

Orally ingested pancreatic enzymes survive the hydrochloric acid in the stomach, the alkaline environment of the duodenum, and can then pass into the systemic circulation, with little loss along the way. Enzyme molecules are absorbed by the intestines using special transport mechanisms. Interestingly, evidence now suggests that oral enzymes can be absorbed and re-secreted by the pancreas, in a manner similar to the liver's recycling of bile salts and hormones.[39, 40, 41] This active performance by the intestinal cells is highly dependent on individual factors, and on the actual conditions in the intestines of the individual. It is important to recognize that cytotoxic chemotherapy, like antibiotics, greatly disrupts the intestinal mucus membrane, destroys much of the beneficial gut bacteria, and impairs the digestive process. It is usually necessary to include probiotic cultures together with oral enzyme therapy. Though this article has been mostly about oral proteolytic enzymes, it should be noted that regular

supplementation with other digestive enzymes (amylase, lipase) taken with food improves digestion and reduces the metabolic stress on the pancreas and liver.

Specific Proteolytic Enzymes and their Properties

Bromelain is another cysteine protease enzyme derived from the stems of pineapples, although it exists in all parts of the fresh plant and fruit. The extract has a history of traditional use and has a wide range of health applications. *In vitro* and some *in vivo* studies demonstrate its anti-inflammatory properties.[42,43] Bromelain reduces serum fibrinogen levels, supports fibrinolysis and has been investigated for its debriding effects on burn wounds.[44] In addition, it may be useful for treating some skin conditions.[45] Bromelain reduces mild, acute knee pain in a dose-dependent fashion.[46] It was also shown to reduce post-operative pain and swelling, and improve quality of life.[47] Studies of bromelain's pain-relieving effect on patients with arthritis yielded mix results.[48, 49, 50]

Studies done *in vitro* and in mice have shown that bromelain has chemopreventive[51, 52] and antitumorigenic effects.[53, 54] Bromelain and other proteolytic enzymes were used as adjuvants in cancer treatments.[55, 56, 57] It also increased the survival indices of animals bearing leukemia, sarcoma, lung, breast, and ascetic tumors.[58] However, the anticancer effects of bromelain has not been thoroughly evaluated in clinical trials.

Allergic reactions may occur in individuals who are sensitive or allergic to pineapples or who may have other allergies. Theoretically, bromelain may enhance anticoagulation therapy and thereby increase bleeding risk due to its antithrombotic effects. Bromelain also inhibits cytochrome P450 (CYP) 2C9 activity.[59]

Papain is another cysteine protease enzyme that is found in the leaves, latex, roots, and fruit of the papaya plant (*Carica papaya*), that has enzymatic properties like bromelain. It is well known for its use as a meat tenderizer, since, as a natural proteolytic enzyme, it breaks down the proteins in meat. Papain is used as a digestive enzyme, as well as proteolytic enzyme formulations. Like other proteolytic enzymes, if papain is taken between meals, much of it enters the bloodstream where it helps reduce inflammation, as well as fluid retention following trauma and surgery.[60, 61, 62] In fact, studies have shown that papain possesses strongly marked anti-inflammatory activity, and this ability is no less than that of the pharmaceutical drugs, butadion and indomethacin.[63] Preliminary research shows papain holds promise as an adjuvant enzyme in proteolytic enzyme formulations in the treatment of tumors.[64, 65]

Nattokinase is a serine proteinase that is extracted and purified from a traditional Japanese food called natto. This vegetable cheese-like food is extremely popular in Japan, with a history extending back over 1,000 years. Natto is made from boiled or steamed soybeans fermented with the beneficial bacteria, *Bacillus subtilis*.[66, 67] *In vitro* and *in vivo* studies have consistently demonstrated the potent pro-fibrinolytic effect of the enzyme.[68, 69, 70]

Blood clots (fibrin) are formed from fibrinogen by thrombin and are lysed (broken down) by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). Although fibrin clot formation and fibrinolysis are maintained in balance by the biological system, thromboses, such as myocardial infarction, occur when clots are not lysed as a result of a disorder of the balance. Nattokinase has been reported to enhance fibrinolytic activity in plasma and the production of tissue plasminogen activator (tPA).[71]

Nattokinase has greater thrombolytic activity than plasmin, a natural thrombolytic protease in blood, and increases the production of plasmin from plasminogen due to its action on plasminogen activator. These observations, together with the fact that it can be absorbed across the intestinal tract after oral administration and induce fibrinolysis, make nattokinase a potential clot-dissolving agent for the treatment of cardiovascular disease.

Intravenous fibrinolytic agents such as streptokinase, urokinase and tissue plasminogen activator (t-PA) have been widely used in clinical practice for thrombolytic therapy since the 1960s. However, the biological activity of these substances is short lived in the circulation after intravenous administration, and there is a significant risk of hemorrhagic complications. Additionally, these pharmaceuticals have a low specificity to fibrin and are generally expensive. Nattokinase rivals many blood thinning pharmaceuticals, such as warfarin, but without any side effects making it of value to everyone, not just heart disease patients.

Numerous studies have also shown that nattokinase also helps correct hypertension as it acts as an ACE inhibitor to help lower blood pressure.[72, 73, 74, 75] A 2008 study conducted over eight weeks found that nattokinase was able to moderately lower both systolic and diastolic blood pressure in hypertensive human subjects.[76]

Studies have also shown that nattokinase has the ability to dissolve amyloid fibrils (amyloids), which means it may help prevent Alzheimer's disease.[77, 78] Hence, nattokinase may help dissolve or disrupt pre-formed or pre-deposited amyloid fibrils

and/or inhibit amyloid formation, deposition, accumulation, or other forms of amyloidosis.

Serrapeptase, also known as serratia peptidase, is a proteolytic enzyme produced by a bacterium that lives in the intestinal tract of the silkworm. This enzyme allows the emerging moth to dissolve its cocoon. However, there is no method to extract enough serrapeptase from silkworms to support the world's consumption of serrapeptase. What is used to meet the global demand for serrapeptase is serratia peptidase. Serratia peptidase is a proteolytic enzyme isolated from the non-pathogenic enterobacteria Serratia E15. This bacterium is cultured to produce, through batch fermentation, the necessary amount of serratia peptidase to meet global demand. Hence, silkworms are not harmed.

Clinical studies show that serrapeptase induces fibrinolytic, anti-inflammatory and anti-edemic activity, and that its anti-inflammatory effects are superior to other proteolytic enzymes. Serrapeptase has been shown to break down non-living tissue, such as blood clots, cysts, scar tissue, arterial plaque, as well as reduce inflammation and pain. It also has a remarkable ability to help clear mucous from the lungs by thinning the mucoprotein in the lungs of patients with chronic airway diseases.

The clinical use of serrapeptase as an anti-inflammatory in Europe and Asia spans over twenty-five years. In the 1980s and early 1990s, Japanese and European research compared several proteolytic enzymes and their studies indicated that serrapeptase was one of the most effective in reducing inflammation. Hence, serrapeptase became widely used in Japan and Europe as the anti-inflammatory and pain treatment of choice.

Research in other treatable conditions included chronic sinusitis[79, 80, 81], elimination of bronchopulmonary secretions[82, 83, 84, 85] (the enzyme breaks down protein fibers, allowing mucous to thin), sprains and torn ligaments, and other traumatic injuries,[86] edema,[87] as well as postoperative inflammation.[88, 89] Besides reducing inflammation, one of serrapeptase's most profound benefits is reduction of pain, due to its ability to block the release of pain-inducing amines from inflamed tissues. In a 70-patient, double-blind controlled trial treating breast engorgement, serrapeptase improved breast pain and swelling in significant numbers of the treatment group with no adverse reactions.[90]

The late Hans A. Nieper, M.D., an internist from Hannover, Germany, studied the effects of serrapeptase on plaque accumulations in the arteries. Excessive plaque results in partial or complete blockage of blood flow through an artery, resulting in arteriosclerosis,

or hardening of the arteries, and a potential stroke or heart attack. Dr. Nieper's results are documented in a paper published by the Brewer Science Library, Wisconsin. In treating nearly completely blocked arteries without surgery or stents, Dr. Nieper, along with serrapeptase, included magnesium orotate, bromelain, L-carnitine, thiamine chloride (Vitamin B1) and selenium. Actually, there is a lot more to the Nieper protocol than a few supplements. The Brewer Library91 will sell a copy of Nieper's therapy for a few dollars. Further clinical studies are called for in this area as Nieper's preliminary research indicated that the protein-dissolving action of serrapeptase will gradually break down atherosclerotic plaques.

Oral proteolytic enzyme dosages vary greatly depending on the formulation, the individual and of course the condition being treated. In the treatment of tumors, some experts recommend up to 10 or more proteolytic enzymes, three times daily, between meals. However, the total number of proteolytic enzyme tablets taken per day by each cancer patient on the Gonzalez regimen typically was much more than this. There are proteolytic enzyme manufacturers in Europe that make higher enzyme dose tablets, so that it is not necessary to take 30 or more tablets per day to achieve the dosage Dr. Gonzalez recommended. Most European doctors who utilize proteolytic enzymes in their cancer treatment protocols recommend doses between meals every four hours while awake. This allows the enzymes a better chance of absorption through the intestinal walls and ultimately into the bloodstream. Usually, physicians prescribe the proteolytic enzymes to be taken in cycles; i.e. three weeks on, one week off. During "rest periods", other metabolic therapies are utilized.

Oral proteolytic enzymes are one of the most effective therapies for inflammation, antithrombosis, hypertension, and as an adjuvant treatment for tumors of all types. Due to their lack of side effects and unique properties, proteolytic enzymes and their formulations are a bio-logical choice to replace harmful NSAIDs and steroids, and may be used to improve abnormal blood viscosity.

References

1. Desser L, Rehberger A, Paukovits W. Proteolytic enzymes and amylase induce cytokine production in human peripheral blood mononuclear cells *in vitro*. Cancer Biother. Fall 1994;9(3):253-263.
2. Desser L, Rehberger A. Induction of tumor necrosis factor in human peripheral-blood mononuclear cells by proteolytic enzymes. Oncology. 1990;47(6):475-477.
3. Beuth J, Braun JM. Modulation of murine tumor growth and colonization by bromelaine, an extract of the pineapple plant (Ananas comosum L.). In Vivo. Mar-Apr 2005;19(2):483-485.

4. Desser L, Rehberger A, Kokron E, et al. Cytokine synthesis in human peripheral blood mononuclear cells after oral administration of polyenzyme preparations. Oncology. Nov-Dec 1993;50(6):403-407.
5. Tysnes BB, Maurer HR, Porwol T, et al. Bromelain reversibly inhibits invasive properties of glioma cells. Neoplasia. Nov-Dec 2001;3(6):469-479.
6. Wald M, Olejar T, Sebkova V, et al. Mixture of trypsin, chymotrypsin and papain reduces formation of metastases and extends survival time of C57Bl6 mice with syngeneic melanoma B16. Cancer Chemother Pharmacol. Jul 2001;47 Suppl:S16-22.
7. Wald M, Závadová E, Poucková P, Zadinová M, Polyenzyme preparation Wobe-Mugos inhibits growth of solid tumors and development of experimental metastases in mice.Boubelik M.Life Sci. 1998; 62(3):PL43-8.
8. Dittz D, Figueiredo C, Lemos FO, Viana CT, Andrade SP, Souza-Fagundes EM, Fujiwara RT, Salas CE, Lopes MT.Antiangiogenesis, loss of cell adhesion and apoptosis are involved in the antitumoral activity of Proteases from V. cundinamarcensis (C. candamarcensis) in murine melanoma B16F1. Int J Mol Sci. 2015 Mar 27; 16(4):7027-44. Epub 2015 Mar 27.
9. Beard J: The action of trypsin upon the living cells of Jensen's mouse tumor. Br Med J 4, 140-141, 1906.
10. Beard J: The Enzyme Treatment of Cancer and its Scientific Basis. London: Chatto & Windus, 1911.
11. Moskvichyov BV, Komarov EV, Ivanova GP: Study of trypsin thermodenaturation process. Enzyme Microb Tech 8, 498-502, 1986.
12. Gotze H, Rothman SS: Enteropancreatic circulation of digestive enzymes as a conservative mechanism. Nature 257(5527), 607-609, 1975.
13. Liebow C, Rothman SS: Enteropancreatic circulation of digestive enzymes. Science 189(4201), 472-474, 1975.
14. Wolf, M. Anwendung proteolytischer Enzyme bei der Behandlung metastasierter Melanomalignome. Munch Med Wochenschr, 108: 1614-1617, 1966.; Karl Ransberger worked with Wolf and together they formed the Medical Enzyme Research Foundation.
15. http://www.wobenzym.com/en/how-wobenzym-works/the-story-behind-wobenzym/
16. http://www.drkelley.com/CANLIVER55.html - Dr. William Donald Kelley, DDS, One Answer to Cancer, Published By Cancer Coalition for Alternative Therapies, Inc. P.O. Box 222, Mount Pearl, NF A1N 2C2 CANADA
17. http://www.dr-gonzalez.com.
18. Gonzalez NJ, Isaacs LL: Evaluation of pancreatic proteolytic enzyme treatment of adenocarcinoma of the pancreas, with nutrition and detoxification support. Nutr Cancer 33 (2): 117-24, 1999.
19. Sakalová A, Bock PR, Dedík L, Hanisch J, Schiess W, Gazová S, Chabronová I, Holomanova D, Mistrík M, Hrubisko M.Retrolective cohort study of an additive therapy with an oral enzyme preparation in patients with multiple myeloma. Cancer Chemother Pharmacol. 2001 Jul; 47 Suppl:S38-44.
20. Popiela T, Kulig J, Hanisch J, Bock PR.Influence of a complementary treatment with oral enzymes on patients with colorectal cancers--an epidemiological retrolective cohort study.Cancer Chemother Pharmacol. 2001 Jul; 47 Suppl:S55-63.
21. Beuth J, Ost B, Pakdaman A, Rethfeldt E, Bock PR, Hanisch J, Schneider B.Impact of complementary oral enzyme application on the postoperative treatment results of breast cancer patients--results of an epidemiological multicentre retrolective cohort study.Cancer Chemother Pharmacol. 2001 Jul; 47 Suppl:S45-54.
22. Novak JF, Trnka F. Proenzyme therapy of cancer. Anticancer Res. 2005 Mar-Apr;25(2A):1157-77.
23. Chabot JA, Tsai WY, Fine RL, et al.: Pancreatic proteolytic enzyme therapy compared with gemcitabine-based chemotherapy for the treatment of pancreatic cancer. J Clin Oncol 28 (12): 2058-63, 2010.
24. In their official determination letter appearing on their website after a two-year investigation, the Office of Human Research Protections, the NIH agency in charge of investigating mismanagement on

government-funded studies, found that Dr. Chabot, who was in charge of admissions of patients, had improperly approved 42 out of a total of 62 patients, including 40 for whom he had failed to obtain appropriate written informed consent. Furthermore, the determination letter states that the Principal Investigator (Dr. Chabot) admitted he committed the managerial lapses, and in their letter the OHRP requires Columbia to set up a program for training in appropriate research methodology – a serious indictment of a major academic medical center.
25. Gonzalez, Nicholas: Journal of Clinical Oncology Article Rebuttal. New York, NY, 2009. For more information see Dr. Gonzalez' book What Went Wrong: The Truth behind the Clinical Trial of the Enzyme Treatment of Cancer.
26. Gunji Y, Gorelik E: Role of fibrin coagulation in protection of murine tumor cells from destruction by immune cells. Cancer Res 48: 5216–5221, 1988.
27. Costantini V, Zacharski LR, Memoli VA, Kisiel W, Kudryk BJ, Rousseau SM, Stump DC: Fibrinogen deposition and macrophage-associated fibrin formation in malignant and non-malignant lymphoid tissue. J Lab Clin Med 119: 124–131, 1992.
28. Atagi S, Sone S, Fukuta K, Ogura T. Inhibition by fibrin coagulation of lung cancer cell destruction by human interleukin-2-activated killer cells. Jpn J Cancer Res. 1992 Oct; 83(10):1088-94.
29. Gunji Y, Lewis J, Gorelik E. Fibrin formation inhibits the *in vitro* cytotoxic activity of human natural and lymphokine-activated killer cells. Blood Coagul Fibrinolysis. 1990 Dec; 1(6):663-72.
30. Zacharski LR, Howell AL, Memoli VA: The coagulation biology of cancer. Fibrinolysis 6 (Suppl): 39–42, 1992
31. Zavadova E, Desser L, Mohr T. Stimulation of reactive oxygen species production and cytotoxicity in human neutrophils *in vitro* and after oral administration of a polyenzyme preparation. Cancer Biother. 1995 Summer;10(2):147-52.
32. Desser L, Rehberger A Induction of tumor necrosis factor in human peripheral-blood mononuclear cells by proteolytic enzymes. Oncology. 1990; 47(6):475-7.
33. Desser L, Rehberger A, Kokron E, Paukovits W. Cytokine synthesis in human peripheral blood mononuclear cells after oral administration of polyenzyme preparations.Oncology. 1993 Nov-Dec; 50(6): 403-7.
34. Desser L, Rehberger A, Paukovits W. Proteolytic enzymes and amylase induce cytokine production in human peripheral blood mononuclear cells *in vitro*. Cancer Biother. 1994 Fall;9(3):253-63.
35. Blobe GC, Schiemann WP, Lodish HF (May 2000). Role of transforming growth factor beta in human disease. N. Engl. J. Med.342 (18): 1350–8. doi:10.1056/NEJM200005043421807.PMID 10793168.
36. Desser L, Holomanova D, Zavadova E, Pavelka K, Mohr T, Herbacek I. Oral therapy with proteolytic enzymes decreases excessive TGF-beta levels in human blood. Cancer Chemother Pharmacol. 2001 Jul; 47 Suppl:S10-5.
37. Ernst E., Matrai A.: Oral Therapy with proteolytic enzymes for modifying blood rheology. Klin Wschr. 65 (1987), 994.
38. http://www.mucos.de/
39. Leibow C, Rothman SS: Enteropancreatic circulation of digestive enzymes. Science 189 (4201): 472-4, 1975.
40. Rothman S, Liebow C, Isenman L: Conservation of digestive enzymes. Physiol Rev 82 (1): 1-18, 2002.
41. Isenman L, Liebow C, Rothman S: Transport of proteins across membranes--a paradigm in transition. Biochim Biophys Acta 1241 (3): 341-70, 1995.
42. Onken JE, Greer PK, Calingaert B, et al. Bromelain treatment decreases secretion of pro-inflammatory cytokines and chemokines by colon biopsies *in vitro*. Clin Immunol. Mar 2008;126(3): 345-352.
43. Secor ER, Carson WF, Singh A, et al. Oral Bromelain Attenuates Inflammation in an Ovalbumin-induced Murine Model of Asthma. Evid Based Complement Alternat Med. Mar 2008;5(1):61-69.

44. Klasen HJ. A review on the non-operative removal of necrotic tissue from burn wounds. Burns 2000;26:207-22.
45. Massimiliano R, Pietro R, Paolo S, et al. Role of bromelain in the treatment of patients with pityriasis lichenoides chronica. J Dermatolog Treat. 2007;18(4):219-222.
46. Walker AF, et al. Bromelain reduces mild acute knee pain and improves well-being in a dose-dependent fashion in an open study of otherwise healthy adults. Phytomedicine 2002 Dec;9(8):681-6.
47. Majid OW, Al-Mashhadani BA. Perioperative bromelain reduces pain and swelling and improves quality of life measures after mandibular third molar surgery: a randomized, double-blind, placebo-controlled clinical trial. J Oral Maxillofac Surg. 2014 Jun;72(6):1043-8.
48. Klein G, Kullich W, Schnitker J, et al. Efficacy and tolerance of an oral enzyme combination in painful osteoarthritis of the hip. A double-blind, randomised study comparing oral enzymes with non-steroidal anti-inflammatory drugs. Clin Exp Rheumatol. Jan-Feb 2006;24(1):25-30.
49. Kerkhoffs GM, Struijs PA, de Wit C, et al. A double blind, randomised, parallel group study on the efficacy and safety of treating acute lateral ankle sprain with oral hydrolytic enzymes. Br J Sports Med.Aug 2004;38(4):431-435.
50. Brien S, Lewith G, Walker AF, et al. Bromelain as an adjunctive treatment for moderate-to-severe osteoarthritis of the knee: a randomized placebo-controlled pilot study.QJM. Dec 2006;99(12):841-850.
51. Bhui K, Prasad S, George J, Shukla Y. Bromelain inhibits COX-2 expression by blocking the activation of MAPK regulated NF-kappa B against skin tumor-initiation triggering mitochondrial death pathway.Cancer Lett 2009 Sep 18;282(2):167-76.
52. Hale LP, Chichlowski M, Trinh CT, Greer PK. Dietary supplementation with fresh pineapple juice decreases inflammation and colonic neoplasia in IL-10-deficient mice with colitis. Inflamm Bowel Dis.2010 Dec;16(12):2012-21.
53. Kalra N, Bhui K, Roy P, et al. Regulation of p53, nuclear factor kappaB and cyclooxygenase-2 expression by bromelain through targeting mitogen-activated protein kinase pathway in mouse skin.Toxicol Appl Pharmacol. 2008 Jan 1;226(1):30-7.
54. Bhui K, Tyagi S, Prakash B, Shukla Y. Pineapple bromelain induces autophagy, facilitating apoptotic response in mammary carcinoma cells. Biofactors. 2010 Nov-Dec;36(6):474-82.
55. Desser L,et al. Oral therapy with proteolytic enzyes decreases excessive TGF-beta levels in human blood. Cancer Chemother Pharmacol 2001;47:S10-5.
56. Desser L, Zavadova E, Herbacek I. Oral enzymes as additive cancer therapy. Int J Immunotherapy. 2001;17(2-3-4):153-161.
57. Maurer HR. Bromelain: biochemistry, pharmacology and medical use. Cell Mol Life Sci 2001;58:1234-45.
58. Baez R, Lopes MT, Salas CE, et al. *In vivo* antitumoral activity of stem pineapple (Ananas comosus) bromelain. Planta Med. Oct 2007;73(13):1377-1383.
59. Hidaka M, Nagata M, Kawano Y, et al. Inhibitory effects of fruit juices on cytochrome P450 2C9 activity *in vitro*. Biosci Biotechnol Biochem. Feb 2008;72(2):406-411.
60. Pandey S et al. Anti-inflammatory and immunomodulatory properties of Carica papaya. J Immunotoxicol. 2016;13(4),590-602.
61. Amri E, Mamboya. Papain, a Plant Enzyme of Biological Importance: A Review. Am J Biochem Biotechnol. 2012;8(2),99-104.
62. da Silva CR, et al. Genotoxic and Cytotoxic Safety Evaluation of Papain (Carica papaya L.) Using *In Vitro* Assays. J Biomed Biotechnol. 2010; 2010,197898.
63. Rakhimov MR. Anti-inflammatory activity of domestic papain]. Eksp Klin Farmakol. 2001 Jul-Aug; 64(4):48-9. http://www.ncbi.nlm.nih.gov/pubmed/11589110.
64. Müller A, et al. Comparative study of antitumor effects of bromelain and papain in human cholangiocarcinoma cell lines. Int J Oncol. 2016;48(5),2025-34.

65. Bellelli A, et al. Inhibition of tumor growth, invasion and metastasis in papain-immunized mice. Invasion Metastasis. 1990;10(3),142-69.
66. Fujita M., Nomura K., Hong K., Ito Y., Asada A. and Nishimuro S. Purification and Characterization of a Strong Fibrinolytic Enzyme (Nattokinase) in the Vegetable Cheese Natto, a Popular Soybean Fermented Food in Japan. Biochemical and Biophysical Research Communications, Vol. 197, Issue 3, 30 December 1993, pp. 1340-1347. http://www.ncbi.nlm.nih.gov/pubmed/8280151
67. Peng Y, Huang Q, Zhang RH, Zhang YZ Purification and characterization of a fibrinolytic enzyme produced by Bacillus amyloliquefaciens DC-4 screened from douchi, a traditional Chinese soybean food. Comp Biochem Physiol B Biochem Mol Biol. 2003 Jan; 134(1):45-52.
68. Sumi H, Hamada H, Tsushima H, Mihara H, Muraki H. A novel fibrinolytic enzyme (nattokinase) in the vegetable cheese Natto; a typical and popular soybean food in the Japanese diet. Experientia. 1987 Oct 15; 43(10):1110-1.
69. Pais E, Alexy T, Holsworth RE Jr, Meiselman HJ: Effects of nattokinase, a pro-fibrinolytic enzyme, on red blood cell aggregation and whole blood viscosity. Clin Hemorheol Microcirc2006; 35: 139–142.
70. Fujita M, Hong K, Ito Y, Fujii R, Kariya K, Nishimuro S. Thrombolytic effect of nattokinase on a chemically induced thrombosis model in rat. Biol Pharm Bull 1995;18:1387-91.
71. Sumi H, Hamada H, Nakanishi K, Hiratani H. Enhancement of the fibrinolytic activity in plasma by oral administration of nattokinase. Acta Haematol 1990;84:139-43.
72. Murakami K1, Yamanaka N, Ohnishi K, Fukayama M, Yoshino M. Inhibition of angiotensin I converting enzyme by subtilisin NAT (nattokinase) in natto, a Japanese traditional fermented food. Food Funct. 2012 Jun;3(6):674-8. http://www.ncbi.nlm.nih.gov/pubmed/22453301.
73. Maruyama M, Sumi H (eds): Effect of natto diet on blood pressure, in Basic and Clinical Aspects of Japanese Traditional Food Natto II. Japan Technology Transfer Association (JTTAS), 1998, pp 1–3.
74. Okamoto A, Hanagata H, Kawamura Y, Yanagida F: Antihypertensive substances in fermented soybean, natto. Plant Foods Hum Nutr 1995; 47: 39–47.
75. Kuba M, Tanaka K, Tawata S, Takeda Y, Yasuda M: Angiotensin I—converting enzyme inhibitory peptides isolated from tofuyo fermented soybean food. Biosci Biotechnol Biochem 2003; 67: 1278–1283.
76. Kim JY, Gum SN, Paik JK, et al. Effects of nattokinase on blood pressure: a randomized, controlled trial. Hypertens Res. 2008;31(8):1583-1588. http://www.ncbi.nlm.nih.gov/pubmed/18971533
77. Hsu, R. L., Lee, K. T., Wang, J. H., Lee, L. Y. L., & Chen, R. P. Y. (2008). Amyloid-degrading ability of nattokinase from Bacillus subtilis natto. Journal of agricultural and food chemistry, 57(2), 503-508.
78. Chen, Rita P-Y., and Kung-Ta Lee. Nattokinase for degrading and reducing amyloid fibrils—associated with Alzheimer's disease, prion diseases and other amyloidoses. U.S. Patent No. 8,137,666. 20 Mar. 2012.
79. Mizukoshi, D. et al. A double-blind clinical study of serrapeptase in the treatment of chronic sinusitis. Igaku Ayrni 109:50-62.1979.
80. Odagiri, J. et al. Clinical applications of serrapeptase in sinusitis. Med. Consult. New Remedy 6:201-209, 1979.
81. Majima Y, Inagaki M, Hirata K. Takeuchi K, Morishita A, Sakakura Y. The effect of an orally administered proteolytic enzyme on the elasticity and viscosity of nasal mucus. Arch Otorhinolaryngol. 1988;244(6):355-9.
82. Carratu, L. et al. Physio-chemical and rheological research on mucolytic activity of serrapeptase in chronic broncho-pneumopathies. Curr.Ther. Res. 28(6):937-951. 1980.
83. Braga, P.C. et al. Effects of serrapeptase on muco-ciliary clearance in patients with chronic bronchitis. Curr. Ther. Res. 29(5):738-744,1981.
84. Tomoda, K. and Miyatam K. Some information on the composition of tracheal secretions before and after the administration of serrapeptase. Exper. Ther. 477:9-16, 1972.
85. Tago. T. and Mitsui, S. Effects of serrapeptase in dissolution of sputum, especially in patients with bronchial asthma. Jap. Clin. Exp. Med. 49:222-228, 1972.

86. Marly, M. Enzymotherapie anti-inflammatoire a l'aide de la serrapeptase: resultats cliniques en traumatologie et en ORL. C RTherapeut. 3:9-19,1985.
87. Matsudo, A. et at. Effect of serrapeptase (Danzen) on inflammatory edema following operation for thyroid disease. Med. Consult. New Remedy 18:171-175, 1981.
88. Esch PM, Gemgross H. Fabian A. Reduction of postoperative swelling. Objective measurement of swelling of the upper ankle joint in treatment with serrapeptase-a prospective study (German). FortschrMed. 1989; 107(4):67-8, 71-2.
89. Harada, Y. Clinical efficacy of serrapeptase on buccal swelling after radical operation for chronic sinusitis. Igaku Ayumi 123:768- 778.1982.
90. Kee WH, Tan SL, Lee V, Salmon YM. The treatment of breast engorgement with Serrapeptase (Danzen): a randomized double-blind controlled trial. Singapore Med J. 1989;30(1):48-54.
91. http://www.mwt.net/~drbrewer/

The information in this monograph is intended for informational purposes only, and is meant to help users better understand health concerns. Information is based on review of scientific research data, historical practice patterns, and clinical experience. This information should not be interpreted as specific medical advice. Users should consult with a qualified healthcare provider for specific questions regarding therapies, diagnosis and/or health conditions, prior to making therapeutic decisions.

For more information, visit www.brmi.online.