Chapter 1: Types of Enzymes

The recognition of the importance of enzymes in biological phenomena has been a prominent feature of the current surge in scientific progress. Proteolytic enzymes have been used therapeutically in various areas [1]:

1. as oral agents for specific gastrointestinal disorders;
2. as local agents to debride or solubilize collections of proteinaceous material, which either cause or foster disease;
3. as anti-inflammatory agents;
4. as thrombolytic agents in the treatment of thromboembolic disorders;
5. as a treatment for specific connective tissue disorders, such as Dupuytren’s Contracture and Peyronie’s Disease.

Over the years various proteolytic enzymes have been employed (papain, ficin, streptokinase, streptodornase, trypsin-chymotrypsin, sutilain, collagenase, etc.) for the debridement of wounds. This section provides an overview of these various enzymes.

The concept of using proteolytic enzymes to digest dead tissue as an adjunct to the management of dirty, infected wounds is an old one, probably stemming from the observation of the natives of tropical countries where the papain-rich latex obtained by scratching the skin of the green fruit of the papaw tree (Carica papaya) has long been used for the treatment of eczema, warts, ulcers and other types of foul sores [1]. It is also known that in addition to applying papain-rich latex to a wound, the wounds were at times exposed to urine, then wrapped in green leaves from the same plant. This is interesting as these 3 naturally occurring materials -- papain, urea and chlorophyllin (a derivative of chlorophyll) -- are the active ingredients of one of the most well-known enzymatic debriders ever used (Panafil®). Urea is a component of mammalian urine and chlorophyll is a component of green leaves. In this formulation the urea acts as a denaturant assisting in the degradation of various proteins in necrotic tissues. Chlorophyllin is an anti-agglutinating/anti-inflammatory agent, which helps to counter some of the less desirable effects of papain-urea on tissue. Chlorophyllin is also felt to have odor controlling properties [2,3].

Before the turn of the 20th century, literature on the use of papaya latex preparations for treating sloughing ulcers, removing impacted cerumen and dissolving diphtheritic membranes became available [4,5]. More recently, it has been found that the major insoluble constituents of inflammatory exudates, fibrin and desoxyribonucleoprotein derived from the nuclei of dead degenerating cells, could be rapidly lysed by the local application of a mixture of enzymes obtained from the secretory products of certain strains of hemolytic streptococci. The major constituents of this enzyme mixture, streptokinase (an activator of plasminogen, the naturally occurring precursor of a proteolytic and fibrinolytic enzyme of human plasma) and streptodornase (streptococcal desoxyribonuclease) provided the basis for an enzymatic debridement [6,7].

Specific enzyme preparations that have been used or are in current use for purposes of local debridement include, but are not limited to:
Microbe derived enzymes

**Sutilain:** a water-soluble mixture of serine proteases derived from the bacteria *Bacillus subtilis* that is relatively nonspecific in its action and is capable of breaking down a variety of necrotic tissue types within an optimal pH range of 6.0-7.5 [8].

**Clostridial collagenase:** a water-soluble enzyme that specifically attacks and breaks down undenatured (natural) collagen. In actuality, collagenase is known to degrade denatured collagen as well. Collagenase is commercially derived from bacterial (*Clostridium histolyticum*) sources. Collagenase is active over a pH range of 6.0-8.0. Bacterial collagenase, although a zinc metalloproteinase that uses calcium bears little structural relationship to mammalian collagenase. Bacterial collagenase rapidly attacks human collagen at many points, degrading it into small peptides. The commercially available collagenase is made up of proteolytic enzymes that break collagen into small peptides (oligopeptides) of differing molecular weights, most of which are tripeptides [9,10]. However, more recent work has shown that the oligopeptides may be larger [11]. Two genes, colG and colH transcribe for two *C. collagenases*. These collagenases uniquely cleave the interstitial collagens and exhibit both endopeptidase and tripeptidylcarboxypeptidase activities. The combined activity of endo- and tripeptidyl-C-peptidase makes these enzymes ideally suited for rapid collagen degradation. Clostripain is a cysteine-activated protease also found in culture filtrates of *Clostridium histolyticum*. However, the level of this enzyme is low and the effects on collagen may not be as pronounced as for *C. collagenases*. In contrast, the mammalian collagenase-1 (MMP-1) acts differently by cleaving interstitial collagen at a single locus within the triple helical structure, giving rise to 2 large fragments, TC_A and TC_B. These portions of the helix are then attacked by other less specific proteases, released by connective tissue cells, to be further degraded into small peptides [12].

**Streptokinase-streptodornase mixtures:** This preparation is only partially purified and contains a number of other streptococcal enzymes, such as a ribonuclease, hyaluronase, nucleotidase and nucleosidase, all of which may contribute to the effects observed. The enzyme mixture is essentially free of streptolysin and streptococcal proteinase. It does not contain any proteolytic enzymes in the conventional sense. The mixture contains enzymes, which act upon non-protein substrates; much of its virtue lies in its content of streptodornase, which rapidly reduces the viscosity of purulent exudates. Plasmin, the proteolytic enzyme formed from the latter precursor, is active at neutral pH and, though distinct from trypsin, resembles it in many respects (pH optima, types of links split, etc.). The major attribute of streptokinase lies in its special fibrin-dissolving properties. In contrast to the rapid inhibition of proteolytic enzymes by naturally-occurring humoral antiproteolytic substances, streptokinase is inactivated at a relatively slow rate (except in the presence of an excess of a specific antibody, antistreptokinase).

StreptokinaseStreptodornase preparations are the agents of choice for liquefying clotted blood, loculated effusions and purulent exudate in closed body cavities. A significant incidence of pyrogenic [pyogenic] and inflammatory reactions to the locally administered enzyme mixture has limited its usefulness since the therapeutic procedure may be complicated by the patient’s discomfort and the need for frequent and repeated drainage [1].

**Streptodornase:** (streptococcal desoxyribonuclease) acts directly upon deoxyribonucleic acid (DNA), rapidly depolymerizing this highly complex substance into smaller units [1,13]. The activity of streptodornase is enhanced by the presence of Mg²⁺ or other divalent metallic ions and inhibited by the presence of substances, such as citrate, which form complexes with the metallic cofactor (i.e., chelating agents).
**Fungal:** Fungal proteases have also been employed as topically applied enzymatic debriders.

**Animal-derived enzymes**

**Fibrinolysin:** commercially obtained from bovine plasma, then activated by chloroform, it specifically attacks and breaks down the fibrin component of blood clots and fibrinous exudates.

**Desoxyribonuclease:** obtained from bovine pancreatic tissue, acts specifically on the nucleoprotein components of purulent exudates.

**Trypsin:** Crystalline trypsin preparations of bovine pancreatic origin have been used in the past. Trypsin is a serine protease and can directly hydrolyze a large number of naturally-occurring proteins. It is thought not to affect living cells or require any cofactors, and its action on denatured proteins is usually more extensive than on native proteins. Trypsin has advantages over streptokinase for surface wound debridement since it does not require additional factors for its action, acts upon a greater number of proteins than plasmin, and degrades them more extensively [1].

**Chemotrypsin:** This preparation is of bovine pancreatic origin and is the other major serine protease of the pancreas. Pancreatic enzymes are usually standardized in terms of their proteolytic activity. Though chymotrypsin acts upon different bonds in proteins than does trypsin or plasmin, its spectrum of activity on whole proteins is somewhat similar to that of trypsin [1].

**Hyaluronidase:** This is another common animal-derived enzyme used for topical enzymatic debridement.

**Plant-derived enzymes**

**Bromelain:** A mixture of water-soluble, cysteine proteases derived from the stem or fruit of the pineapple plant. This mixture of proteolytic enzymes is reported to be effective in breaking down a variety of different necrotic tissue substrates over a fairly wide pH range (5.5-8.5). It should be noted that cases of anaphylactic shock have been reported with enzymes derived from the pineapple plant, as well as with other plant-derived enzymes.

**Papain:** A latex protein obtained from the skin and green fruit of the papaw tree (Carica papaya). Papain is a cysteine protease and acts upon a wide variety of proteins; its activity can be considerably enhanced by the addition of cysteine or other reducing agents or by protein denaturants, such as urea. Indeed, without the presence of urea, papain displays lower proteolytic activity. The enzyme’s activity is optimal over a pH range of 3-9. It has been stated that at low pH, papain is capable of digesting collagen. Though papain preparations have been used occasionally in acetic acid solutions to digest collagenous tissue, the success of this method has not been established [1]. Other literature sources have described papain as having no effect on collagen. In 1958, J. Miller et al. [14], showed that papain-urea lacks the ability to degrade native collagen & states that only clostridial collagenase was able to adequately digest collagen.

Some feel that purified papain preparations eventually may prove to be the most practical for surface debridement. Others feel this is unlikely, given the mode of action of papain, its aggressive attack on viable tissue, and the associated stinging and burning reported in some literature sources. Miller [15], and Morrison et al. [16] all describe prolonged and intensified inflammatory responses as a result of treatment with papain-urea systems. Langer et al. 2013 [17], found in a prospective descriptive study on burns (mean TBS = 33.17%) that the combination of papain and urea caused so much pain (and fever) that only 2 of the 34 patients involved were able to complete the study.

Why were papain-urea based enzymatic deriders removed from the market?
As per the Federal Register, in 2008 the U.S. Food and Drug Administration (FDA) [18] ordered companies to stop marketing unapproved drug products that contain papain in a topical dosage form. Under this ruling, firms marketing any unapproved topical papain products had to stop manufacturing these products by November 24, 2008. Companies or others engaged in shipping these products had to stop shipping them by January 21, 2009. After these dates, all topical products containing papain must have FDA approval to be manufactured or shipped interstate. The FDA went on to state that topical drug products containing papain have historically been marketed without approval; there are no approved topical drug products containing papain. FDA took this action because adverse events with use of topical papain drug products reported to the agency raised serious safety concerns regarding these products. The FDA found that these drugs can produce harmful or near fatal effects including hypersensitivity resulting in anaphylactic reactions. Such cases have required emergency rooms visits, some requiring treatment with epinephrine. Hypersensitivity manifestations have also resulted in cardiovascular symptoms such as hypotension (low blood pressure) and tachycardia (rapid heart rate). Additionally, reports in the medical literature suggest that patients who are allergic to latex may also be allergic to papaya, the source of papain. Furthermore, the effectiveness of these products is not supported by scientifically sound studies in the medical literature.

The FDA pointed out that papain is in fact a latex protein and sites cases of cross reactivity between latex and papaya have been documented in medical literature, and one of the cases reported to FDA involved anaphylactic shock in a patient with a history of allergy to latex. In addition, papain-containing drug products in topical form historically have been marketed without approval, and because no firm obtained an application for them prior to passage of the Drug Amendments of 1962, they were not included in the Drug Efficacy Study Implementation (DESI) review. Adverse events associated with the use of topical papain products reported to FDA raise serious safety concerns regarding these products. Through January 2008, FDA had received 37 reports of adverse events associated with topical papain products. In addition to several complaints that the products were ineffective, the reports include cases of potentially life threatening hypersensitivity reactions. Reactions described include serious cases of anaphylaxis and anaphylactic shock that started within 15 minutes of topical papain use and resulted in hospitalizations, including admissions to the intensive care unit. Finally, the FDA was particularly concerned about adverse events associated with the use of papain-containing topical drug products in light of the dearth of published, well-controlled studies demonstrating the effectiveness of those products. Given the absence of the kinds of scientific studies routinely conducted by sponsors and submitted for agency review as part of the FDA approval process, it was impossible for the agency to assess either the amount of risk associated with these products or the extent to which their benefits might justify their risks.

Products affected (by name) were Accuzyme®, Allanfil®, Allanzyme®, Ethezyme®, Gladase®, Kovia®, Panafil®, Pap Urea®, and Ziox®. Other formulations were marketed under the names of the active ingredients, for instance papain-urea ointment. At the time of the FDA’s determination there are approximately 35 unapproved topical products containing papain on the market. This ruling in effect ended the use of papain-urea based enzymatic debriding agents in the US.

Actinidin: A member of the papain-like family of cysteine proteases, is abundant in kiwifruit. Chalabi et al. 2014 [19], investigated the proteolytic activity of actinidin compared to papain on several different fibrous and globular proteins under neutral, acidic and basic conditions. The findings showed that actinidin has no or limited proteolytic effect on globular proteins such as immunoglobulins including sheep IgG, rabbit IgG, chicken IgY, and fish IgM, bovine serum albumin (BSA), lipid transfer protein (LTP), and whey proteins (α-lactalbumin and β-lactoglobulin) compared to papain. In contrast to globular proteins, actinidin could hydrolyze collagen and fibrinogen perfectly at neutral and mild basic pHs. Moreover, this enzyme could digest pure α-casein and major subunits of micellar casein especially at acidic pHs. Taken together,
the data (in this particular *in vitro* study) indicated that actinidin has narrow substrate specificity with the highest enzymatic activity for the collagen and fibrinogen substrates. Hafezi et al. 2010 [20], found that debridement and scar contraction occurred faster in the kiwi-treated group than in the untreated group in acute burn wounds. Following rapid enzymatic debridement, healing appeared to progress normally, with no evidence of damage to adjacent healthy tissue. However, information on the clinical application as a topical enzymatic debrider is limited. In a randomized controlled clinical study on 17 neuropathic diabetic foot ulcers Mohajeri et al. 2014 [21], found that the mean reduction in surface area of foot ulcer in the experimental group was significantly higher than the control group (168.11 ± 22.31 vs. 88.80 ± 12.04 mm² respectively, P < 0.0001). The amount of collagen and granulation tissues was significantly higher in the experimental groups than the control group (P value < 0.0001). Significantly higher levels of angiogenesis and vascularization were found in the kiwifruit treated patients (P value < 0.0001). No significant antibacterial effect was observed for kiwifruit in this study. However, in this particular study, all patients were surgically debrided prior to initiation of the study period.

**Ficin:** Ficin is another plant-derived cysteine protease found in figs.

Additional sources for enzymes such as avian, larva and crustaceans have been investigated, as well.

Effective collagen breakdown appears to be essential to optimum eschar removal. Collagen is a major component of chronic wound eschar, as collagen makes up 70%-80% of the dry weight of skin and is a major component of the extracellular matrix.

The history of enzymatic debriders has been a turbulent one, with only one enzymatic system currently used widely in clinic, clostridial collagenase. One reason for this turbulent history may be related to an enzyme’s ability to degrade collagen. Howes et al [22], and Rao et al. [23], have demonstrated that necrotic tissue is anchored to the wound surface by strands of undenatured collagen. Until these fibers are severed, débridement cannot take place, granulation is slowed, and thus no supportive base is available for proper epithelialization. Consequently, the wound fails to heal. It should be noted that though limited, studies suggest that actinidin (found in kiwi fruit) may have the ability to degrade collagen, which may warrant further study. Another aspect may be the fact that most enzymes used historically have not been highly selective in their catalytic activity. Non-selective being the inability to distinguish between healthy and necrotic tissue. The one exception would be clostridial collagenase, which is felt to be more selective than the enzymes mentioned, previously.

**References**


