Kirk-Othmer Encyclopedia of Chemical Technology.

ENZYME APPLICATIONS, THERAPEUTIC

Advances in the knowledge of therapeutic uses of enzymes have heightened interest in the manufacture and processing of these macromolecules. Therapeutic uses of proteolytic enzymes were attempted in the early nineteenth century; in 1902, Emmerich first demonstrated the therapeutic application of a nuclease capable of degrading nucleic acids (1). This milestone study opened the way for the use of enzymes, first as crude topical and oral preparations with proteolytic and hydrolytic activity, and later as highly purified proteins for use in cancer chemotherapy, genetic metabolic deficiencies, clotting disorders, and as antidotes to treat poisons, drug toxicities, and kidney failure (2–7). Immobilized, modified, and entrapped enzyme preparations have become available to circumvent some of the side effects inherent in the use of foreign, or immunogenic, proteins.

1. Physical and Chemical Properties

Enzymes are protein catalysts of remarkable efficiency and specificity. Lipid, carbohydrate, nucleotide, or metal-containing prosthetic groups may be attached to these enzymes and serve as essential components of their catalyses by enhancing specificity and/or stability (8–13). Each enzyme has a specific temperature and pH range where it functions to its optimal capacity; the optima for these proteins usually lie between 37–47°C, and pH optima range from acidic, ie, 1.0 in the case of gastric pepsin, to alkaline, ie, 10.5 in the case of alkaline phosphatase. However, enzymes from extremely thermotolerant bacteria have become available; these can function at or near the boiling point of water, and therapeutic use of these ultrastable proteins can be anticipated.

Crude enzyme extracts are often unsuitable for therapeutic uses because of their antigenicity, contamination with endotoxins, and rapid inactivation under physiological conditions or in fluids intended for intravenous infusion over several hours. When the enzyme used is a foreign protein, it can elicit an immune response that alters the clearance rate or induces severe allergic reactions in the host. After an intravenous injection of an enzyme, its activity in plasma decreases with time due to distribution to other fluids and tissues, and as a consequence of proteolysis or excretion. Distribution is related to molecular size, charge, and lipophilicity; surface charges attributable to the availability of free amino, amido, or carboxyl groups may affect the rate of inactivation of some enzymes.

Hydrolases represent a significant class of therapeutic enzymes [Enzyme Commission (EC) 3.1–3.11] (14) (Table 1). Another group of enzymes with pharmacological uses has built-in cofactors, eg, in the form of pyridoxal phosphate, flavin nucleotides, or zinc (15). The synthases, and other multisubstrate enzymes that require high energy phosphates, are seldom available for use as drugs because the required co-substrates are either absent from the extracellular space or are present in prohibitively low concentrations.

It is essential to maintain high maximal velocities of enzymatic activity for the attainment of optimal therapeutic efficacy. As a general rule, only enzymes whose Michaelis-Menten constants lie between 1–100 μM are effective as drugs (16) because most substrates for therapeutically useful enzymes are present in body fluids and cells at submillimolar concentrations.

Table 1. Therapeutic Enzymes

Enzyme	CAS Registry Number	Enzyme commission number	Catalysis	Use
neuraminidase	[9001-67-6]	3.2.1.18	hydrolysis of terminal acylneuraminyl residues	antineoplastic
ribonuclease	[9001-99-4]	2.7.7.16	RNA $\longrightarrow$ oligoribonucleotides	antineoplastic
L-α-arabinofuranosidase	[9067-74-7]	3.2.1.55	L-α-arabinofuranoside $\longrightarrow$ alcohol+ L-arabinose	antineoplastic
brinase	[9074-07-1]	3.4.21.15	fibrinogen $\longrightarrow$ fibrin	fibrinolytic
α-glucosidase	[9001-42-7]	3.2.1.20	D-1, 4α-glucoside $\longrightarrow$ α-D-glucose	metachromatic leukodystrophy
β-glucosidase	[9001-22-3]	3.2.1.21	D-1, 4β-glucoside $\longrightarrow$ β-D-glucose	type A glycogenosis
arylsulfatase	[9016-17-5]	3.1.6.1	phenolsulfate $\longrightarrow$ phenol +sulfate	metachromatic leukodystrophy
α-galactosidase	[9025-35-8]	3.2.1.22	α-D-galactoside $\longrightarrow$ α-D-galactose	Fabry's disease
β-galactosidase	[9031-11-2]	3.2.1.23	β-D-galactoside $\longrightarrow$ β-D-galactose	Fabry's disease
bromelain	[37189-34-7]	3.4.22.4	protein $\longrightarrow$ amino acids and peptides	antiinflammatory
collagenase	[9001-12-1]	3.4.24.3	collagen $\longrightarrow$ amino acids and peptides	dermal ulcers
papain	[9001-73-4]	3.4.22.2	protein $\longrightarrow$ amino acids and peptides	reduction of edema after dentalsurgery
L-asparaginase	[9015-68-3]	3.5.1.1	L-asparagine $\longrightarrow$ L-aspartic acid + NH_3	antineoplastic
streptokinase	[9025-51-8]	3.4.22.10	plasminogen $\longrightarrow$ plasmin	thrombolytic
arvin	[9046-56-4]		fibrinogen $\longrightarrow$ fibrin	fibrinolytic
urokinase or tissue plasminogen activator	[9039-53-6]	3.4.21.31	plasminogen $\longrightarrow$ plasmin	thrombolytic
coagulation factor VIII	[9001-27-8]		prothrombin $\longrightarrow$ thrombin	hemophilia
glucocerebrosidase	[37228-64-1]		glycolipid glucocerebroside $\longrightarrow$ $\alpha-$D$-$glucose+ ceramide	Gaucher's disease
lipase	[9001-62-1]	3.1.1.3	carboxylic ester $\longrightarrow$ alcohol+ carboxylic acid	pancreatic deficiency
L-glutaminase	[9001-47-2]	3.5.1.2	L-glutamine $\longrightarrow$ L-glutamic acid + NH_3	antineoplastic
L-arginase	[9000-96-8]	3.5.3.1	L-arginine $\longrightarrow$ L-ornithine + urea	antineoplastic
L-tyrosinase	[9002-10-2]		L-tyrosine + O_2 $\longrightarrow$ dihydrophenylalanine + H_2O	antineoplastic
L-serine dehydratase	[9014-27-1]	4.2.1.13	L-serine $\longrightarrow$ pyruvate + NH_3	antineoplastic
L-threonine deaminase	[9024-34-4]	4.2.1.16	L-threonine $\longrightarrow$ 2-ketobutyric acid + NH_3	antineoplastic
L-tryptophanase	[9024-00-4]	4.1.99.1	L-tryptophan $\longrightarrow$ indole + pyruvate + NH_3	antineoplastic
deoxyribonuclease	[9003-98-9]	3.1.4.5	DNA $\longrightarrow$ oligodeoxyribonucleotides	chronic bronchitis
trypsin	[9002-07-7]	3.4.21.4	protein $\longrightarrow$ peptides	athletic injuries
chymotrypsin	[9004-07-3]	3.4.21.1	protein $\longrightarrow$ peptides	athletic injuries
superoxide dismutase	[9054-89-1]	1.15.1.1	$O_2^- + O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2$	antiinflammatory

Commercial enzymes are available in oral form, sometimes formulated with appropriate stabilizers and excipients. However, such preparations are seldom suitable for parenteral use. Therefore, dry preparations devoid of high salts, excipients, or reducing agents have been adopted for the final formulation of therapeutic enzymes intended for systemic administration. The most commonly used method for the formulation of therapeutic enzymes involves lyophilization in the presence of mannitol and a physiological buffer. Since many enzymes can be denatured by heat even in the dry state, refrigeration or freezing during transit or storage is customary.

The therapeutic utility of an enzyme preparation is largely dependent on its stability as finally formulated. A number of chemical modifications have been employed and include binding to inert surfaces and encapsulation to increase the resistance of these intrinsically labile macromolecules to decomposition. Unfortunately, some of these modifications can interfere with the optimal kinetic performance of the enzyme. The development and deployment of recombinant DNA technologies have made significant contributions toward meeting the goal of mass production of human enzymes for human use. However, it must be stressed that post-translational modifications of a given enzyme may not occur in the foreign organism or cell used to manufacture it. Some of these modifications may prove to be essential for catalytic activity, or for imparting other necessary therapeutic characteristics, and it may be necessary to carry out these modifications by chemical means (17).

2. Uses

Therapeutic enzymes have a broad variety of specific uses, ie, as oncolytics, thrombolytics, or replacements for inherited deficiencies. Additionally, there is a growing group of miscellaneous enzymes of diverse function.

2.1. Oncolytic Enzymes

An early report of cancer chemotherapy using an enzyme, pepsin [9001-75-6], was published in 1922 (18); its clinical use was surrounded by controversy.

2.1.1. Enzymes Degrading Amino Acids

One principal stimulus for the successful enzymic therapy of cancer came from the observation that L-asparaginase from guinea pig serum had striking antilymphoma activity (4). This enzyme decomposes L-asparagine [56-86-0] to L-aspartic acid [73-22-3] and ammonia [7664-41-7]. Subsequent clinical trials with L-asparaginase from bacterial sources, principally *Escherichia coli* and *Erwinia carotovora*, showed pronounced antileukemic activity in the therapy of acute lymphoblastic leukemia (19). The attachment of poly(ethylene glycol) to proteins protracts their plasma half-lives, and reduces their immunogenicity (20); the covalent attachment of poly(ethylene glycol) to L-asparaginase yields a polymer–enzyme conjugate whose plasma half-life ranges from 2 to 3 weeks, with minimal immunogenicity. In contrast, unconjugated bacterial L-asparaginase preparations have plasma half-lives of approximately 24 hours, require repetitive dosing (21, 22), are strongly immunogenic, and its use is complicated by the rapid emergence of L-asparaginase-resistant tumor cells. The poly(ethylene glycol) conjugate of L-asparaginase exhibits activity even in patients with non-Hodgkin's lymphoma, a disease ordinarily refractory to the unmodified enzyme (21).

Therapy with L-asparaginase is most successful against tumors exhibiting a deficiency in the synthesis of L-asparagine. Most normal cells exhibit a healthy capacity to synthesize this nonessential amino acid and are not damaged by exposure to L-asparaginase (23). This finding demonstrates that biochemical differences between normal and cancer cells can be exploited for successful cancer chemotherapy.

L-Asparaginase is used for the treatment of appropriate lymphoproliferative disorders; in two clinical trials, L-asparaginase was used in combination with chemotherapy for the treatment of refractory acute

nonlymphocytic leukemia in children (24) and adults (25). A moderate efficacy, attributable to the enzyme, was demonstrated in both trials.

A review of the synthetic pathways and requirements for amino acids of eukaryotic cells shows that tumor cells require abundant supplies of L-glutamine, L-arginine, L-cysteine, L-tryptophan, and other amino acids for growth and survival (26). An L-glutaminase–asparaginase preparation from *Acinetobacter,* which hydrolyzes L-glutamine [70-47-3] or L-asparagine to L-glutamic acid [56-84-8] or L-aspartic acid, respectively, shows activity against cultured tumor cells (27–29). In addition, the chemical modification of this protein with succinic anhydride prolongs its plasma half-life in humans (30) in much the same way as the poly(ethylene glycol) modification. L-Arginine desimidase from *Streptococcus faecalis*, mammalian L-arginase coupled to poly(ethylene glycol), and L-arginine decarboxylase, which converts L-arginine to agmatine, have all shown antitumor activity vs experimental neoplasms (27). In a study where 17 commercially available amino acid degradative enzymes were tested, L-asparaginase and L-lysine decarboxylase [9024-76-4], which decarboxylates L-lysine [63-68-3] to form cadaverine [462-94-2], were found to be effective inhibitors of lymphocyte growth, followed by arginase which hydrolyzes L-arginine [7004-12-8] to L-ornithine [70-26-8] and urea [57-13-6], and L-tyrosinase which converts L-tyrosine [63-91-2] to dopaquinone [4430-97-1]; other enzyme preparations were ineffective (31).

Although essential amino acids are required by both host and tumor, deprivation of select essential amino acids for 2–3 weeks is tolerated by the host yet exerts a pronounced antiproliferative effect on the tumor. Thus, treatment of mice with indole-3-alkane-α-hydroxylase [63363-76-8] from *Pseudomonas,* which transforms L-tryptophan [73-22-3] to 3-indolylglycaldehyde, lowers the concentration of L-tryptophan in plasma, brain, and lungs, and inhibits the growth of a variety of tumors (32–34).

Certain tumor cells cannot synthesize L-methionine from L-homocysteine. For this reason, L-methionine degrading enzymes from *Clostridium sporogenes* and other sources have been used successfully against a variety of murine tumors (32–34). L-Phenylalanine ammonia lyase [9024-28-6], an enzyme which deaminates both L-phenylalanine [63-91-2] and L-tyrosine, is effective in the therapy of transplantable tumors of mice (35, 36) but has yet to be studied in a systematic way against human cancer. Antimetastatic activity was observed after treatment of mice bearing Lewis lung carcinoma with L-lysine-α-oxidase (37). Other amino acid-degrading enzymes with oncolytic activity in animal tumors include L-tyrosinase (38); L-serine dehydratase, which decomposes L-serine [56-45-1] to form pyruvate (39); L-threonine deaminase, which deaminates L-threonine [56-45-1] to form 2-oxobutanoate [600-18-0] (40); L-tryptophanase (41); and L-cysteine–cystine degrading enzymes (42).

A different kind of enzyme, translocase [80700-39-6], which transfers a fragment of NAD to the protein–synthesis factor (elongation factor 2), is catalyzed by diphtheria toxin, thereby inhibiting protein synthesis (43). In tumor cells, the rate of protein synthesis is 100 to 1000 times more sensitive to diphtheria toxin than the analogous process in normal cells (41); therefore, diphtheria toxin is selectively toxic to tumor cells.

2.1.2. Enzymes Degrading Macromolecules

Enzymes that degrade macromolecules such as membrane polysaccharides, structural and functional proteins, or nucleic acids, have all shown oncolytic activity. Treatment strategies include the treatment of inoperable tumors with pepsin (1); antitumor activity of carboxypeptidase G_1 (44); cytotoxicity of ribonuclease (45–47); oncolytic activity of neuraminidase (48–52); therapy with neuraminidase of patients with acute myeloid leukemia (53); antitumor activity of proteases (54); and hyaluronidase treatment in the management of human solid tumors (55).

Carboxypeptidases, especially carboxypeptidase G_1 [9054-73-3] from *Pseudomonas,* hydrolyze peptide bonds at the carboxyl terminus of proteins and, in an analogous process, hydrolyze the terminal ^{L}-glutamic acid moieties of folic acid, which is naturally polyglutamylated in cells. This action induces a state of folic acid deficiency deleterious to the tumor cell. Systemic administration of carboxypeptidase G_1 also hydrolyzes polyglutamylated methotrexate [82334-40-5], an analogue of folic acid with antitumor activity, in blood but

not in cerebrospinal fluid because its molecular mass prevents it from penetrating the blood brain barrier (44). This selectivity prolongs the action of methotrexate in brain while the drug is polyglutamated in other compartments. A pilot human trial of carboxypeptidase G_1 in a patient with a brain tumor showed that the enzyme was excluded from the cerebrospinal fluid by the blood brain barrier. Such selectivity permitted the antifolate, methotrexate [59-05-2] to exert its antiproliferative action on the brain tumor; at the same time the patient's normal tissues were protected from the side effects of the drug (56).

The ribonucleases, a family of enzymes that degrade RNA, are abundant in plasma, urine, and tissues (45). Studies with seminal ribonuclease have shown that it exerts antitumor activity against several murine plasmacytomas (46); antitumor activity was also observed after administration of a ribonuclease from *Bacillus intermedius* to mice bearing a transplantable lymphocytic leukemia (47).

Neuraminidase removes sialic acid residues from the surface of cancer cells, thereby altering their immunogenicity and in certain cases rendering them susceptible to the host's immune response (48–52). Although this treatment alters the physical, biological, and immunological properties of tumor cells, it does not change the cells' viability. Injection of these treated cells regularly induces a specific immune reaction and improves the survival of animals subsequently inoculated with untreated tumor cells. Treatment with neuraminidase of mice bearing solid metastasizing tumors, ie, Lewis lung carcinoma and mammary carcinoma, yields noteworthy increases in life-span (55, 57, 58). Significantly longer remissions and survival in leukemic patients were demonstrated when they were immunized with allogenic myeloblasts previously treated with neuraminidase from *Vibrio cholerae* (53).

A second family of carbohydrate-degrading enzymes, the lysozymes, produces synergistic antimetastatic activity when co-administered with cisplatin [15663-27-1] to mice whose primary tumor had been surgically removed (51).

Mucopolysaccharide levels are increased in many cancer cells; this increase is accompanied by a decreased vascular supply. Hyaluronidase [9001-54-1], obtained from bovine testis, dissolves the mucopolysaccharides surrounding a tumor, thereby allowing cytotoxic agents to penetrate the neoplasm with enhanced facility. In clinical studies hyaluronidase was given to patients with malignant tumors, alone or in combination with 5-fluorouracil [51-21-8]; significant decreases in tumor mass were observed (55).

Protease has also been demonstrated to exhibit antitumor activity. Intratumoral microinjection of proteases from *Serratia marcescens* into mice with solid tumors resulted in necrosis and solubilization of the tumor mass (53).

Ricin [9009-86-3], a phytotoxin found in the seeds of the castor oil plant *Ricinus communis*, conjugated to murine monoclonal antibody (Immunogen Corp.), has been approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with B-cell leukemia and lymphoma (59).

2.2. Thrombolytic Enzymes

Although atherosclerosis and the accompanying vascular wall defects are ultimately responsible for such diseases as acute pulmonary embolism, arterial occlusion, and myocardial infarction, the lack of blood flow caused by a fibrin clot directly results in tissue injury and in the clinical symptoms of these devastating diseases (54). Thrombolytic enzyme therapy removes the fibrin clot by dissolution, and has shown promise in the treatment of a number of thrombo-occlusive diseases (60).

The most important use of thrombolytic therapy is in acute myocardial infarction, a leading cause of death in the United States. The fibrin cross-links that form the structural skeleton of a thrombus can be effectively degraded by an endogenous serine protease, plasmin [9001-90-5], which is released following activation of its zymogen precursor, plasminogen [9001-91-6]. Human plasminogen is an inactive plasma protein secreted by the liver and circulating at levels of about 20 mg/dL. This single-chain glycoprotein has a molecular weight of about 90,000, with 790 amino acids and 22 disulfide bonds that serve to maintain its convoluted three-dimensional structure (61, 62). Plasminogen is converted to plasmin by a specific proteolytic cleavage yielding two chains,

bound together by two disulfide linkages; this product is the catalytically active form of the protease, and the overall process is customarily denoted plasminogen activation.

Since the blood level of plasminogen is rarely a rate-limiting factor in its activation, thrombolytic therapy is ideally designed to activate this precursor at the site of thrombus decomposition. The thrombolytic agents currently being used clinically include streptokinase; anistreplase [81669-57-0], an anisoylated-plasminogen–streptokinase-activator-complex; urokinase; scuPA, [82657-92-9], ie, single-chain urokinase plasminogen activator (Saruplase, prourokinase); and tissue plasminogen activator, tPA (Alteplase).

2.2.1. Streptokinase

The fibrinolytic activity of streptokinase, isolated from strains of hemolytic *Streptococci,* was first demonstrated in 1933 (63). Streptokinase is a secreted protein product inasmuch as filtrates free of demonstrable bacteria were found to dissolve fibrin clots with rapidity. Streptokinase has a molecular weight of about 47,000 with a single chain of 415 amino acids; there are no intramolecular disulfide bonds (64). The complete nucleotide sequence of the gene encoding the RNA for this protein has been reported (65, 66).

The fibrinolytic activity of streptokinase, mediated by generation of plasmin from plasminogen, is initiated by binding to the β-chain, ie, Val 561-Asp 790, of plasminogen (67, 68). This results in a conformational change in the plasminogen molecule which, in turn, acts as a specific protease in activating a second plasminogen molecule to plasmin (69, 70). To achieve optimal thrombolysis, the ratio of streptokinase to plasminogen should be approximately 1:10 (71).

Indications for treatment with streptokinase include acute occlusion of arteries, deep vein thrombosis, and pulmonary embolism. Streptokinase therapy in coronary thrombosis, which is the usual cause of myocardial infarction (54, 71, 72), has proved to be valuable. In this frequently fatal condition, the enzyme is administered intravenously at a dose of 1.5 million units over 60 min, or given by intracoronary infusion at a 20,000- to 50,000-unit bolus dose followed by 2000 to 4000 units/min for 60 min; therapy must be instituted as soon as practicable after the diagnosis of heart attack is made. For deep vein thrombosis, pulmonary embolism, or arterial occlusion, streptokinase is infused at a loading dose of 250,000 units given over 30 min, followed by a maintenance dose of 100,000 units over a 60-min period.

Streptokinase has an initial plasma half-life ($t_{1/2}\ \alpha$) of 18 min, and a β half-life of 83 min (73); it is well recognized that the thrombolytic efficacy of the enzyme decreases as the age of the thrombus increases; thus, thrombolysis is significantly decreased when therapy is initiated more than three hours after an occlusion (74).

Several clinical trials have been conducted with streptokinase administered either intravenously or by direct infusion into a catheterized coronary artery. The results from 33 randomized trials conducted between 1959 and 1984 have been examined (75), and show a significant decrease in mortality rate (15.4%) in enzyme-treated patients vs matched controls (19.2%). These results correlate well with an Italian study encompassing 11,806 patients (76), in which the overall reduction in mortality was 19% in the streptokinase-treated group, ie, 1.5 million units administered intravenously, compared with placebo-treated controls. The trial also shows that a delay in the initiation of treatment over six hours after the onset of symptoms nullifies any benefit from this type of thrombolytic therapy. Conversely, patients treated within one hour from the onset of symptoms had a remarkable decrease in mortality (47%). The benefits of streptokinase therapy, especially in the latter group of patients, was still evident in a one-year follow-up (77). In addition to reducing mortality rate, there was an improvement in left ventricular function and a reduction in the size of infarction. Thus early treatment with streptokinase is essential.

In a more extensive international trial, 17,187 patients were treated intravenously with streptokinase alone, aspirin alone, a combination of streptokinase and aspirin, or placebo (78). Streptokinase and aspirin were equally effective in treating acute myocardial infarction, each decreasing mortality by 25%; their combination further reduced mortality by 42%. A significant reduction in mortality was seen even in those patients treated up to 24 hours after the onset of symptoms.

In addition to the intravenous route, streptokinase is also administered by the direct intracoronary route. In a Dutch study with 533 patients treated intracoronarily, a significant reduction (12%) in mortality was noted (79). Of the two methods of administration, the intravenous route appears to be the method of choice because of ease of administration and the shorter lag period before therapy is initiated; the lag period observed in studies utilizing the intracoronary route is attributable to the time required for catheterization.

The thrombolytic efficacy of streptokinase treatment may be compromised by the presence of antibodies to the enzyme in the patient's blood. These neutralizing antibodies may arise because of a prior streptococcal infection, or prior streptokinase treatment (80–82). Titers of antibodies sufficient to neutralize a complete dose of 1.5 million units of streptokinase may be present even one year after enzyme treatment (83).

Since streptokinase has no clot-selective property, its therapeutic levels cause extensive activation of circulating plasminogen to plasmin. The increased plasmin levels not only cause degradation of the offending thrombus (therapeutic effect), but also cause degradation of coagulation factor V, factor VIII, and fibrinogen, and a reduction in α_2-antiplasmin (84). All of these factors produce a systemic lytic state that significantly increases the potential for hemorrhage, a serious hazard of thrombolytic therapy. Bleeding complications, including hemorrhagic strokes, have occurred, although infrequently, after administration of this enzyme. Another important side effect of therapy is related to the antigenic potential of streptokinase. Since the enzyme is obtained from bacterial sources, it can induce immune responses from allergy (fever, rash) to life-threatening anaphylactic shock (0.1% in one study (76)). However, the benefits of fibrinolytic activity with this enzyme clearly outweigh such side effects as allergic reactions, hypotension, or minor bleeding. Moreover, in extensive clinical trials, serious side effects have proven to be rare; eg, there were no significant differences in the total number of strokes in the treated vs the placebo groups.

2.2.2. Anistreplase (APSAC)

The general drawbacks of streptokinase therapy are its short plasma half-life (18–20 min) and its poor clot selectivity. These drawbacks have been addressed by making conjugates of the enzyme. Anistreplase is a complex of streptokinase and acylated human Lys-plasminogen, synthesized by a noncovalent attachment of an acyl group to the active center of a streptokinase-Lys-plasminogen complex (85, 86). Streptokinase on its own is inactive as a plasminogen activator, and must first form a complex with circulating plasminogen; this complex converts the second plasminogen molecule to plasmin. Anistreplase, on the other hand, is a fully active plasminogen activator. More importantly, it activates plasminogen at the site of deposition of the thrombus (85, 86).

Anistreplase has a considerably longer α half-life than streptokinase, ie, 90 min compared to 20 min (87, 88). Moreover, it does not require prolonged infusion to achieve its thrombolytic effects. Anistreplase was found to be highly effective after a single intravenous dose of 30 units over a 5-min period compared to a 60-min infusion of 1.5 million units of streptokinase (89–94). In direct comparative studies, anistreplase was as effective as intracoronary (95, 96) and intravenously (96–100) administered streptokinase. In a randomized, double-blind, placebo-controlled study (AIMS trial) with 1004 patients given this modified enzyme, the 30-day mortality rate was 12.2% for patients receiving placebo, compared to 6.4% for patients who received 30 units of anistreplase intravenously within six hours of the onset of symptoms (101).

The side effects of anistreplase appear to be similar to those of streptokinase, including immune reactions and a systemic lytic state conductive to hemorrhage.

2.2.3. Urokinase

This trypsin-like serine protease consists of two polypeptide chains held together by a single disulfide bond. Urokinase exists in two molecular forms, ie, a high mol wt form of about 55,000, and a low mol wt variant of 33,000 (102, 103). The half-life of urokinase, 10 min, is shorter than that of streptokinase (104). One of the more prominent disadvantages of streptokinase or anistreplase therapy is the antigenic potential; urokinase is

a naturally occurring enzyme present in human urine, plasma, and tissues, and does not provoke an immune response directed against it. Urokinase can directly activate plasminogen, but is a nonspecific plasminogen activator, ie, it lacks clot selectivity, although the presence of fibrin does slightly stimulate the activation of plasminogen by urokinase (105). In early studies, urokinase was isolated from kL of human urine; in the early 1990s, recombinant DNA technology is used to manufacture the enzyme in cultured bacteria.

Compared to streptokinase, urokinase has been less extensively studied because of its high cost, ie, about 10 times that of a comparable treatment with streptokinase. In addition to the indications described for streptokinase, urokinase is indicated for use in patients with prior streptokinase treatment, or prior *Streptococcal* infection. Urokinase is commonly used at a loading dose of 4400 units/kg, with a maintenance intravenous infusion dose of 4400 units/kg/h for thromboses other than acute myocardial infarction. In the latter case, a much larger dose, ie, 0.5–2.0 million units/h or a bolus dose of 1.0 million units followed by a 60-min infusion with 1.0 million units, has been found optimal (106). An intracoronary dose of 2000 units/min for two hours was used in one comparative study with intracoronary streptokinase (107). In this study, urokinase exhibited efficacy equivalent to streptokinase with fewer side effects. Other studies with intracoronary urokinase have administered doses ranging from 2,000 to 24,000 units/min with a reperfusion efficacy of 60–89% (108–112). In another urokinase trial, 2.0 million units were administered intravenously, resulting in a thrombolytic efficacy of 60% (113). Effectiveness in terms of reduction in mortality rate has not been determined because of the small number of patients studied.

Overall, urokinase is better tolerated than streptokinase with no significant hypotension or allergic reactions. Intracoronary administration of urokinase, at a dose comparable in efficacy to streptokinase, also causes less reduction of fibrinogen, resulting in significantly fewer bleeding complications (107). It is generally accepted that, apart from the immune reactions associated with streptokinase therapy, the two enzymes are essentially the same as regards other side reactions.

2.2.4. Single-Chain Urokinase Plasminogen Activator (scuPA)

This is the single-chain precursor of urokinase, with an enhanced fibrin specificity and clot selectivity (114–117). scuPA (pro-urokinase) was first purified from human urine using a fibrin-Celite column (118). By selective proteolysis, it activates only those plasminogen molecules directly associated with a fibrin clot. This results in a lag period until additional plasminogen molecules are exposed by the released plasmin at the clot surface (119). scuPA is a relatively new thrombolytic agent; its first clinical trial was reported in 1986 using a preparation derived from a cultured human renal adenocarcinoma cell line (120). Four of the six patients in this study showed beneficial therapeutic effects. This was followed by a multicenter trial in which patients were treated intravenously with 15–60 mg scuPA, urokinase, or its combination, ie, 200,000 units of bolus urokinase followed by a 60-min infusion with 48 mg scuPA (121–124). The patency rate, used as an index of reopening of a thrombosed vessel, was 55% for scuPA; that of the combination was 82%. Inclusion of urokinase in the regimen increases the patency rate without significantly changing the fibrinogen, plasminogen, or α2-antiplasmin degradation profile observed with scuPA alone. Pretreatment with urokinase reduces the lag period of the first phase of scuPA action, and therefore acts synergistically with it. Although several studies have demonstrated the fibrinolytic specificity of scuPA, the overall side effects of this agent in clinical trials are quite similar in nature to those of its nonspecific analogue, urokinase.

2.2.5. Tissue Plasminogen Activator (tPA)

While streptokinase and urokinase can effectively induce clot dissolution in the majority of patients if given early, they lack clot specificity. Treatment with these enzymes results in a systemic lytic state attributable to their degradative action on circulating fibrinogen. Tissue plasminogen activator (tPA) was developed to achieve rapid and specific thrombolysis.

tPA was first identified in 1966 as a naturally occurring serine protease (125). However, it was not characterized until 1979 when a sufficient quantity of tPA was purified from human uterine tissue (126). A more extensive characterization of tPA was performed following its purification from a human melanoma cell line that produces large quantities of the molecule (127, 128). Subsequent to these studies, human tPA was cloned and purified by recombinant DNA technology (129). The molecular weight of tPA is about 70,000, with 527 amino acids in its single-chain form. The single-chain molecule is converted to its active two-chain form by hydrolysis of the Arg275–Ile276 peptide bond (130). At the clot surface, single-chain tPA generates small quantities of plasmin by plasminogen activation; these then initiate the thrombolytic cycle by generating additional plasmin at the clot surface, resulting in dissolution. The presence of fibrin dramatically increases the catalytic activity of tPA (131, 132). The initial half-life of exogenous tPA in humans is about 5 to 8 min (133).

In the first clinical trial of tPA for acute myocardial infarction, the molecule purified from melanoma cell cultures was used (134). A reperfusion rate of 85%, ie, six of seven patients, was observed after infusion of 6 to 14 mg of tPA at the rate of 12–24 mg/h. More importantly, no changes in fibrinogen, α2-antiplasmin, or circulating plasminogen levels were detected following treatment. With the availability of human tPA produced by recombinant DNA methods, additional clinical studies have been conducted. The *in vitro* thrombolytic activity of recombinant tPA and of tPA obtained from melanoma cells was identical (135). In clinical trials with recombinant tPA for coronary thrombosis, a 73% reperfusion rate was observed after the intravenous infusion of 0.5 mg/kg for 60 min (136); an 87% rate was observed when the dose was raised to 0.75 mg/kg given over 120 min (137). The mean time to recanalization averaged 46 ± 18 min. These trials show that recombinant tPA is efficacious, and that its use is accompanied by minimal side effects. Another clinical trial, designed to compare the efficacy of recombinant tPA with streptokinase, yielded a 70% patency rate of the occluded artery in the recombinant tPA group, given 0.75 mg tPA/kg over 90 min, compared to a 55% patency rate in the streptokinase group, given 1.5 million units of enzyme over 60 min (138). Although there was no significant difference in the percentage patency achieved in these studies, there was a significantly greater degradation of fibrinogen in the streptokinase treated group. Thus, fibrinogen levels fell below 0.5 g/L in 90% of the streptokinase treated patients, but in only 5% of the recombinant tPA patients. In another comparative trial, 80 mg recombinant tPA over three hours was compared to 1.5 million units of streptokinase. The recombinant tPA treatment resulted in a 60% reperfusion rate at 90 min vs a 35% rate when streptokinase was used alone (137). Additional randomized clinical trials show recombinant tPA to be more efficacious than streptokinase in coronary thrombolysis, with an average of 75% patency for recombinant tPA and about 50% with streptokinase (138–143). In one controlled, randomized study with 5013 patients, a significant decrease in mortality was observed with recombinant tPA compared to patients given placebo (144). However, in two more recent (ca 1990) large-scale trials no significant differences between streptokinase and the tPA treatments were observed: in a study of 12,490 patients (145), in-hospital mortality and severe left ventricular damage were used as end points; in the International Study Group's SK/tPA trial in which 8401 patients were randomly assigned, mortality was used as the sole end point. In a more recent and larger ISIS-3 trial (147) comprising 46,092 patients, direct comparisons were made between the three thrombolytic agents, streptokinase, recombinant tPA, and APSAC; no significant differences among the three agents could be demonstrated. A 35-day mortality rate was used as the primary end point of this trial, and the mortality rates were streptokinase, 10.5%; recombinant tPA, 10.3%; and APSAC, 10.6%. In addition, patients in the streptokinase group had a significantly lower incidence of strokes than patients in the recombinant tPA and the APSAC groups. This is paradoxical since the development of recombinant tPA was based on the premise that it is clot-specific and thus should not have produced bleeding complications.

2.2.6. Combination of Plasminogen Activators

Since each of the five agents discussed achieve thrombolytic activity in a unique fashion on the basis of individual molecular structures, various combinations of these agents are being evaluated with the goal of

minimizing side effects, especially the risk of hemorrhage, and of augmenting efficacy (148). For example, administration of a small amount of urokinase prior to treatment with scuPA results in an increase in patency rate with no further decrease in circulating fibrinogen (122, 123). The most extensively studied combination is of scuPA and tPA. Synergistic thrombolytic activity of this combination has been shown both *in vitro* (149–151), and *in vivo* (152, 153). In an initial clinical study with nine patients, combination of low doses of scuPA (10 mg) and tPA (10 mg) infused over a 60-min period resulted in a patency rate of 78% (102). In a slightly larger patient group (38 patients), the combination of recombinant tPA (12 mg) and scuPA (48 mg) infused over 30- and 40-min, respectively, resulted in a patency rate of 61% at 60 min and 82% at 90 min, with minimal side effects (154). Similar results have been obtained in two other human trials (155, 156). Combination studies have also been designed to reduce the amount of tPA used in these treatments because of its high cost. Combination of one-half the usual dose of recombinant tPA, ie, 50 mg instead of 100 mg, with a full dose of streptokinase (1.5 million units) resulted in a 75% patency rate (157); this rate is comparable to that achieved in other clinical trials using single thrombolytic agents.

One drawback of thrombolytic therapy is a high incidence of reocclusion. In a report using a canine model, inclusion of heparin [9005-49-6] (anticoagulant therapy) in the treatment prevented this side effect (158). The combination of aspirin [50-78-2] (antiplatelet therapy) and streptokinase (thrombolytic therapy) has also shown significant therapeutic advantages (78). Although additional work is needed to establish the thrombolytic advantage of various combinations, preliminary results in this area indicate promise in terms of increased efficacy and reduced side effects.

2.3. Replacement Therapy for Inherited Enzyme Deficiencies

Correction of inborn errors of metabolism is one of the principal goals of enzyme treatment. For example, lysosomal storage diseases arise from a genetically determined deficiency in the activity of a single specific enzyme, resulting in the accumulation of substrates in the lysosomes of affected cells; this accumulation in turn results in cellular engorgement, and death. Kinetic studies predict that clinical disease will not occur until enzyme activity falls below 10% of normal (159, 160). Well-known examples of disorders in this group of thesauroses, ie, storage disorders, are TaySachs disease (hexosaminidase deficiency), Gaucher's disease (glucocerebrosidase deficiency), Krabbe's disease (galactocerebrosidase deficiency), the mucopolysaccharidoses (various eponyms), and mannosidosis (159). With the recognition that many of these disorders are the result of inadequacies of lysosomal enzymatic catabolism, it was anticipated that administration of the deficient enzyme might be used to dispose of the respective pathologic accumulations. Such treatment has yielded symptomatic improvement in select cases, but the task of forcing exogenous enzymes to enter the cytoplasm or nucleus of affected cells has not yet been successfully addressed.

One of the principal problems in cystic fibrosis is malnutrition (161); because malnutrition affects growth, and contributes to the severity of pulmonary involvement, it plays a decisive role in shortening the survival of subjects with this condition. Fat malabsorption in these patients has been attributed to pancreatic insufficiency. The use of pancreatic enzymes in these patients helps in the correction of malabsorption, thereby restoring a more normal pattern of growth (162). Pancreatin [8049-47-6], cotazym capsules, or Viokase tablets, alone or in combination with bicarbonate and/or cimetidine to reduce gastric acidity, administered to patients with cystic fibrosis result in increased fat absorption, normal levels of lipid-soluble vitamins, and improved growth in $\sim$70% of the patients given these regimen (163–165). However, the poor dose-response relationship between different preparations (166) and the correction of malabsorption is peculiarly weak. A pancreatic preparation (Creon) having an improved lipase/trypsin ratio was tested in 15 patients with severe pancreatic insufficiency; this formulation produced a dose-related increase in fat absorption as well as in weight gain (167). The combination of pancreatin and calcium gluconate (168) has been shown to be useful in reducing steatorrhea resulting from chronic pancreatitis. Enteric-coated preparations sensitive to pH can be used to ensure that a given enzyme is released only in the alkaline medium of the duodenum or proximal jejunum (169). Microsphere

preparations, consisting of enteric-coated spheres of lipase and protease inside a gelatin capsule, were superior to conventional enzyme therapy for treating fat malabsorption in patients with cystic fibrosis (170–173). In these patients, such microsphere preparations were able to reduce both steatorrhea and symptoms such as abdominal pain and frequency of evacuation (170–172).

Adenosine deaminase [9026-93-1] (ADA) deficiency in children causes profound immunodeficiency, analogous to that seen in AIDS, leading to frequent infections. This condition has been successfully treated in a few subjects with poly(ethylene glycol)-modified bovine ADA (173, 174). These patients had 1% or less of ADA activity in their erythrocytes and circulating mononuclear cells. Following treatment, the patients showed normal circulating T-lymphocytes and marked improvement in weight gain as well as in the incidence of infection.

Lactose [63-42-3] intolerance is a genetic deficiency of lactase [9031-11-2] which is responsible for hydrolyzing lactose to form D-glucose [50-99-7] and D-galactose [59-23-4]. In affected infants, ingestion of lactose, primarily from milk, causes diarrhea, cramping, bloating, and abdominal pain (175). It has been demonstrated that the addition of β-galactosidase to milk at mealtime hydrolyzes lactose effectively and reduces lactose malabsorption and intolerance to milk (175, 176). β-Galactosidase from *Aspergillus oryzae* (Lactrase) administered along with milk resulted in temporary reversal of lactose malabsorption in five of nine recipients (176).

Algucerase [9001-22-3] (β-glucocerebrosidase, β-glucosidase, ceredase), produced by Genentech, was approved by the FDA for the treatment of Gaucher's disease (177). This enzyme is obtained from human placenta. Gaucher's disease is a functional deficiency of β-glucocerebrosidase in tissue macrophages which become engorged, and thus are termed Gaucher's cells; they are found in the liver, spleen, and bone marrow. This deficiency leads to hepatomegaly. Skeletal complications including osteoporosis and osteonecrosis with secondary pathological fractures are a common feature of Gaucher's disease.

Antihemophilic factor [9001-28-9] (AHF) is a protein found in normal plasma that is necessary for clot formation. It is needed for transformation of prothrombin to thrombin. Administration of AHF by injection or infusion can temporarily correct the coagulation defect present in patients with hemophilia. Antihemophilic factor VIII (Alpha Therapeutic) has been approved by the FDA as replacement therapy in patients with hemophilia B to prevent bleeding episodes, and also during surgery to correct defective hemostasis (178).

2.4. Depolymerizing Enzymes

Depolymerizing enzymes belong to a special class of hydrolases which cleave peptide bonds using water as co-substrate. They are used widely by the topical route as antiinflammatory agents. They reduce inflammation and edema by digesting and dissolving the proteinaceous debris found in inflammatory exudates (179).

Another subclass of proteases attacks internal peptide bonds and liberates large peptide fragments. Bromelain, a plant protease derived from the stem of the pineapple plant, can even produce detectable serum proteolysis after oral administration (180). Oral therapy with bromelain significantly reduces bruising that stems from obstetrical manipulations (181). Bromelain–pancreatin combinations have been more effective in digestive insufficiency compared to either pancreatin or placebo (182, 183). Bromelain may also enhance the activity of antibiotics, especially tetracycline, when administered concurrently (184).

Chymopapain [9001-90-6], derived from the latex of the papaya tree, produces improvement in lower back pain and sciatica in the majority (75%) of recipients (185–189) when injected into the lumbar intervertebral disks of patients suffering from herniated disk (the nucleus pulposus). This treatment degrades the proteoglycans of the diseased nucleus pulposus, resulting in shrinkage of the disk and reduction of pressure on the nerve roots (190).

Collagenase specifically catalyzes the hydrolysis of collagen, and is used in debridement of dermal ulcers and burns (191). It, like chymopapain, is also useful in the treatment of herniated lumbar disks (192, 193). The rationale for collagenase treatment in this instance is based on the preponderance of collagen in herniated disk tissue, and the inability of other enzymes to dissolve collagen (194).

Papain has been shown to produce marked reductions of obstetrical inflammation and swelling, and of edema resulting from dental surgery (195–197). In a controlled experiment in dogs with gunshot wounds, a neutral protease of bacterial origin was shown to proteolyze devitalized tissue, cleaning the wounds, and promoting granulation and epithelialization (198). Streptokinase–streptodornase [37340-82-2], containing both protease and nuclease activities, has long been used in the treatment of inflammatory exudates. The streptokinase component hydrolyzes plasminogen resulting in the lysis of fibrin clots, while the streptodornase component depolymerizes deoxyribonucleic acid (DNA), leading ultimately to liquefaction of exudates. This combination also was found to be useful in the treatment of empyema, hemothorax, and loculated or clotted pleural effusions (199, 200). Streptokinase also has been used in patients maintained on continuous peritoneal dialysis who have developed recurrent bacterial peritonitis resistant to therapy. Bacterial sequestration within fibrin clots located on indwelling catheters contributes to the resistance of this type of infection to standard antibiotic therapy. Streptokinase dissolves such fibrin clots, thereby permitting effective antibiotic therapy (201).

The use of fibrin [9001-31-4] to seal wounds in ophthalmic surgery was first demonstrated in 1945 (202). Fibrin has also been used along with conventional sutures as a sealant in lamellar keratoplasty (203). Such fibrin glue effectively reduces pulmonary air leakage in animals and humans with ruptured lungs, and can also be utilized for adhesion of prematurely ruptured membranes during pregnancy (204–207). Other applications include repair of traumatically ruptured spleen in children (208), sealing of chronic gastric ulcers in patients with repeated episodes of bleeding (209), repair of leakage of lymph from a femoral cutdown site in a neonate (210), rejoining of severed Achilles' tendon (211), and repair of delicate peripheral nerves in microneurosurgery (212).

Deoxyribonuclease (DNAase), an enzyme that degrades deoxyribonucleic acid, has been used in patients with chronic bronchitis, and found to produce favorable responses presumably by degrading the DNA, contributed by cell nuclei, to inflammatory mucus (213). Lysozyme [9001-63-2] hydrolyzes the mucopeptides of bacterial cell walls. Accordingly, it has been used as an antibacterial agent, usually in combination with standard antibiotics. Topical applications are also useful in the debridement of serious burns, cellulitis, and dermal ulceration.

The proteolytic enzymes, trypsin, chymotrypsin, and chymoral [8076-22-0], in combination, have been used for the treatment of post-operative hand trauma, athletic injuries, and sciatica (214–216). Trypsin has also been used successfully in treating hyaline membrane disease of newborn babies, a condition usually fatal without treatment (217). Immobilized preparations of trypsin are useful in treating acute radiation cystitis following pelvic x-irradiation therapy (218).

Superoxide dismutase has been approved by the FDA for preventing reperfusion injury or damage to donor organ tissue (178). This enzyme is prepared by recombinant DNA technology and marketed by Bristol-Myers and Pharmacia-Chiron.

Improvements of the electrocardiograms of patients with acute myocardial infarction have been demonstrated following hyaluronidase treatment (219). Hyaluronidase destroys hyaluronic acid, a mucopolysaccharide, thus allowing vital molecules to penetrate the normally impermeable connective tissue barrier. Accordingly, hyaluronidase is believed to limit the extension of a myocardial infarct by increasing the diffusion of nutrients through the liquefied ground substance. Hyaluronidase also has been used to reduce intraocular pressure during cataract surgery (220, 221). Intrathecal treatment with hyaluronidase has been used in pediatric practice for the management of hydrocephalus resulting from tubercular meningitis (222). In extravasation injuries of children, hyaluronidase, injected after an intravenous infiltration, significantly reduces tissue damage (223).

2.5. Enzymes as Antidotes.

Rhodanese [9026-04-4] given along with thiosulfate to counteract cyanide poisoning in mice (224) was the first enzyme used as an antidote. This combination raised the LD_{50} of potassium cyanide in mice by eightfold (224).

Superoxide dismutase, an enzyme that decomposes the highly toxic oxygen free radical $O^{-}{}_{2}$, has been put into veterinary use as an antiinflammatory agent with efficacy in the treatment of traumatic arthritis of horses (225). The identification of free-radical toxicity as being operative in poisonings with an increasing variety of pharmacologic agents is expected to increase the use of superoxide dismutase (225, 226). Superoxide dismutase has also been shown to exert antiinflammatory effects on uveitis (227–230), as has catalase, and to protect murine and human bone marrow progenitor cells from the effects of radiation (231). Liposomal superoxide dismutase has been used to treat radiofibrosis caused by excessive radiation and leading to fibrosis at a local target site (232). Superoxide dismutase prepared by recombinant technology prevents reperfusion injury of the ischemic spinal cord in dogs (233).

Glutathione peroxidase [9013-66-5] oxidizes glutathione, and helps to remove inorganic and organic hydroperoxides (227). It exhibits antiinflammatory activity in experimental uveitis of rats (234).

Bilirubin oxidase [80619-01-8], derived from *Myrothecium verrucaria*, was modified with polyethyleneglycol; when this conjugate was injected intravenously to jaundiced rats, the plasma bilirubin dropped to normal levels. This approach might have potential in the treatment of hyperbilirubinemia, fulminant hepatitis, and neonatal bilirubin encephalopathy (177).

In some patients with IgA nephropathy (IgAN), intraglomerular coagulation plays a role in depositing fibrinogen (235, 236). IgAN patients treated with urokinase show a marked improvement in urinary protein concentration, serum creatinine, and blood urea nitrogen levels (237).

3. Immobilized, Derivatized, and Entrapped Enzymes

3.1. Immobilized Enzymes

Immobilized catalase was shown in the early 1970s to correct the enzyme deficit in genetically acatalasemic mice (238). The ideal goals of enzyme modification are to increase enzyme stability *in vivo* by rendering them resistant to the action of endogenous proteinases, inhibitors, and antibiotics; increase their stability during storage; decrease their immunogenicity, antigenicity, and toxicity; decrease the dose of a given enzyme preparation; and prolong enzyme action as indicated by the enzyme's pharmacokinetic profile (239–241). With the development of techniques for binding enzymes to insoluble supports, immobilized enzymes have been used not only in clinical analysis but also for therapeutic purposes. For example, L-asparaginase therapy with an extra-corporeal device in patients with malignant lymphoma has resulted in repeated remissions of metastases (242). This technique utilizes L-asparaginase covalently bound to the surface of a battery of plates contained in a portable chamber which has been inserted between an artery and vein.

3.2. Enzyme Conjugates

One approach used to prolong residence time of a given enzyme in the circulation is to conjugate that enzyme with albumin [103218-45-7], a natural plasma protein. Cross-linked preparations of albumin and either uricase [9002-12-4], which oxidizes urea to form allantoin [97-59-6], or L-asparaginase, using the bifunctional reagent glutaraldehyde, were first described in 1974 (243). Conjugates of albumin with L-asparaginase or α-1,4-glucosidase were shown to be 4 to 10 times more stable to denaturation by either heat or trypsin than the native enzyme (244). Albumin conjugates of α-1,4-glucosidase, superoxide dismutase, and uricase were also shown to have notably longer plasma halflives than their unconjugated counterparts (245).

One limitation of enzyme replacement therapy is the targeting of enzyme proteins to appropriate sites of substrate accumulation. Administration of a cholesterol esterase conjugated to albumin results in the degradation of pathologic cholesterol ester accumulations within the lysosomes of fibroblasts from a patient with cholesterol ester storage disease (246).

Hemodialysis with microencapsulated urease and an ammonia ion adsorbent, zirconium phosphate [13772-29-7], has been used (247) to delay the onset of dialysis therapy in patients retaining some renal function, and to reduce the time between dialysis treatment.

Poly(ethylene glycol) (PEG) molecules attached to adenosine deaminase (ADA) have been used in patients exhibiting symptoms of the severe combined immunodeficiency syndrome (SCID) caused by ADA deficiency. The modified enzyme has a plasma half-life of weeks as compared to the unmodified enzyme (minutes) (248). PEG-L-asparaginase has induced remissions in patients with non-Hodgkin's lymphoma (248). However, one disadvantage of PEG-enzyme treatment is its expense, ie, a year's treatment costs about $60,000 (248).

3.3. Erythrocyte Entrapment of Enzymes

Erythrocytes have been used as carriers for therapeutic enzymes in the treatment of inborn errors (249). Exogenous enzymes encapsulated in erythrocytes may be useful both for delivery of a given enzyme to the site of its intended function and for the degradation of pathologically elevated, diffusible substances in the plasma. In the use of this approach, it is important to determine that the enzyme is completely internalized without adsorption to the erythrocyte membrane. Since exposed protein on the erythrocyte surface may elicit an immune response following repeated sensitization with enzyme loaded erythrocytes, an immunologic assessment of each potential system in animal models is required prior to human trials (250).

Chemically or enzymatically modified preparations of enzymes present another potential immunologic problem since modifications designed to stabilize enzyme activity may produce new antigenic determinants. For example, the attachment of carbohydrate moieties to enzymes using glycopeptides, carbohydrate polymers, or individual sugars may alter the *in vivo* fate of a given enzyme, causing it to become immunogenic (250). Moreover, addition of carbohydrate residues may also expose new antigenic sites by inducing conformational changes in the protein; additionally, the carbohydrate moiety itself might act as a hapten or, if large enough, as a neo-antigen in its own right (250).

As of this writing there are eight methods of erythrocyte entrapment. Six methods depend on loading via hypotonic exchange, one method depends on chlorpromazine-induced endocytosis, and one depends on voltage-step induced transitory permeation. Although only minor leakage of entrapped enzyme is detected by these methods, up to 11% of entrapped glucose is released within three hours of entrapment (251).

Erythrocyte-entrapped β-glucuronidase has been retained in the circulation and in the liver of β-glucuronidase-deficient mice for longer periods of time than intravenously administered free enzyme (251). Entrapment in autologous erythrocytes may provide an effective means of optimizing the delivery and protection of exogenous enzymes in the treatment of selective lysosomal storage diseases, such as Fabry disease and Type I Gaucher disease.

4. Economic Aspects

There has been a steady growth in the economic importance of therapeutic enzymes, with sales reaching hundreds of millions of dollars per year as of 1992. Table 2 lists the trade names and costs of some of the more commonly prescribed enzyme preparations. Despite the magnitude of use of these products, the manufacture and sale of therapeutic enzymes represents a comparatively small fraction of the production and profits of the pharmaceutical houses that market them.

Table 2. Economic Importance of Therapeutic Enzymes

Trade name	Cost,[a] $	Contents	Manufacturer
		Thrombolytic enzymes	
Activase	550/20 mg	lyophilized recombinant tPA	Genentech
Abbokinase	263/250,000 IU	lyophilized urokinase	Abbott
Kabikinase	600/250,000 IU	streptokinase	SKF
Streptase	157/250,000 IU	streptokinase	Hoechst-Roussel
Eminase		anisoylated plasminogen	Beecham
		Oncolytic enzymes	
Elspar	40/10,000 IU	L-asparaginase	Merck
		Ophthalmic enzymes	
Catarase	22/150 IU	chymotrypsin	Lolab
Zolyse	28/750 IU	chymotrypsin	Alcon
		Topical enzymes[b]	
Travase	34/14 g	sutilains	Boots
Santyl	28/15 g	collagenase	Knoll
Elase	11/10 g	fibrinolysin/deoxyribo-nuclease	Fugisawa
Granulex[c]	2.10/10 g	trypsin/papain	Hickam
Panifil[d]	2.30/10 g	papain/urea	Rystan
Panifil White[e]	5.60/10 g	papain/urea	Rystan
		Digestive enzymes[f]	
Entozyme	18/100	pancreatin/pepsin/bile salts	Robins
Viokase	15/100	pancreatin/pepsin/bile salts	Robins
Pancrease	30/100	pancreatin/pepsin/bile salts	McNeil
Cotazyme-S	24/100	pancreatin/pepsin/bile salts	Organon
Festal II	17/100	pancreatin/pepsin/bile salts	Hoechst-Roussel
Creon	32/100	pancreatin/pepsin/bile salts	Reid-Rowell
Ilozyme	77/250	pancreatin/pepsin/bile salts	Adria
Hi-Vegi-Lip	15/100	pancreatin/lipase/protease/amylase	Freeda
Dizymes	9/100	pancreatin/lipase/protease/amylase	Recser Labs
Digestozyme	8/100	pancreatin/pepsin/HCl	Various
Acro-Lase	5/50	amylase/protease/lipase/cellulose	Arco
Enzobile	13/100	pancreatic enzyme/cellulase/pepsin	Mallard
Kanulase	14/50	amylase/protease/lipase/pepsin/bile extract/cellulase/glutamic acid	Sandoz
Digestalin	3/100	pancreatin/pepsin/papain/activated charcoal/bismuth/barberis/hydrastis	Vortech
Phazyme-PB	15/100	amylase/protease/lipase/simethicone /phenobarbital	Reed and Carnrick
Kutrase	29/100	amylase/pancrease/lipase/cellulase /hyoscyamine/phenyltoloxamine	Schwarz
Donnazyme	20/100	pancreatin/pepsin/homatropine/hyoscyamine /atropine/scopolamine/phenobarbital	Robins
Panase	21/100	pancreatic enzymes	Qualitest

[a] Costs (per units indicated) obtained from Ref. 252.
[b] Used to selectively digest necrotic tissue in wounds and burns.
[c] 12/2 oz.
[d] 103/1 lb.
[e] 16/1 oz.
[f] Available in tablet or capsule formulations.

5. Health and Safety Factors

Repetitive doses of foreign proteins may produce severe immunologic reactions, ranging from mild allergy to anaphylactic shock and death. Parenteral administration of metabolically active enzymes can be acutely toxic on account of their biochemical effects (253, 254). However, large metabolically effective doses of these products may be less toxic than small doses, due to immune paralysis, wherein large doses of antigen repress the expression of the complementary antibody. It is essential to circumvent such allergic responses in the enzyme therapy of genetic diseases, because replacement enzyme therapy may be required over a lifetime. By contrast, treatment of cancer with enzymes may be somewhat less problematic because of the attenuation of the immunologic reactivity of patients in many cases. A significant effort is being directed toward the use of human sources of enzymes, or of human-type enzymes produced in cultures of prokaryotic or eukaryotic organisms, with a goal of reducing or eliminating the problems of antigenicity.

For purposes of dosage, the specific activity of an enzyme is usually expressed as International Units (IU) rather than in terms of weight. However, unit measurements do not provide information on the absolute purity of a given product. Moreover, purity is not as critical an attribute for oral enzymes, as opposed to those administered parenterally, inasmuch as the gastrointestinal tract is capable of disposing of most inert contaminants.

For enzymes intended for parenteral use, the manufacturer must assure that the enzyme preparation is essentially pure and free of endotoxins. Electrophoretic and immunologic tests provide the requisite evidence of purity and homogeneity. Most importantly, the manufacturer must remove toxic impurities, eg, bacterial lipopolysaccharide (endotoxins) which might cause severe toxic reactions such as anaphylactic shock, fever, and vascular collapse.

All preparations of enzymes intended for parenteral use are tested for safety in lower animals under the conditions anticipated in clinical trials; ie, their use must be nonpyrogenic in the USP rabbit assay (255), and must be sterile. Such toxicologic studies are usually a prerequisite for approval by the FDA for the sale of such pharmaceuticals.

Since many therapeutic enzymes are still derived from bacterial sources, FDA requirements can serve to make the commercial preparations more expensive. However, toxicological examination of each lot may not be necessary when the purification procedures yield reproducible preparations.

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