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ENZYME–PROTEIN CONJUGATES: NEW POSSIBILITIES FOR ENZYME THERAPY

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1. INTRODUCTION

The ability of a group of complex proteins, namely enzymes to catalyze the degradation of a wide range of both complex and simple molecules such as polypeptides, polysaccharides, lipids and nucleic acids has stimulated interest in the use of enzymes as tools for a wide range of processes. The food industry has been interested in enzymes to help accelerate chemical or biochemical processes such as fermentation important to the dairy and beverage industries (Reed, 1975). The clinical chemist is concerned with the use of enzymes for a variety of assay procedures (Klibanov, 1979); the ELISA or Enzyme Linked Immuno Assay becoming an increasingly important tool in immunology and analytical and clinical biochemistry. The use of enzymes and especially immobilized enzymes has been appreciated as an important tool in preparative organic chemistry (Jones *et al.*, 1976). The potential use of enzymes as therapeutic agents in the medical clinical has also not been ignored. The specificity and low general toxicity of enzymes make them ideal drug candidates. As early as 1870, Purdon demonstrated the use of the proteolytic enzyme pepsin during a number of surgical procedures for controlling and cleaning absceses, varicose ulcers and ulcerating cancers, to inhibit bacterial growth and other contamination. In 1902, Sir Achibald Garrod, described a new class of diseases "The Inborn Errors of Metabolism" and suggested the possibility of using enzyme replacement for the treatment of a series of *Enzyme Deficiency Diseases*. By 1960 while enzymes were not yet in any important widespread clinical use their potential was being investigated for the treatment of a wide range of enzyme deficiency diseases (then numbering some 40 and now identified as greater than 400) (Stanbury *et al.*, 1983), as antineoplastic agents and as a means of controlling enzymatic activation of fibrinolysis in the treatment and/or control of thromboembolytic vascular disease (Holcenberg and Roberts, 1981).

In recent years pharmacologists have recognized the need to devise drug and/or enzyme delivery systems to direct the therapeutic agents to specific sites while avoiding normal tissue which may also be sensitive, possibly in an adverse fashion, to the therapeutic agent. The concept of a *magic bullet* or *Trojan Horse* to introduce drug or enzymes to specific sites is not new. In 1898 Paul Ehrlich (1906) foreshadowed the possible use of "bodies which possessed a particular affinity for a certain organ . . . as a carrier by which to bring therapeutically active groups to the organ in question". Ehrlich thus predicted the existence of antibodies, which were to be defined many years later, and foresaw their use as site specific carriers of therapeutic agents. While antibodies have yet to be used clinically in a widespread fashion to target or deliver drugs, a number of possibilities are currently being investigated (Möller, 1982).

2. THERAPEUTIC POTENTIAL OF ENZYMES

2.1. ENZYME REPLACEMENT THERAPY

Since Garrod's first description of inborn errors of metabolism and the probable cause as the deficiency of specific enzymes we seem to have been uncovering new metabolic

diseases with enzyme defects at their root cause at what seems to be an exponential rate. This is perhaps best seen by observing the list appearing in the various editions of "*Inherited Basis of Metabolic Disease*" by Stanbury *et al.*, (1983). The earliest edition in 1960 listed some 40 specific enzyme defects of catalogued diseases whereas the most recent edition in 1983 lists more than 400. A great many of these enzyme deficiency diseases are lysosomal in nature and this area has been ably reviewed (Desnick *et al.*, 1976; Brady, 1982, 1983) on several occasions.

The possibility of treating these diseases by enzyme replacement has not been encouraging. To date most attempts at enzyme replacement have proven disappointing. Hug and co-workers attempted to treat infants with Pompe's disease (Type II glycogen storage disease) by the administration of the enzyme α-1,4-glucosidase (Hug, 1967, 1978; See also de Barsy *et al.*, 1973; Tager *et al.*, 1980). No clinical improvement in the condition of the infants could be detected. In at least one case a severe hypersensitivity reaction to the enzyme, (fungal in origin) occurred and at autopsy, whereas some decrease in liver glycogen was observed, large deposits of glycogen in muscle tissue of the cardiac and respiratory systems remained. Brady and co-workers have attempted to treat several infants with Tay Sachs disease by administering the deficient enzyme hexosaminidase in several different forms (Brady *et al.*, 1975; Johnson *et al.*, 1973). While they observed evidence of enzyme uptake and substrate depletion by the liver and significant decreases in liver GM_2 (substrate) levels, they were not able to demonstrate any clinical improvements. Furthermore they saw no decrease in the number of foam cells in the central nervous system of the affected infants and as expected no apparent penetration of the blood brain barrier by the administered enzyme. Brady (1982) discusses more recent attempts and new approaches to enzyme replacement therapy trials in lipid storage diseases.

In 1941 Linus Pauling stated that "in as much as the whole body is made up of molecules, then all diseases are molecular". Nowhere is this more evident as in the examination of the course of discovery of the inherited metabolic diseases (Stanbury *et al.*, 1983). Most of these conditions are currently without viable means of treatment and many of them are at least potentially amenable to enzyme replacement therapy.

2.2. ENZYMES IN CANCER THERAPY

The first suggestion that certain enzymes might possess antineoplastic activity came through a somewhat chance discovery by Kidd in the early 1950's (Kidd, 1953) when he showed that guinea-pig serum was able to cause the regression of a murine lymphoma. In fact it was then thought that the guinea-pig serum was being used as a source of complement to enhance a form of 'Immunotherapy' when the tumor cells were being treated with a rabbit antisera to the lymphoma. By the early sixties it became clear (Broome, 1961; Mashburn and Wriston, 1964) that the component of the serum responsible for the antitumor activity was the enzyme L-asparaginase. The enzyme derived from *E. coli* proved equally effective in producing complete regression of not only a number of other murine lymphomas but also against a spontaneously occurring canine lymphosarcoma. By the late 1960's it had been demonstrated that at least one important human cancer, Acute Lymphocytic Leukemia (ALL), the major leukemia in children, was L-asparaginase sensitive and many clinical trials were initiated using mainly the *E. coli* enzyme (Burchenal and Karnovsky, 1970; see *Cancer*, February 1970 for detailed reviews). The basis for this therapy appears to be the requirement of these cells for an exogenous source of the amino acid L-asparagine. It is suggested that L-asparaginase sensitive tumor cells lack the enzyme asparagine synthetase and thereby require an exogenous source of the amino acid (Horowitz *et al.*, 1968). The bottom line in the current therapeutic use of L-asparaginase however has been a disappointing one. The only disease that appears to have been widely responsive to L-asparaginase therapy has been ALL. Complete remission rates of between 30–60% have been reported. Unfortunately, moderate to severe hypersensitivity reactions to the foreign enzyme have been encountered in as many as 40% of the patients receiving the enzyme (Crowther, 1971; Cooney and Rosenbluth, 1975).

L-asparaginase from *Erwinia cartovora* shows little cross reactivity with the *E. coli* enzyme, but problems with immunogenicity are just as severe. A second limitation to the common use of L-asparaginase as an anticancer agent is the fact that resistant tumors and normal tissue respond to the drop in plasma L-asparagine levels by inducing endogenous asparagine synthetase activity thereby increasing plasma asparagine levels (Haskell *et al.*, 1969). These problems have relegated L-asparaginase to a secondary or tertiary role as an antitumor agent even in the treatment of ALL where it is used only in combination chemotherapy and only where the leukemia has failed to respond to more commonly used chemotherapy such as oral prednisone and i.v. vincristine (Pratt and Ruddon, 1979).

The fact that L-asparaginase was shown to have some important toxicity towards certain cancers led to an investigation of other amino acid degrading enzymes and their potential as anticancer agents. The potential of such enzymes/drugs, like L-asparaginase is exciting because their cytotoxicity is based on a fundamental biochemical difference between the tumor cells and resistant normal cells and we might anticipate the enzyme will be without effect on the latter. Holcenberg (1981) and Roberts (1981) have reviewed this area in some detail and catalogued a number of other potential enzyme therapy strategies for depleting amino acids (mostly nonessential) from tumor cells which lack the mechanism to synthesize or induce synthesis of that specific amino acid.

2.3. Proteolytic Enzymes and their Medical Application

Since Purdon (1871) first used the enzyme pepsin to inhibit bacterial infection and inflammation during surgery, the possibility of using proteolytic enzymes in therapy to aid in protein degradation under abnormal circumstances has been most enticing. The most obvious is the possibility of enzyme therapy in digestive disorders such as pancreatic insufficiency common in conditions of cystic fibrosis, chronic pancreatitis and pancreatic resection brought about by a number of different causes. The use of pancreatic enzyme supplements for the treatment of a number of these conditions has proven most successful (see Graham, 1981 for review). The oral route of administration is especially attractive since it does not suffer from the serious limitations of enzyme therapy via the intravenous route, such as rapid clearance and hypersensitivity reactions, topics to be dealt with in following sections.

Because of the importance of thromboembolytic vascular disease as one of the common causes of morbidity, much attention has been given to the possibility of using proteolytic enzymes to degrade or retard the development of emboli (see Fletcher and Alkjaersig, 1981 for review). Several different approaches and enzymes have been utilized and many have already been considered in clinical trials. The most accepted enzyme treatments, which have now been licensed by the FDA are the plasminogen activators streptokinase and urokinase. The rationale for this type of therapy is that the enzymes potentiate the plasmin–plasminogen enzyme system which is apparently responsible for lysing both intra and extravascular thrombi, clots and fibrinous deposits (Fletcher and Alkjaersig, 1981). Clinical trials to date, however, have not been conclusive in showing that these enzymes reduce thromboembolytic vascular disease. While there have been some hopeful signs (Collen, 1980), the high antigenicity of the enzymes, especially streptokinase, and the presence of a relatively high antibody titre in most of the population as a result of streptococcal infection (Fletcher *et al.*, 1958) induces problems of interaction of the administered enzyme with pre-formed antibodies (50% plasma clearance in under 15 min) as well as dangers associated with hypersensitivity reactions. These limitations have made it especially difficult to ascertain whether this approach to treating thromboembolytic vascular disease is feasible. Additional interest arose in the use of defibrinating enzymes from snake venom (Kwaan, 1973). While clinical interest was high, problems associated with the high antigenicity of these preparations and problems with greatly increased coagulation times have temperated the interest in this and similar thrombolytic agents.

Perhaps the most successful common clinical use of proteolytic enzymes in medicine has been that of clotting factor replacement used to treat a wide range of disorders, both inherited and acquired, of the plasma coagulation system (Moncada and Vane, 1979;

Walsh, 1977). Present clinical approaches involve the use of whole plasma concentrates in the treatment of hemorrhagic disorders. Attempts at using specific factors for replacement are being considered as purification procedures for these factors become available. Defects in the clotting mechanism in each of the more than 12 factors have been identified (Lazerson, 1981). For example Factor XIII represents the final step in the enzymatic clotting sequence that ends in the formation of a stable fibrin clot. The final enzyme called fibrinolygase (Lorand, 1977) is a transglutaminase that covalently cross-links the fibrin molecules to stabilize the clot. The disease associated with the deficiency in Factor XIII is a relatively rare autosomal recessive trait which can be relatively easily treated with a small dose of a plasma cryoprecipitate although attempts at replacement of the specific factor (Lazerson, 1981) has been deomonstrated with some success.

A number of other proteolytic enzymes may prove to have important uses as therapeutic agents. An important thrust in the area of transplantation immunology has been the possible use of proteolytic enzymes to manipulate cell surface proteins or antigens. For example it has already been shown that β-galactosidase is capable of converting blood group A red blood cells to blood group O red cells; a process that has obvious benefits in transfusions (Yatziv and Flowers, 1971). The possibility of using proteolytic enzymes to enhance cell surface antigenicity of tumor cells and thereby increase the possibility of having the tumor cell rejected (see Jelsema *et al.*, 1981 for discussion) is especially attractive. Defects associated with the complement system represent yet another area where enzyme therapy using proteolytic enzymes might prove useful. The complement system represents a series of serum proteins, some of which are enzymes which function to initiate, modulate, regulate and amplify immunological responses to injury, bacterial or viral infections or simply membrane damage. The complement pathway has been implicated in a number of diseases ranging from specific complement component deficiencies (Gelfand *et al.*, 1976) to atherosclerosis where complement has been postulated to be responsible for the initiation of the damage to the arterial endothelium (Geertinger and Sorenson, 1975), to different types of cancer where deficiencies in complement factors have been documented (Kassel *et al.*, 1977).

3. PROBLEMS ASSOCIATED WITH ENZYME ADMINISTRATION

While the potential use of enzymes in the treatment of a wide range of disorders has long been recognized, a number of serious limitations has mitigated against the common use of enzyme therapy as the preferred mode of treatment for most of these diseases.

1. Enzymes, once introduced into the organism are subject to the same biodegradative processes responsible for turnover of endogenous proteins. Introduction of exogenous proteins into the circulation often results in their rapid denaturation by proteolysis. This may necessitate repeated administration of the enzyme.
2. The source and degree of purification of the enzyme has to be considered. We can anticipate the production of pure enzymes by genetic engineering and/or solid state synthesis should their usefulness warrant such a development: but for the moment the purification of sufficient quantities of enzyme for enzyme replacement therapy studies remains a serious limitation. The sources of enzymes are more often than not either bacterial or fungal in origin although more human sources (often placental) are being demonstrated and used (Brady, 1983).
3. Perhaps the major limitation to enzyme therapy and its clinical evaluation is the highly immunogenic nature of most enzyme preparations currently available as therapeutic agents. Typically enzymes available in sufficient quantity have been derived from bacterial or fungal sources (e.g. L-asparaginase from *E. coli* and α-glucosidase from *A. niger*) and have resulted in moderate to severe hypersensitivity reactions (Desnick *et al.*, 1976). The potential use of enzymes from human sources may alleviate the problems of immunogenicity, dependent on the nature of the enzyme deficiency. It has been recognized that patients with Tay Sachs disease lack hexosaminidase activity but possess

a proper number of copies of the enzyme (proper immunological cross-reactivity. Stanbury *et al.*, 1983). Under such conditions, it may be that the individual will recognize the exogenous normal human hexosaminidase as 'self' and thus not mount an immune reaction. If however the genetic defect is as a result of a deleted or significantly altered code for a specific enzyme, then administration of the human enzyme may still result in the mounting of a rejection reaction. The cross-reactivity of most such enzymes between even normal individuals has not been investigated in any systematic manner. The immunological consequences of administering a foreign protein, from whatever source remains one of the most significant drawbacks to enzyme replacement therapy.

4. The site of substrate accumulation and hence the route of administration of enzymes is another drawback to the common use of enzyme therapy., The enzyme must be administered in such a way that it has access to substrate. When the substrate accumulates in plasma, it may not be crucial where the enzyme replacement is effected in the case of many of the lysosomal storage diseases such as Pompe's disease where glycogen accumulates and remains in secondary lysosomes, enzyme replacement will be effective only if the enzyme is delivered to those lysosomes of cells and tissue that are affected. In this case hepatocytes of the liver and muscle cells of the cardiac and respiratory tissue must be the target sites. In the lipid storage defect, Tay–Sachs disease, consideration must be given to crossing the blood brain barrier to deliver enzyme to cells of the central nervous system which is most affected by substrate accumulation. Not only must the specific targeting of the enzyme be considered but also the length of time the enzyme will have to be effective in that region. These are considerations which give rise to the concepts of enzyme carriers and targeting agents to be discussed at length in the following sections.
5. The number of animal models of specific diseases available to test the concepts of enzyme therapy are limited (Desnick *et al.*, 1976). In the past few years several new model systems have been developed. The fact that a number of murine lymphomas (Mashburn and Wriston, 1964) are L-asparaginase-sensitive made this system fairly easy to develop and test in spite of the subsequent problems that have arisen in human use of this therapy. The acatlasemic mouse (Chang and Poznansky, 1968) could be used as a model for substrate accumulation of a permeable molecule. For the carbohydrate storage disease there is an animal model system which has been studied in Australia in cattle which appears to resemble closely Pompe's disease or Type II Glycogenosis (Howe *et al.*, 1981). Other models for lysosomal storage disease have been developed including: the β-glucuronidase deficient mouse (Fiddler and Desnick, 1977) and GM_2 ganglioside storage disease in cats as a result of hexosaminidase deficiency (Rattazzi *et al.*, 1981). This latter model may prove especially useful for investigating the possibilities of enzyme replacement therapy for lipid storage diseases with important central nervous system involvement and with consideration to penetrating the blood brain barrier.

4. BIOLOGICAL MACROMOLECULES AS ENZYME CARRIERS

It is now evident that carrier systems may have important functions in enzyme therapy; firstly as a protective agent to avoid immunological consequences to administration of a foreign protein, secondly to retard bioinactivation and thirdly as a mechanism to target or direct enzymes to specific sites. While Ehrlich as early as 1898 had forecast the potential use of 'antibodies' as a drug delivery system, it was not until 1958 that Mathe and colleagues (1958) demonstrated that antibodies could be used to direct therapeutic agents to specific cells. He conjugated the antimetabolite, methotrexate by a diazo linkage to immunoglobulins directed against the murine leukemia L1210 cells and showed that the resultant conjugate was effective as an antitumor agent . Since then there have been many different attempts at targeting drugs/enzymes to specific sites and at protecting enzymes from immunological reactivity and bioinactivation using a variety of different macromolecular agents (see Poznansky and Cleland, 1980 for review).

Many of the new approaches to drug/enzyme delivery are described in the accompanying chapters. The possibility of encapsulating enzymes within synthetic (nylon, colloidion, etc.) semipermeable aqueous microcapsules (Chang, 1964; Chang and Poznansky, 1968) was first envisaged in the early sixties followed by the concept of liposomes as vehicles for enzyme replacement (Gregoriadis, 1976; Juliano and Layton, 1980). The approaches may be different in concept dependent on whether it is intended that the enzyme remain within the enclosure to act upon permeant substrate with subsequent release of product or whether the enzyme is encapsulated only to be protected and then released from the vehicle upon delivery to some specific site. Similarly the potential use of red cell ghosts (Humphreys and Ihler, 1982; Green *et al.*, 1980) magnetically targeted albumin microspheres (Widder *et al.*, 1982) permits the delivery and potential release of encapsulated enzyme. In the following pages I will discuss the use of 'other' sorts of biological macromolecules that might be used as carrier molecules and/or targeting agents to effect enzyme replacement therapy.

4.1. SYNTHETIC BIOPOLYMERS

Abuchowski and Davis (Abuchowski *et al.*, 1977, 1979; Abuchowski and Davis, 1981) expanded upon the work of Landsteiner and van der Scheer (1932) to demonstrate that the attachment of certain synthetic polymers could change the antigenic nature of protein molecules. They extended this observation to show that the covalent attachment of polyethylene glycol (PEG) to a number of different enzymes could greatly diminish both the immunogenicity (ability to induce an immune reaction) and the antigenicity (ability to react with preformed antibodies) of the native enzyme. They have succeeded in cross-linking many different enzymes to PEG in each successfully diminishing the immunological reactivity of the enzyme while retaining the enzyme activity. They have shown increased circulation half-lives for the PEG-enzymes for arginase, uricase, asparaginase and a number of other enzymes. Some of this data is summarized in Table 1. These authors have studied the physical properties of their conjugates in detail. In a number of cases although the enzyme yields appear poor to start with, the advantages in terms of circulation half-lives and resistance to bioinactivation (both by heat denaturation and resistance to proteolytic enzymes) easily compensate for the loss in activity during the cross-linking procedure. The fear that the eventual breakdown products of the PEG–enzymes conjugates would themselves form a 'storage' problem due to their nonphysiological character appears to be dispelled by the observation that all products of the PEG–protein conjugate are eliminated in the urine and feces within a 30 day period (Abuchowski and Davis, 1981).

While PEG has been perhaps the most extensively studied of the synthetic polymer carrier systems, a number of other systems have been studied. Some of these along with

TABLE 1. *Examples of Biological and Synthetic Macromolecules as Enzyme Carriers*

Carrier	Enzyme	Method of Conjugation	Reference
Polyethylene Glycol	Arginase, Uricase, L-Asparaginase, etc.	Cyanuric Chloride	Abuchowski and Davies (1981)
Poly-DL-Alanine	L-Asparaginase	Mixed Anhydride	Uren and Ragin (1979)
Poly(*N*-vinylpyrrolidone)	β-D-*N*-acetyl hexosaminidase A	Imidoester	Geiger *et al.* (1977)
Poly L-Lysine	Horseradish Peroxidase	Carbodiimide	Shen and Ryser (1978)
Lipoproteins	α-1,4-Glucosidase		Williams and Murray (1980)
Albumin	Superoxide Dismutase	Glutaraldehyde	Wong *et al.* (1980)
Albumin	Uricase, α-Glucosidase	Glutaraldehyde	Poznansky *et al.* (1982)
	L-Asparaginase, Superoxide Dismutase		Poznansky and Bhardwaj (1981)
			Remy and Poznansky (1978)
Polymaleic Acid	Indolyl-3-alkane-α-hydroxylase	Carbodiimide	Schmer and Roberts (1979)
Polyacrylic Acid	Indolyl-3-alkane-α-hydroxylase	Carbodiimide	Schmer and Roberts (1979)
Collagen	L-Asparaginase	Glutaraldehyde	Olanoff *et al.* (1977)
Dextrans	Superoxide Dismutase	Cyanogen Bromide	McCord and Wong (1979)

For a more complete list of Immobilized Therapeutic Enzymes see Weetall and Cooney (1981).

a number of biological macromolecules are listed in Table 1. Sela (1966) demonstrated that multichain poly-DL-alanine preparations provided a large degree of protection against immune reaction to proteins conjugated with the polyamino acid. Uren and Ragin (1979) used this property to prepare nonimmunogenic preparations of L-asparaginase from both *E. coli* and *Erwinia cartovora* that also had increased plasma half-lives ranging from 4 hr for the unprotected enzyme to 28 hr for the polymer conjugated enzymes. The conjugated preparations were much more effective in lowering plasma L-asparagine levels than equivalent amounts of free enzyme. Poly(*N*-vinylpyrrolidone) has been conjugated to the enzyme β-D-*N*-acetyl-hexosaminidase A (Geiger *et al.*, 1977) resulting in a conjugated enzyme with a greatly enhanced circulation half-life. The preparation was, however clearly antigenic since a weak but definite reaction was seen with antibodies produced against the enzyme alone. No clear testing of the immunogenicity of the preparation was reported. A number of other synthetic polymers have been used as enzyme carriers including polyvinyl alcohol (Hixson, 1973), carboxymethylcellulose (Mitz and Summaria, 1961), polyacrylic acid and polymaleic acid (Schmer and Roberts, 1979). While these preparations exhibit certain advantages over free enzyme with respect to stability and resistance to biodegradation, no testing of either immunogenicity or antigenicity has been reported.

Preparations of polyamino acid or polyanion conjugated enzymes may have another important advantage over free enzymes. Dependent on the source of the enzyme (as well as the site of potential enzyme therapy) it might be important to be able to alter the pH optimum characteristics of the enzyme to be administered. For instance uricase from hog liver has a pH optimum of 10.5. By cross-linking it with albumin (e.g. see Poznansky, 1977) we have been able to shift the pH optimum to a more physiological pH (Fig. 3). By conjugating the enzyme indolyl-3-alkane-α-hydroxylase with polyacrylic acid it was possible to produce a pH optimum change from 3.5 for the native enzyme to 6.5–7.0 for the conjugated preparation (Schmer and Roberts, 1979) allowing for use of the enzyme under more physiological (non-lysosomal) conditions. This property was demonstrated much earlier for enzymes conjugated to insoluble supports (Goldman *et al.*, 1968) but may have great advantages in enzyme therapy to allow enzymes which are not normally active at neutral pH to act in a physiological medium. Alternatively enzymes that are normally inactive under acidic conditions may be modified to act at pH 4.5, for example for enzyme replacement therapy involving lysosomal storage diseases. Abuchowski and Davis (1981) have demonstrated similar changes in pH optimum following conjugation of a number of different enzymes with PEG.

4.2. Lectins and Specific-Receptor Ligands

The previous section dealt exclusively with attempts to protect enzymes from inactivation either by immunological attack or proteolysis. No mention was made of attempts to target the enzymes to specific sites other than to increase circulation half-times in order to allow the enzyme to act on plasma-borne substrates. A number of systems have been devised for the enhanced targeting of proteins either in their free or conjugated forms to specific sites. The most obvious is the use of antibodies (either monoclonal or polyclonal) directed against cell-surface specific antigens. Ashwell and Morell (1974) demonstrated that rat liver hepatocytes could recognize and remove from the circulation certain polysaccharides that contain galactose at the nonreducing terminus. This receptor has been termed the galactose or 'Ashwell' receptor and Rogers and Kornfeld (1971) succeeded in enhancing the clearance of albumin from the circulation utilizing this receptor. They conjugated fetuin to albumin and then treated the conjugate with *N*-acetyl neurominidase to remove the terminal sialic acid residue and expose the terminal galactose residue. This was then recognized by the appropriate receptor on hepatocytes causing rapid clearance of the conjugated albumin from the circulation in less than 10 min. The normal circulation of albumin can be as high as 40 hr. The use of these and similar receptors as potential sites for targeting 'glycoconjugates' have been described by Ponpipon *et al.*, 1981.

Lectins such as concanavalin A (con A) and Wheat Germ Agglutinin are protein molecules which generally have high specific affinities for one or more sugar residues

situated on either glycolipids or glycoproteins on cell surfaces. Con A is the lectin that has been most seriously investigated for the possibility of localizing enzymes at specific sites. Con A–dextranase conjugates have been prepared to localize dextranase at sites of tooth decay (tissue containing mannoside residues, one of the receptors for con A) as a means of preventing dental caries (Barker *et al.*, 1974). Con A–L-asparaginase conjugates have been made as a means of allowing L-asparaginase to bind to tumor cells bearing terminal mannose derivatives. While the enzyme–lectin conjugates retained enzyme activity and were able to localize on cells containing the mannose receptors, no increase in therapeutic index was demonstrated (Shier, 1979). The reason for this lack of success is not understood. It is not known whether the conjugate simply remained on the surface of the cell, whether it was internalized and suffered from a meeting with lysosomal processes, whether it was shuttled through the golgi-apparatus for recycling or whether it simply was compartmentalized within the cell giving it little or no access to the substrate L-asparagine.

4.3. PLASMA PROTEINS

Plasma proteins including albumin (discussed in detail in the following section) offer attractive possibilities as enzyme carrier systems. Most importantly they are natural constituents of the plasma and so their conjugation to other proteins may allow the foreign enzymes to be accepted into the plasma either as a family member of the plasma proteins (at best) or else in the best tradition of the 'Trojan Horse'. The question of which is in fact true: whether the carrier proteins mask the antigenic sites of the foreign enzyme, or whether the carrier imparts upon the foreign protein the notion of 'self', as in tolerance, is covered in some detail in a following section.

Serum proteins other than immunoglobulins and albumin have been used primarily as a means of carrying certain cytotoxic antitumor agents in order to avoid rapid removal from the body via the urinary system. A number of different authors (Szekerke *et al.*, 1972; Isliker *et al.*, 1964) have examined the potential use of the plasma protein fibrinogen as a carrier of drugs such as phosphoramide dichloride or methotrexate and have demonstrated an increased therapeutic index of the drug due supposedly to a preferential deposition of the conjugate in the region of tumor tissue. Although by no means confirmed, it has been suggested that this increased targeting of the fibrinogen–drug complexes to regions of tumor cell growth occurs as a result of the fibrin content increasing along with the newly formed vascular bed associated with rapidly growing tumors. Collagen has been proposed as a possible matrix for the immobilization of enzymes in an insoluble form in such a fashion that it might be implanted or else used in conjunction with an extracorporeal shunt. Several techniques have been used to either deposit or covalently link (Venkatasubramanian and Vieth, 1977) enzymes to solid supports, some of which have been implanted to sit as a part of the vascular system. Olanoff and co-workers (1977) demonstrated a dramatic and sustained drop in serum asparagine levels following attachment of an extracorporeal system, containing a collagen-L-asparaginase matrix, to the vascular system of healthy dogs. These and other studies with collagen–enzyme reactors (Venkatasubramanian and Vieth, 1977) appeared to be attractive alternatives to the *in vivo* administration of soluble enzyme–carrier complexes.

Several different groups have proposed the possibility of using low density lipoproteins (LDL) to direct drugs to specific cell types (extrahepatic and nonreticuloendothelial tissue) possessing specific high affinity receptor sites for the LDL molecule. Williams and Murray (1980) have not only demonstrated the feasibility of using LDL bound α-glucosidase as a means of enzyme replacement therapy in patients with Pompe's disease but have actually performed some preliminary human trials. Although their clinical trial was not successful in terms of increasing the life span of the terminally ill patient or in fact seeing any significant decrease in muscle (either respiratory or cardiac) glycogen levels, they did make several interesting observations. They detected no antibody formation either to the LDL molecules or to the enzyme (α-glucosidase from human liver) although the number of infusions was limited. Following the third infusion the authors suggest that a three-fold increase in muscle enzyme could be detected although this represented only 13% of normal