RESEARCH & DEVELOPMENT

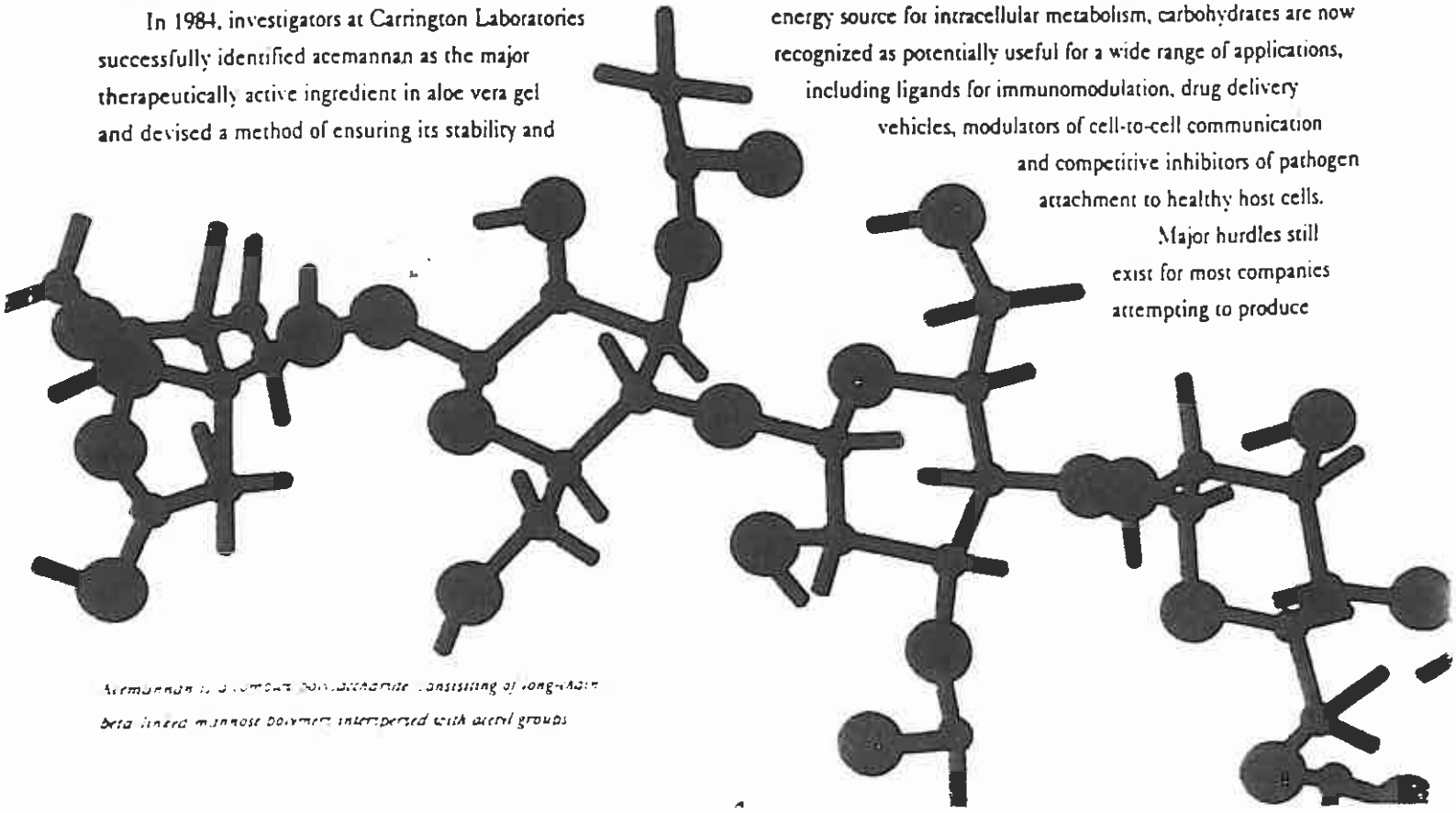
I. BACKGROUND

Although the use of medicinal plants has not been highly regarded in this country, there is a general consensus among lay people that the clear sap of the aloe vera plant is of benefit in healing wounds and reducing inflammation. Despite this consensus, until recently there had been very little success in identifying the active principle. The major impediment to these studies was that aloe vera gel lost its effectiveness within hours of being taken from the plant. Good results seen with fresh aloe were always counterbalanced by lack of results in later tests. As recently as 1983, aloe vera had not been proven effective in the treatment of any condition and the aloe vera industry could, therefore, not make health care claims. Nevertheless, in 1983, the FDA's Advisory Panel for over-the-counter drugs reviewed a hundred reports on aloe vera products and found that not one adverse effect had been reported. They concluded that "clearly the substance is safe." (Zimmerman DR. Essential Guide to Non-prescription Drugs. Harper and Row. 1983)

In 1984, investigators at Carrington Laboratories successfully identified acemannan as the major therapeutically active ingredient in aloe vera gel and devised a method of ensuring its stability and purity. In solving the stability problem, they opened the way for studies on its therapeutic activities. On June 5, 1986, Carrington applied for a patent on this compound and its stabilization. The patent was issued on April 5, 1988.

Initial studies revealed that the active ingredient of the aloe vera gel was a complex carbohydrate. Thus of necessity, the Company entered the field of glycobiology, the study of carbohydrates and their biological activities. (Carbohydrates are compounds such as sugars and starches.) The study of the biology of carbohydrates dates back to the very foundations of biochemistry. Until recently however, the chemistry of these molecules was not widely studied because of their complexity and technical difficulties. Since the early 1980s however, new techniques and methodologies have resulted in an explosion in the number of studies conducted in this area. The field of glycobiology has experienced remarkable growth. (Hart GW, editorial, Glycobiology, 1990) At the same time, many small biotechnology companies have been established in an attempt to use the newly acquired knowledge for therapeutic benefit. This is evidenced by trade publications and the emergence of at least 15 companies developing carbohydrate-based drugs. No longer considered merely an energy source for intracellular metabolism, carbohydrates are now recognized as potentially useful for a wide range of applications, including ligands for immunomodulation, drug delivery vehicles, modulators of cell-to-cell communication and competitive inhibitors of pathogen attachment to healthy host cells.

Major hurdles still exist for most companies attempting to produce



Acemannan is a complex polysaccharide consisting of long-chain beta linked mannose polymers interspersed with acetyl groups

carbohydrate-based drugs. Low yields of pure carbohydrate and high costs for virtually all synthetic methods can be formidable barriers. Thus, most of the carbohydrates under investigation are short-chain saccharides – not true polysaccharides – and clinical drug development at these companies tends to lag behind the preclinical science. Because of its natural source, Carrington Laboratories is in the unique position of having available large quantities of a pure, chemically-defined carbohydrate. As a result, the production of acemannan faces none of the availability, cost and molecular configuration problems faced by other companies and provides Carrington with a unique therapeutic franchise.

The task of Carrington Laboratories research group has been to study the properties of acemannan, to determine its therapeutic applications, to ensure its lack of toxicity, and to make this remarkable compound available to the public in as timely a manner as possible. Progress towards each of these goals has been impressive. To this end, Carrington has 53 patents in 9 broad categories carrying 1,534 claims in 21 countries.

II. SCIENTIFIC RATIONALE

The properties of acemannan

Acemannan is a complex polysaccharide consisting of long-chain, beta-linked mannose polymers interspersed with acetyl groups. Carrington scientists have shown that acemannan is extremely stable. Due to its beta linkage and other structural features, acemannan is not metabolized until it reaches the target cell. Acemannan interacts with the immune system and thus acts as an immunomodulator. Immunomodulators are compounds that stimulate immune cell function and cytokine synthesis and have a wide variety of potential uses in the treatment of human and animal diseases. Acemannan also possesses significant antiviral activity which makes it potentially useful in the treatment of infectious diseases.

Cytokine release

Macrophages are cells that patrol throughout the body. Among other functions, they seek out, ingest and destroy bacteria, viruses, tumor cells and other foreign material. They present foreign material to the cells of the immune system and in this way regulate the immune response. Macrophages secrete a number of potent chemicals that assist not only in their scavenger role, but also in performing their regulatory activities. Products of the macrophage include enzymes, growth factors, coagulation factors, prostaglandins and, most importantly, cytokines. At least two macrophage-derived cytokines are of significant therapeutic interest: tumor necrosis factor (TNF) which destroys tumor cells, promotes the growth of fibroblasts in wound healing, and stimulates the production of other molecules involved in the immune response; and interleukin-1 (IL-1) which enhances the response mounted against infection, increases natural killer cell (NK-cell) activity, and promotes the growth of new blood vessels. Acemannan is readily taken up by macrophages and subsequently triggers the release of the major macrophage cytokines such as IL-1, interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α). Their therapeutic effect is modulated by the simultaneous release of the interleukin-1 receptor antagonist (IL-1RA) as well as macrophage inflammatory protein (MIP-α). Acemannan can also exert more subtle effects on macrophage functions by priming them for enhanced release of nitric oxide and superoxide anion when exposed to cytokines or a phagocytic stimulus. It also promotes functional changes such as increased phagocytosis and intracellular destruction, antiviral activity, enhanced antigen processing, and the increased release of nitric oxide and superoxide anion. Acemannan may also have a direct effect on T-cell and NK-cell function.

Release of a mixture of many different cytokines by activated macrophages can be of therapeutic benefit in many

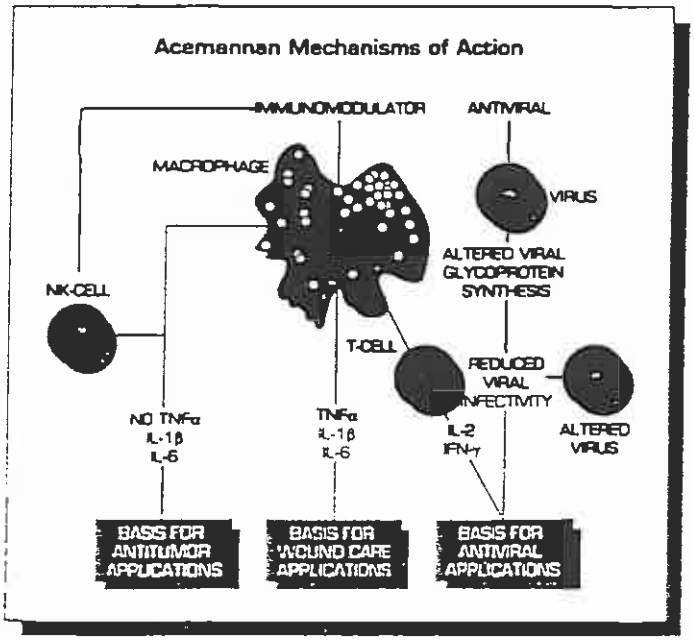
different situations. Thus the release of TNF or nitric oxide from activated macrophages may result in significant destruction of nearby cancer or virus-infected cells. The release of IL-1 and other cytokines in damaged tissues may enhance the rate of wound healing and increase resistance to bacterial and viral infections. Secondary release of cytotoxic products and interferons may be of benefit in antiviral immunity since it may result in inhibition of viral replication and destroy virus-infected cells. Most importantly however, activation of macrophages may enhance their antigen processing ability and so stimulate the immune responses to antigens associated with them. This "adjuvant" activity is readily observed with acemannan.

The use of a macrophage-stimulating immunomodulator such as acemannan will be of much greater therapeutic benefit than the use of isolated, recombinant cytokines. One of the characteristic features of cytokines is that they do not act alone but normally function together with a mixture of other cytokines. These molecules have complex interactions with the other cytokines in a mixture showing both synergism and antagonism. There is tremendous redundancy among the biological activities of these molecules while the activities of mixtures may be distinctly different from the activities of the purified, isolated molecules. For this reason, the use of single purified recombinant cytokines has produced unexpected or unsatisfactory responses. Clearly their use in this way is unphysiological. The problems associated with the use of isolated cytokines has been compounded by their very significant toxicity as well as their extraordinary expense. Acemannan possesses none of these disadvantages. Stimulation of macrophages stimulates the release of a mixture of cytokines that are subjected to the normal physiologic control processes of the body. As a result, the effectiveness of the therapy is superior while toxicity is kept to a minimum.

Antiviral activity

Although the great variety of therapeutic applications of acemannan therapy is derived from its effects on macrophages, it is important to note that it also possesses direct antiviral activity. Enveloped viruses, such as lentiviruses (HIV, FIV), orthomyxoviruses (influenza), paramyxoviruses (Newcastle disease) and herpesviruses, grown in the presence of acemannan show a significant loss of infectivity. Analysis of these viruses has shown that changes have occurred in their surface glycoproteins. These changes include alterations in the size of the carbohydrate side chains and blockage of viral assembly. The precise mechanism of this antiviral effect remains unclear but it is believed that either the acemannan interferes with the activities of glycosidases or alternatively, it alters carbohydrate transport within the cell. The net result is decreased infectivity of the virus. This direct antiviral activity suggests that acemannan may be of significant benefit as an aid to the treatment of diseases such as AIDS that are mediated by a virus with envelope glycoproteins. In addition,

Acemannan's interaction with the body's immune system and its antiviral activity bring about a very wide variety of clinical indications.



acemannan also appears to act synergistically with AZT resulting in a significant enhancement of the antiviral effects of AZT at low, nontoxic doses.

Vaccine adjuvant

As a result of these effects on macrophage function, acemannan has a very large variety of potential therapeutic uses. For example, it can act as a vaccine adjuvant. Currently acemannan is licensed by Solvay Animal Health, Inc. (Solvay) for use in poultry as an adjuvant for Marek's disease vaccine. Marek's disease is a virus disease that results in significant death loss and condemnations. The addition of acemannan to the vaccine significantly enhances its effectiveness. More interestingly perhaps, the immunomodulatory effect of acemannan on the immune system as a whole has a positive effect on the health of these birds. As a result, the birds show decreased morbidity and mortality, as well as decreased condemnations at slaughter. In addition, their improved overall health results in improved feed conversion. All of these features can result in a significant economic gain to the poultry industry.

Wound healing

Acemannan and other complex carbohydrates also have the ability to promote wound healing, not only in animals where normal healing is impaired, but also in normal healthy animals. It is believed that this effect is also related to the release of cytokines from macrophages within the wound. Macrophages are known to play a key role in normal wound healing. Defects in macrophage function result in a delay in healing while stimulation of macrophage function results in accelerated healing. Studies with local inhibitors suggest that the activity of acemannan is primarily mediated through local release of TNF-α and possibly also nitric oxide. Its lack of toxicity makes preparations containing acemannan potentially very important in the area of wound healing. Clinical studies in Europe indicate that acemannan may significantly accelerate healing of bed sores (decubitus ulcers) and venous stasis ulcers in human patients.

Anticancer activity

Because it has the ability to stimulate local release of TNF-α and nitric oxide, acemannan has found a use in the treatment of spontaneous tumors of dogs and cats. Acemannan also directly enhances NK-cell activity. When acemannan is injected into fibrosarcomas it causes a rapid swelling of the tumor as a result of local destruction and inflammation. In a significant proportion of cases the tumor may subsequently undergo significant shrinkage. Acemannan has proven to be especially effective when administered in association with surgery and radiation.

Antiinflammatory activity

Acemannan also possesses significant antiinflammatory activity. It stimulates macrophage production of IL-1RA, an antagonist of IL-1 function. In addition, moderate doses of acemannan can suppress nitric oxide production by macrophages (although stimulating nitric oxide production at low doses). Since nitric oxide probably plays a significant pathogenic role in inflammatory states, judicial application of acemannan may be of significant clinical benefit. For example, nitric oxide is a mediator of inflammation and tissue destruction in ulcerative colitis. It is therefore not surprising that acemannan can significantly ameliorate the clinical signs of ulcerative colitis. Acemannan has also found a use in reducing the inflammatory reaction induced by local radiation therapy.

The "panacea problem"

One problem that the Company has had to overcome is the idea that acemannan is a panacea. As the list of activities described above makes clear, acemannan appears to be effective against an extraordinarily wide range of pathologic conditions. It is not normally expected that a single compound will effectively promote wound healing, while at the same time have anticancer

activity, antiinflammatory, antiviral and adjuvant effects. However this concept may be more easily accepted by pointing out that the principal activity of acemannan is simply to stimulate macrophage activity. Macrophages have long been known to have the potential to be readily stimulated into greater activity. Even the initial event of migration of a monocyte from a blood vessel into inflamed tissues leads to structural changes in these cells and enhanced microbicidal behavior. Given the key role of macrophages in the defense of the body, we should not be surprised that compounds that activate these cells exhibit a very wide variety of clinical activities.

Further Development of Acemannan Molecule

In 1993, two new derivatives of acemannan were synthesized. Preliminary *in vitro* data indicate that these molecules may demonstrate more antitumor activity when compared to the parent molecule, acemannan. This is a very exciting development in view of the potential for these synthetic products to act as highly potent antitumor and antiviral drugs.

III. THERAPEUTIC APPLICATIONS

Given the research results reported above, it is clear that Carrington Laboratories has a unique opportunity to develop acemannan as a therapeutic agent for a remarkably wide variety of conditions. Much thought and planning has therefore gone into determining the Company's priorities and its principal research directions. This has involved not only the therapeutic potential of acemannan, but also the regulatory constraints that exist as well as the size and profitability of potential markets. Taking all these considerations into account, primary emphasis for research in 1993 has been in the area of wound care and related disease, including ulcerative colitis. Excellent progress has also been made in antiviral studies as well as in the area of cancer therapy and vaccine adjuvants.

Wound Care Studies

Carrington entered the wound care market with a hydrogel product in 1984. This first product was for the management of minor wounds, and was marketed solely to nurses. In 1990, Carrington obtained the following label claims: pressure ulcers (stages I-IV), stasis ulcers, first and second degree burns, cuts, abrasions, irritations of the skin, and skin conditions associated with periostomal care. By 1992 Carrington had sufficient scientific data to support marketing to clinicians in hospitals, especially vascular surgeons, plastic surgeons and radiation oncologists in addition to enterostomal therapists.

As a nonprescription product, acemannan hydrogel is marketed as an ingredient in Carrasyn™ Hydrogel Wound Dressing. The product is available in tubes, spray and impregnated gauze pads for use in a wide variety of skin conditions. All available data from extensive *in vitro* and animal studies as well as human trials and clinical use experience indicate that this product can accelerate the healing of wounds as well as enhance the quality of healing in terms of cosmetic acceptability and wound integrity.

Work by Carrington's researchers resulted in thirty-three (33) papers on acemannan, with fifteen (15) in wound healing, being published and/or presented at major conferences in the U.S., Canada and Europe in 1993.

In 1993, Carrington's efforts to broaden the claims for wound care products containing acemannan hydrogel were expanded to include application within the dental field. Two studies were conducted at Baylor College of Dentistry to examine the efficacy and safety of two modified formulations of Carrasyn™ Hydrogel Wound Dressing in the treatment of oral aphthous ulcers (canker sores). The first study involved Carrasyn™ Hydrogel Wound Dressing modified for intraoral use versus a leading product. The second trial involved the modified oral formulation that had been freeze-dried. The results obtained were highly encouraging. In short, Carrasyn™ Hydrogel Wound Dressing reduced the pain of these ulcers and significantly accelerated their healing rate. As a result of this, a premarket notification [510(k)], was submitted to FDA for permission to market the freeze-dried formulation for the management of oral aphthous ulcers. FDA's response to the submission is anticipated in 1994.

The effects of acemannan on macrophage cytokine production in laboratory studies suggested that under some circumstances, it may also be able to suppress inflammation. As a result, studies were initiated at MD Anderson Cancer Center in Houston to determine if acemannan hydrogel was of benefit in treating radiation-induced skin reactions. These studies clearly showed that acemannan hydrogel could significantly reduce radiation-induced inflammation and tissue damage in animals. As a result of this, clinical trials have been designed and planned for 1994 to study the radiation sparing effects of Carrasyn™ Hydrogel Wound Dressing.

Animal studies conducted at Texas A&M University and other sites during 1993 confirmed that acemannan had the ability to enhance the rate of wound healing in normal animals. These studies focused on its mechanism of action and have confirmed that it probably acts through cytokines secreted by macrophages. Studies conducted at Wayne State University in Detroit demonstrated that acemannan increases the rate at which granulation tissue is deposited within the wound. These results were supported by other studies conducted at the University of California in San Francisco and at Auburn University.

Four additional clinical studies were initiated in 1993 to evaluate acemannan hydrogel's effects in indications such as **diabetic foot ulcers, partial-thickness wounds, venous stasis ulcers and dermatological post-biopsy wounds.** All of these trials are controlled comparative studies against standard therapies designed to develop data for market support and to extend the indications and claims for the Company's wound care products. These additional uses represent potential expansion of Carrasyn™ Hydrogel Wound Dressing products for medical specialists such as radiologists, oncologists, surgeons, dermatologists, and geriatricians.

Since the dental wound healing program became a priority, the Company decided to concentrate resources on dental application and put an allergic rhinitis program on hold, which had been started in 1992.

Ulcerative Colitis Studies

Ulcerative colitis is a chronic, recurrent, inflammatory disease arising in the lining of the large intestine. It affects all ages but is

most threatening to people who contract the disease after age 60, and to adolescents, who can experience severely retarded growth. According to the Crohn's Disease and Ulcerative Colitis Foundation, there are some 600,000 people afflicted with ulcerative colitis in the United States. Ulcerative colitis accounts for approximately 35,000 hospitalizations annually, including an estimated 15,000 surgical procedures. Medical management of severe disease is usually not satisfactory and frequently requires large doses of steroids.

Although the pathogenesis of ulcerative colitis is still a matter of debate, it is often convenient to consider it to be a form of non-healing wound. Acemannan appears to coat and protect the gastrointestinal tract, thus preventing the formation of artificially induced ulcers in test animals. In studies of rats with induced gastric lesions, acemannan-treated rats had ulcer scores that were 32% to 12% lower than those of control nimals, a result comparable to the best existing therapies.

Development of acemannan for oral use in ulcerative colitis patients was given a high priority and a trial at seven sites was initiated in 1993 under the Company's IND concurred to by the FDA on December 1, 1992. This study is an open pilot trial in which patients suffering from acute flareups of the disease are being treated with either 800 mg or 1600 mg oral acemannan each day for up to four weeks. The safety of oral acemannan continues to be confirmed and preliminary clinical results are encouraging. Data generated from this study will help determine optimum dose and duration of treatment in subsequent trials.

Electron micrographs of cells infected with Newcastle Disease Virus (NDV), a paramyxovirus, showing the antiviral effects of acemannan. These cells were either sham treated (Figs. A and B) or treated with acemannan (Figs. C and D) at a concentration of 50 µg/ml and harvested at 48 hours post infection. Panel A: untreated NDV-infected cells. Note the numerous virions budding from the plasma membrane (arrows). Panel B: a higher magnification of an untreated NDV-infected cell. Arrows point to budding virions and ribonucleoprotein (RNP) complexes (viral genome), having the appearance of grains of sand aligned beneath the viral membrane (envelope). Panels C and D: NDV-infected cells treated with acemannan at a concentration of 50 µg/ml. Virion budding and maturation appears to be completely inhibited despite the fact that the cells were infected as evidenced by RNP complexes (arrow heads) aligned beneath the plasma membrane of the NDV-infected acemannan treated cells.

AIDS Studies

A 48-week, 60-patient, double-blind, placebo-controlled trial of oral acemannan in patients with advanced AIDS was begun in February of 1991. This early-phase trial was conducted under the auspices of the Canadian HIV Clinical Trials Network, which is supported by Health and Welfare Canada. The principal objective of this study was to assess the effect of acemannan on CD4 decline, the primary indicator of HIV disease progression. Within the study, acemannan was given in addition to AZT. The results demonstrated that early decreases in CD4 cell numbers were offset by statistically significant stabilization between weeks 16 and 48 in the acemannan group compared to placebo. This study confirmed that acemannan is a safe medication. These results are encouraging and provide an impetus for the continued development of acemannan in the treatment of AIDS.

Because the Company recognized that acemannan is minimally absorbed from the gastrointestinal tract, it was clear that acemannan should probably be injected into AIDS patients for maximum therapeutic benefit. As a result studies were initiated on injectable acemannan at the University of Texas, Health Sciences Center in Houston. A Phase I trial entitled "Phase I Single Ascending Dose Safety and Tolerance Study of Intravenous Acemannan in Normal Subjects" was completed in 1993. This study demonstrated the powerful effects of acemannan when injected intravenously. Subjects who received higher doses of acemannan exhibited signs and symptoms that are expected from treatment with biological response modifiers. Pretreatment with ibuprofen (Advil®), an over-the-counter medication, prevented the onset or reduced the intensity of these symptoms. No other signs of toxicity were demonstrated during the trial.

A Phase I/II trial of injectable acemannan in the treatment of HIV patients is planned for 1994. This study will involve short-term treatment to assess the safety and efficacy of injectable acemannan in HIV patients. Data from this trial will be used to design future studies in the treatment of HIV patients with acemannan.

Cancer Studies

The Company's first veterinary product was Acemannan Immunostimulant, an injectable, sterile form of acemannan. The product was conditionally licensed by the USDA in November 1991 to aid in the treatment and management of canine and feline fibrosarcoma. Fibrosarcoma is a form of soft tissue cancer for which there previously was no satisfactory treatment. Carrington has diligently pursued the removal of the conditional restriction on the sale of Acemannan Immunostimulant. The requirements for post marketing safety and efficacy data have been met. The final requirement for removal is the development of an adequate potency assay. This assay is now in the final stages of development and the Company believes this can be completed during 1994.

As described above, preclinical safety and efficacy studies for injectable acemannan in humans were conducted last year. The results of the Phase I trial of injectable acemannan will be used to support safety for any injectable use in humans including cancer. Since most cancer treatments are too toxic to be tested on normal, healthy volunteers, the fact that the FDA gave Carrington permission to test injectable acemannan on healthy volunteers further confirms the perceived safety of acemannan for use as a treatment for cancer patients.

Several physician INDs, for the treatment of different types of cancer, are being used to design the protocol for Carrington's cancer IND. As a result of the success of Acemannan Immunostimulant in dogs and cats, the Company has reason to believe that injectable acemannan will play a major role in the treatment of cancer in humans.

Adjuvant Studies

An adjuvant is a substance that enhances the immune response to an antigen. The ability to generate a vigorous immune response to an antigen is critical to the effectiveness of a vaccine. While a number of compounds have been demonstrated to be effective adjuvants, most tend to have side effects that make them unacceptable for clinical use. As a result, to the best of the Company's knowledge, only one adjuvant (alum) is currently approved for human use in the United States. Carrington's studies indicate that acemannan, when used as a vaccine adjuvant, produces marked stimulation of the immune system. Acemannan specifically stimulates antigen presenting cells, i.e., macrophages. Acemannan is marketed by Solvay as an adjuvant in their Marek's vaccine (MD-Vac®) in poultry. In 1993, Solvay expanded distribution of MD-Vac® to several countries other than the U.S. Carrington is actively pursuing a research program for the development of acemannan as an adjuvant in mammalian vaccines.