

493

LONG CHAIN
ALOE MANNAN SACCHARIDES

THE BASIC SCIENCE AND PRINCIPLES
FOR THE USE OF ACEMANNAN IN
CLINICAL MEDICINE

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"The purpose of science is to seek to understand the mind of God"
Sir Isaac Newton 1642-1727

Introduction

For more than a century, observers have attempted to comprehend and reduce to scientific expression the fundamental processes of host defense and healing. One goal of this writing is to present a portion of that history as it relates to studies on the nature of host defense. Another aim is to build a bridge from this base of knowledge to introduce a new medication that provides the basis for establishing a new chapter in the book of applied therapeutics. This new therapeutic agent is a biological response modifier (BRM). The molecule works through stimulating the body's innate capacity to defend and heal itself. These functions are mediated through gene activation in response to cell surface molecules that transmit biological signals indicating non-self (infections and transplanted tissue), altered-self (neoplasia and auto immune diseases), or damaged-self (traumatic or ischemic injury).

A brief review focusing on prior contributions to understanding of host defense and the healing process will aid in the comprehension of potential benefits that can be achieved by the use of the BRM acemannan (ACE-M).¹ Potential ACE-M benefits include: enhanced defense against a broad array of microbiological assaults,² correction of inappropriately directed host defenses (allergy and hypersensitivity),^{3,4} optimization of wound healing,⁵ and induction of necrosis in malignant tumors.^{6,7,8,9}

Myths, philosophical interpretations, religious dogma, and secular fantasy regarding the nature of normal body function, illness, disease, and recovery or failure of the body to heal have dominated conventional thinking in the past.¹⁰ Objective observations seeking cause and effect, combined with a willingness to reject "accepted knowledge and entrenched authority" have advanced understanding for the pathology of inflammation and its integration with the healing process. This latter approach has been essential in defining the mechanisms of action of ACE-M and in gaining respect of those who have damned the agent's natural plant origin, its long record of folk medicine usage, and its observed unprecedented broad spectrum of clinical efficacy.¹¹

The Cells, The Signal and The Systems

The components of host defense will be discussed in separate parts for simplicity and a goal of clarity. In actuality, simultaneous interactive, and mutually-dependent biochemical signaling transpires between the cellular and humoral based systems of inflammatory response. We are now in an age that clinical observations, gross tissue changes and cellular microscopic

events revealed by light and electron based instruments are recognized as being the result of highly coordinated sequenced, biochemical events including gene activation, cell organelle synthesis and gene suppression, all initiated appropriately for the conditions in the tissue micro-environment.

"THE CELLS"

The mammalian body has a system of sentinel cells that migrate through the blood to the tissues. These large wandering cells, monocyte/ macrophages (M/M), constantly monitor the condition of organs, tissues and fluids for damage, ageing, infection or transformation to malignant tissue. M/Ms have the capacity to send molecular messages to other cells that have the specialized functional role of attempting to transform any abnormal deviation of the cellular micro-environment back to normal. M/Ms are large, migrating leukocytic cells with pluripotential functional capacity that were formerly known as comprising the reticuloendothelial system. M/Ms respond to phagocytized foreign cell material or to altered host cell surface or internal membrane structure by activating general host defense.¹² This activation is mediated through messenger molecules released from cells. Such molecules are collectively called cytokines. With proper stimulation they are released from M/Ms, other activated leukocytes, and parenchymal cells. Cytokines may be monokines released from M/Ms and lymphokines released from lymphocytes. Cells communicate with one another biochemically. Cells are activated and respond physiologically in response to cytokines which serve as signals targeted to membrane receptors that activate genes for synthesis of molecules essential for life processes, homeostasis, defense, and repair. Cytokines function at the cellular level similar to hormones and neurotransmitters at the somatic level.

It is our observation that the mannan component of cell membrane structure is a molecular core sequence providing the stereochemical conformation that acts as a signal for releasing several monokines from M/Ms. Monokines have an affinity for membrane receptor sites on target leukocytic, stromal, and liver cells. Binding of a monokine can activate target cell genes causing synthesis and release of other cytokines (second-messengers) that initiate and propagate the inflammatory response. Cytokines also serve as signals for leukocytic migration, leukocyte-keratinocyte-endothelial-fibroblast proliferation, and other cell mediated defense activities (phagocytosis, lysosomal enzyme release, cytolysis etc.). These activities are initiated, coordinated and continually modulated by M/Ms. Simply stated, M/Ms are mobile bio-transducers, capable of responding to non-self and damaged-self cell surface macromolecules. When foreign or damaged-self macromolecules come in contact with the M/Ms, there is activation of the cellular

mechanisms for release of monokines that initiate the humoral and cellular cascade of host defense and/or healing.

In our laboratory, ACE-M, a unique non-antigenic complex mannan, is termed an "actogen." It functions as a virtually non-toxic, activating signal that simulates or mimics a "foreign" or alert signal that strongly activates general host defense and healing without inducing antibody production against its structure. This M/M activating signal differs from antigens that stimulate host defense. Antigens induce specific antibody production against the activating molecular structure. Stimulatory signal molecules that are antigenic eventually become ineffective therapy because they are inactivated by neutralizing antibodies induced into production by the antigen's structure.

The history of M/Ms contributes to therapeutic understanding and appreciation for the length of time that it has taken for practical application of this data. In 1874 Eichhorst published the observation that on the blood smears of typhus patients there were large cells that contained 3 to 5, and rarely up to 7, red globules within their cytoplasm.¹³ He cites Gullivier (1842) as having reported similar findings in horses dying of phlebitis and he also reported that Remak (1845) found inclusions of red blood cells in large white cells in calf spleens. Unfortunately, Eichhorst's observations were ignored. His findings could have fostered a more timely recognition of the role cell-mediated host-defense provides. The work of Behring dominated scientific thinking the last quarter of that century. Behring discovered a soluble or humoral activity in blood which he termed "anti-toxins". Anti-toxins were substances in serum, eventually found to be primarily antibodies, complement and acute reactive-phase proteins that protect animals from disease by opsonization and inactivation of bacterial toxins.¹⁴ Despite bitter opposition by Behring's disciples advocating the dominance of humoral host-defense mechanisms in physiology, Metschnikoff, in parallel with Behring's work rediscovered and persistently advanced the importance of cell-mediated host-defense provided by phagocytic leukocytes. Metschnikoff conducted experiments proving that white cells engulfed infectious bacteria invading a host organism and through lytic activity destroyed the microscopic organisms in the leukocyte's cytoplasm.¹⁵

The eventual recognition of the validity of both humoral and cell mediated host defense by Behring and Metschnikoff was a pivotal event. Their findings heralded a total departure from the past. The scientific basis was established to correct all former philosophical paradigms in regard to causes for disease, healing, and understanding of good health. In Western Medicine the causes for disease and healing as a contemplative art steeped in

mythology and authoritative opinions were revised forever. Health and its maintenance became defined as physiological and biological processes that could be understood through experimentation and subject to reproducible proof. This past sequence of events is emphasized because the immune modulator ACE-M commands the position as being the first in a class of therapeutic agents that departs from past belief. ACE-M is destined to launch a new era of safety, efficacy and thinking in therapeutics because this molecule stimulates the innate capacity of humoral and cell mediated host defense.

This paper is based on the premise that M/Ms are the cells of primacy in host defense. The M/Ms provide the first response by determining if a disease process or damaged tissue is present and, if such a condition is present, properly functioning M/Ms activate the cellular and humoral defense mechanisms. It is a hypothesis that mammalian M/Ms have this broad defensive function similar to leukocyte cell function in more simple life-forms. This interpretation is supported by the fact that in lower animal phyla, the hemocyte, a single, large, wandering, ameboid leukocyte is the sole white cell responsible for cellular defense.¹⁶ The pluripotential capacity and morphology of the hemocyte parallels most functions of mammalian and human M/Ms.¹⁷

Numerous activities performed independently by M/Ms or secondarily by monokine-induced target cell synthesis have been extensively reviewed.¹⁸ In excess of 100 target cell activities and cytokine products were cited that are activated by specific stimulation of M/Ms with various agents and under variable conditions. These products and activities demonstrate the vast importance of the role M/Ms assume in health and the recovery process incurred by tissues damaged by physical injury or disease.

In 1986, Carpenter noted in toxicity studies on canine subjects treated orally with ACE-M immunostimulant that up to 12% of circulating leukocytes in peripheral blood were gigantic ameboid cells 20 microns or larger in diameter.¹⁹ (Plate 1) Histochemical staining in combination with monoclonal antibodies against specific cell-membrane antigens determined that these large cells were of M/M cell lineage.²⁰ It should be noted that the classical circulating monocyte routinely reported in clinical hematology is a young or uncommitted M/M cell. When migrating from the blood into tissues, body cavities or organs, the M/Ms assume more specialized biochemical or immunological activities and recognized cytological morphology.²¹ Kupffer cells in the liver containing iron particles and Langerhans cells in the skin with cytoplasmic Birbeck granules are examples of specific M/M migration and specialization.

The environment can markedly alter function and the cytological appearance of M/Ms. Their most apparent alteration is an increase in cytoplasmic affinity for aniline dyes, which may be seen during infection or after administration of ACE-M. On peripheral blood smears there is the development of a perinuclear zone of hyperchromasia representing an increase in cytoplasmic nucleic acid and protein content. (Plate 2) This increase in density progresses until it prevents the penetration of light through the cytoplasm. (Plate 3) In M/Ms showing perinuclear hyperchromia by May-Greenwald Giemsa staining, methylpyronine binding is indicative of an increase in ribose nucleic acid (RNA) content in the darkly staining perinuclear zone. ²² Pyroninophilia is evidence of gene activation and RNA transcription followed by peptide synthesis. (Plate 4)

It is our proposal that M/Ms represent a principle physiological switch for releasing the cascade of cellular and humoral host defense. ACE-M is an effective phalanx to release the first signal, a command for initiating the cellular and humoral actions of host protection. This is supported by data indicating incubation of isolated M/Ms with various doses of ACE-M before addition to purified T-lymphocytes increased lymphocyte incorporation of tritiated thymidine in a dose-related manner. ²³ In experiments, CD4, natural killer and CD8 lymphocytes were activated on a dose-related basis when cultured with M/Ms that had been pre-cultured with ACE-M. Co-culturing the lymphocytes with ACE-M primed M/Ms was essential for T-lymphocyte activation. These experiments provide evidence that ACE-M taken into M/Ms induced immune system activation and modulation.

There is additional information supporting the importance of M/M interactions with lymphocytes and of the stimulatory effect of ACE-M for promoting defense of the body from insult. ACE-M enhanced interleukin-1 (IL-1) and prostaglandin E-2 (PGE-2) production by M/Ms in a dose-related manner. ACE-M increased cytokine production that was up to a twenty-fold above the activity of lipopolysaccharide (LPS), comparing each on an equal weight per volume basis. ²⁴ IL-1 is essential for proliferation of cells necessary for host defense. PGE-2 reduces inflammation and edema in tissues and thus preserves microvascular circulation. Mouse spleen M/Ms cultured with ACE-M displayed dose-related increases in esterase activity, phagocytosis, tritiated thymidine uptake, cell proliferation, and markedly enhanced destruction of target tumor cells, as compared to control, thioglycolate and phytohemagglutinin-stimulated M/Ms. ²⁵ Staphylococcal phagocytosis, chemoluminescence, and tumor cell destruction were enhanced in human M/M cell lines cultured with ACE-M. ²⁶

Chicken mixed-leukocyte cultures verified an ACE-M dose-related increase in phagocytosis (Kemp-Chinnah personal communication). The cited investigators noted that a protein with the electrophoretic mobility consistent with interferon gamma (INF-G) was released into the culture medium in mixed-leukocyte cultures. They also documented that ACE-M enhanced production of neutralizing antibodies to strong and weak viral antigens. This potentiation was systemic and T-lymphocyte dependent. They found that there was enhanced antibody protection when ACE-M was injected into a limb on the opposite side of an animal in which vaccine antigen had been injected. Increased production of neutralizing antibodies was not noted in T-lymphocyte deficient nude mice that were vaccinated and injected with ACE-M. ²⁷

"THE SIGNAL"

ACE-M has proven in our experiments to be a molecule that increases innate and acquired host defense and tissue healing. (Figure 1) This general process has been long described, but it has not been widely recognized or exploited for its therapeutic benefits.

In 1891 William Coley reported a procedure that activated the natural defenses of patients against untreatable and highly malignant soft tissue sarcomas. He boiled, filtered, and injected a mixture of bacterial products into patients with advanced tumors. Coley noted that there was a significant physiological reaction to the material injected. The patients developed high fever and chills. Some patients with advanced malignant tumors experienced a marked reduction in tumor size. In other patients all evidence of their tumor mass was eliminated and the individuals lived for decades. ²⁸ Systemic therapy for malignant tumors and a basis for understanding the mechanism of action for the favorable patient responses, progressed little beyond Coley Toxins for fifty-two years.

In 1943 Coley Toxins were fractionated and purified to identify the active component. ²⁹ The anti-tumor fraction was reported to be the lipopolysaccharide (LPS) component of Gram-negative bacterial cell walls. Injection of LPS into mice incited a systemic reaction that caused hemorrhagic necrosis of tumors, resistance to lethal bacterial injections, and protection from fatal doses of radiation. LPS, which is also known as endotoxin and is responsible for the systemic collapse seen in Gram-negative bacterial sepsis, also killed a number of animals due to its toxicity. The therapeutic use of LPS was limited due to the toxic symptoms that resulted from its administration, poorly defined composition and variable potency. Its production and its usage was ultimately abandoned in the United States.

Radiation therapy, soon followed by cytotoxic chemotherapy, dominated efforts of physicians seeking to improve upon surgical treatment of malignant tumors.

In Czechoslovakia, Lackovic *et al.* studied in great detail the nature of LPS derived from yeast cell walls. ³⁰ They noted that monocytes in culture were stimulated by LPS to produce large amounts of INF-G. Lackovic's team extracted or enzymatically removed serially the lipid, protein, and minute residues of nucleic acid from LPS. The residue was tested for INF-G induction after each extraction step. The residual material continued to induce gene transcription and synthetic translation of INF-G at a constant level. The final product contained only carbohydrate and was chromatographically demonstrated to be greater than 90% cell-wall mannans or polymers of the hexose mannose. This residue continued to induce INF-G production by the cultured monocytes. Incubation of the complex mannans with 2N acetic acid followed by membrane dialysis, lyophilization, and introduction into monocyte cultures doubled the induction of INF-G activity.

Other investigators determined that INF-G has both antiviral ³¹ and antitumor activity. ³² It should be recognized that ACE-M is composed of high molecular weight, polymeric, acetylated mannans. ACE-M, of plant cell origin, is a relatively non-toxic analogue of the bacterial cell wall mannan of LPS reported as inducing M/Ms production of INF-G. ³⁰ Recall that LPS was reported as responsible for the highly desirable biological activities of Coley Toxins. ²⁹ This paper is offered for the reader's consideration as integrating the various fragments of what the authors perceived to be fundamental information that has been accumulating for over a century. This knowledge becomes accessible for practical application and comprehending the importance of ACE-M as a new therapeutic agent. Recognizing the general activating signal to host defense provided by ACE-M is an avenue to understanding and accepting the broad range of efficacy noted in its use for multiple, in no way related diseases, and healing of lesions caused by a variety of agents or conditions.

"THE SYSTEMS"

The inter-related and inter-dependent steps of generalized host defense and how these processes for ameliorating different types of assault against a living organism are systemically integrated is poorly recognized in clinical medicine. For example, the mundane importance for availability of essential biochemical substrates and energy through adequate diet provisions and healthy gastrointestinal function is quite essential to the entire process of maintaining an animal's ability to constantly defend and heal itself. The

complexities of life have fostered narrow specialization in medical practice and the educational process fragments anatomy and life functions for simplification and ease of communication. This communication attempts to unify such artificial division of information.

The hexose sugar, mannose and its polymers, mannans, have not been widely recognized as having great importance in intermediary metabolism. That small, but in this discussion very important point, is being slowly corrected in new editions of biochemistry texts. Appreciation for the importance of mannose-6-PO₄, mannans and other complex carbohydrates is a body of knowledge that is developing in the relatively new field of scientific focus known as glycobiology or glycoscience. In cell structure and function carbohydrates, are a significant component in virtually all ultrastructural cellular constituent parts and are a dynamic participant in biochemical and immunological activities that maintain and protect life.

Mannose molecules are essential constituent and functional components of the surface molecules of cells responsible for the complex biochemical activities essential for cell life. The primary molecular structure of the cellular membrane in plants and animals is synthesized upon a core of mannose molecules (Figure 2) ³³ The chart provided demonstrates the flow of biochemical synthesis and is correlated with ultrastructure for the cytoplasmic organelles where each step in glycoprotein assembly takes place. The importance of mannose molecules is emphasized graphically to stress the role this molecule plays in gene dictated cell synthesis that ultimately results in cell membrane structure that is read, recognized, and responded to immunologically. While figure 2 is complex, it depicts the sequential interaction and serial activities that integrate molecular biology, genetics, biochemistry and immunology. The processes demonstrated are fundamental. Malfunction is a basis for disease and proper function is essential for the activities of maintaining good health, host defense and healing.

The structural orientation of glycoproteins in cell membranes is an important determinant in biochemical function. The peptide segment extended into the cytosol toward the nucleus and the saccharide portion extending outward from the cell surface defines the primary role of carbohydrate molecules in receptor-site structure and cell to cell adhesion and interactions. ³⁴ This remarkably complex molecular cell surface also constitutes the structure and functional capacity of cell-membrane selectivity. Furthermore, the surface molecules provide the molecular code for immunological reading and interpretation. The synthesis of the described molecular structure is determined by nuclear DNA. The mechanism for control of glycoprotein synthesis beyond the ribosome is poorly defined. The cell surface,

constructed on a core of complex mannan molecules, immunologically identifies the organism's species. More specifically these surface molecules determine the tissue transplant antigens of an individual animal. The cell surface mannans constitute the biochemical structure responsible for the gene dictated antigenic expression or "signature" for cell membranes. The complex surface mannan molecules are immunologically read by leukocytes that can accept a cell or call into action the cellular and cytokine components of host defense. Foreign or damaged cell membrane structure provides the binding site for defensive soluble humoral agents, i.e., antibodies, acute reactive-phase proteins, prostaglandins, complement components, etc., that activate and propagate the cascade of the inflammatory response. Cellular membrane composition, self, altered or damaged self and foreign structure is responded to by leukocytes or bound by humoral agents in an appropriate manner to maintain host integrity under optimal circumstances.

The importance in cell biology of cell surface saccharides and particularly mannose with its low molecular weight branched-polymers cannot be over emphasized. Mannans are relatively rare in common western diets.³⁵ However, mannose is such an important hexose in cellular synthesis and function that there is provision in the hemolymph or serum of living creatures, ranking from insects to mammals, for special transport, handling and conservation of this sugar and its low molecular weight polymers. The transport molecules are mannose binding proteins (MBP) produced in the liver of higher-order animals.³⁶ The mannosyl moiety is a critical core component in the oligosaccharide portion of glycoprotein synthesis. MBPs exist to conserve and fastidiously handle this essential cell substrate that is in low concentration in animal and human diets. To conserve and transport this vital family of hexose units, MBPs differ in molecular weight for each hydrolase-produced mannose fragment derived from complex mannose polymers. In mammals there are MBPs for mannose-6-PO₄, the dimer, trimer, quadramer, pentamer and hexamer fragments.³⁷ This transport facilitation, which is not provided for other hexoses, may have evolved due to the fact that trace level mannose is an essential substrate in synthesis, transport and distribution of cell membrane structure, and membrane functional molecules (ion-transport mechanisms, channels, receptor-sites and lysosomal enzymes). Furthermore, plant³⁸ and animal cells have surface membrane MBP receptor sites that utilize an ATP-dependent transport mechanism to foster transfer of mannose-6-phosphate into the cytosol and endoplasmic reticulum (ER).³⁹ More abundant and less essential dietary hexose sugars are not so critically conserved and are less actively transported to a cell's cytoplasmic organelles for use as a substrate in synthesis. Acemannan, extracted from aloe leaf gel, is a rich source of metabolically essential mannan molecules.⁴⁰

The presence of these dedicated MBPs provides the basis for yet another ameliorative action by the administration of ACE-M. In this laboratory it has been demonstrated that adherent human monocytes in culture rapidly phagocytized ACE-M particles which were detected by histochemical stains (Periodic acid-Schiff, Alcian blue and Gram's iodine). Each of these histochemical stains will react with complex carbohydrates. (Plate 5) In serial cultures stained periodically, detection of complex carbohydrates persisted for 5 to 7 days and then no granules were present in the monocyte cytoplasm or medium. These results were interpreted to mean that the M/M hydrolase enzymes digested the macromolecules of ACE-M and cleaved it to mannose or low molecular-weight mannan units. The hexose or mannosyl units produced were then available for synthesis by the cell's organelles. In monocyte and stromal cell-line cultures containing the agent ACE-M, the proliferation of cells compared to those in standard medium significantly exceeded that of controls. ⁴¹ The enhanced cell growth of human stromal fibroblasts and parenchymal cat kidney cells in our experiments strongly suggested utilization of the cleaved mannose units. Mannose-6-PO₄ is an essential substrate in cell membrane synthesis. Mannose polymers produced a dose-dependent increase of cell proliferation when added to culture medium. Based on these observations, administration of mannans or ACE-M would be predicted to provide practical usefulness in therapeutic medicine when cell proliferation for healing is required. This ameliorative potential has been documented as attainable in controlled studies performed *in vivo* through use of an experimental burn model. ⁵ Standardized burns in guinea pigs healed approximately 20 days sooner than controls and treatment with standard medicinal agents used to treat such wounds.

Altering the composition of hexose and hexose amines available in cell cultures alters the glycoprotein synthesis of cells. In tissue cultures of mammalian cell lines, glycosylation of peptide chains in the endoplasmic reticulum (ER) may be altered so that heterologous oligosaccharide complex subunits (synthesis of branched chain oligosaccharides with a mixture of hexoses, i.e., mannose, galactose, fructose, glucosamine, etc.) are produced in altered amounts related to the availability of these hexoses. ⁴² It has been demonstrated that alteration of bacterial cell wall composition can be induced by manipulating the concentration and composition of amino acids and hexose sugars in the culture medium. ⁴³ ER glycosylation and Golgi body tailoring are the organelle processing sites where oligosaccharide synthesis occurs. ⁴⁴ In the Golgi, hexose-amines are substituted on the high mannose content peptide to finalize synthesis and assembly of glycoproteins for cell membrane constitute and functional components. ³³ As described, the addition of ACE-M to monocyte cultures would provide a great excess of an

essential, but relatively scarce substrate through the hydrolysis of this mannose polymer. The increased supply of mannose and the variety of low molecular weight mannan polymer fragments released by hydrolase cleavage of ACE-M fosters altered glycosylation in the ER or Golgi where cell membrane structure is assembled. ACE-M added to viral cultures induced viral envelope glycoprotein dysynthesis (Fig. 3) ⁴⁵. ACE-M was added to HIV-1-infected monocyte cultures and demonstrated that it induced production of multiple molecular weights of HIV-1 envelope glycoproteins rather than only the usual GP-160, GP-120 and GP-41 electrophoretic bands demonstrated in the figure 3 control culture. Alteration in critical viral envelope structure has a therapeutic potential. Forty-two amino acids are in the GP-120 segment of the HIV-1 viral envelope. Deletion of 12 amino acids in this region abolishes virion binding to the target cell CD-4 receptor site and the substitution of one amino acid reduces virion binding. ⁴⁶ GP-120 in the viral envelope is the critical glycoprotein that determines the virion's ability to bind to the CD-4 receptors on susceptible cell membranes. This highly specific viral envelope-receptor site binding is essential for infecting a host cell. ⁴⁷ Alterations in the saccharide composition would be expected to impair the infective capacity of the virions. In other *in vitro* experiments, ACE-M also altered the viral envelope glycoproteins of New Castle and paramyxoviruses in culture and rendered the virions incapable of infecting susceptible target cell lines and animals. ⁴⁸ Thus, ACE-M induced ER and/or Golgi dysynthesis of viral envelope glycoprotein and made the viral particles incapable of infecting previously susceptible target cells. Assessment of the infectivity of HIV-1 virions with altered envelope glycoprotein, as documented by Mitchell, has not been examined *in vivo* due to the lack of a suitable, economical mammalian model.

Responses of symptomatic HIV-1 patients, subsequently presented in this paper, indicate that administration of oral ACE-M results in clinical and laboratory parameter improvement of symptomatic patients infected with the HIV-1 virus. This statement is based, in part, on the following experiment that the authors believe is related to multiple mechanisms of action presented in prior and future paragraphs. Co-cultures of AIDS patients' Ficoll concentrated leukocytes prior and after 6 weeks oral acemannan therapy reduced or eliminated the capacity of the patients' cells to infect previously receptive donor target leukocytes. ⁴⁹ A portion of the documented HIV-1 patient clinical benefit, particularly a rise in CD 4 leukocyte levels (See Table 1), may result from alteration of viral envelope, as has been demonstrated *in vitro* in the three enveloped virus strains cited above.

Mannose-6-PO₄ and its branched polymers have been shown to have additional roles to play in cell biology. Mannosyl units are a component in the structure of lysosomal enzymes and are essential in transport of proenzyme from the Golgi to the lysosomal storage vesicle. Mannose-6-PO₄ and mannose-diphosphate bonds provide an energy source for transport and distribution of glycoproteins completed in the Golgi body.⁵⁰ These activities are essential elements of host defense because activated leukocytes destroy infectious agents by release of lysosomal lytic enzymes on and into the molecular structure of foreign cell material.¹⁷ Lysosomal enzymes cleave or digest the complex carbohydrate, lipid and protein structure of targeted cells or organisms. Importantly, the humoral component of host defense augments this activity by binding of complement, antibodies and other opsins to the surface membrane structure of cells targeted for destruction. This cell lysis process is vital in destroying virus infected cells, tumor cells, scavenging damaged tissue of a wound, remodeling of a scar or callous, and is a deleterious activity in autoimmune diseases.⁵¹

Mannose molecules serve another significant role in cell biology. M/M cells, having emerged from the sinuses of the spleen, liver, and bone marrow, circulate and migrate into tissues or organ cavities. They locate strategically in greater numbers in the skin, mucosa, and other portals of common invasion of the host's inner environment.²¹ The sinus capillaries of the spleen, liver and bone marrow are a generative source and important functional site of these phagocytic cells in their migration stage within an animal. When M/M cells harvested from lung mucus or peritoneal fluid were isotopically labeled and reinjected into the same animal's veins, these cells returned to the tissue or compartment of their origin. M/Ms harvested directly from the blood do not "return home" and are termed to not have "addressin" on their membrane surface.⁵² The addressin on committed phagocytic cells harvested from tissue sites originates in mannosyl and galactosyl molecules arrayed on the cell surface of the committed or experienced M/M cells. Therefore, mannose is a remarkably essential structural and functional hexose in cell physiology. Mannose, as other carbohydrates, is a unit of a complex formed with proteins, lipids and other saccharides providing a vital role in organelle synthesis, biochemical functions, immunologic responses, cellular composition and tissue structural integrity.

One might ask, "What is the molecular mechanism for mannose activation of M/Ms?" French investigators focusing on the carbohydrate portion of the LPS molecule demonstrated that the critical determinant sequence providing the activation signal to M/Ms for IL-1 induction is at the central hinge region of the saccharide portion of the complex moiety.⁵³ This report states that

the essential sequence for activating M/Ms to produce and release IL-1 is the following critical molecule core complexed with at least a disaccharide:

2-keto-3-deoxy-D-Manno*-octulosonic acid, or (KDO)

*bold type for author emphasis

The addition of two heptose units as a side-chain to KDO results in M/M gene transcription and organelle translation for this monokine (IL-1) synthesis. The progressive addition of more hexose units amplifies the induction signal. Investigators have reported that the saccharide fraction of LPS induces production of IL-1, ⁵⁴, INF-G, ⁵⁵ and TNF. ⁵⁶

The molecular biology of the above data is interpreted by the authors to indicate that the constant molecular determinant for M/M sensing essential for humoral and cellular defense activation, is complex glycoprotein and lipoprotein and similar molecular structured biochemical compounds related to the KDO molecular conformation. We propose that the branched oligosaccharide chains of glycoprotein cell surface structure is analyzed by M/Ms and determined to be comprised of a molecular sequence and/or steriomeric configuration recognized as self, non-self, or damaged/alterd self. A molecular sequence recognized as other than "normal-self" can initiate an activation or alert signal to the defense systems of the host. (Figure 4) The released monokines are messenger molecules or stimulating signals that initiate host target cell defensive gene activation resulting in the systemic inflammatory response. ⁵¹ Stimulation or modulation of this activation pathway provides the desirable effects noted in a wide variety of unrelated disease states or lesions responding to the administration of ACE-M. ACE-M is pure saccharide, an amplified, relatively non-toxic, non-antigenic activating molecule (actogen) that we believe mimics the KDO signal. ACE-M and structurally related activator molecules provide a stimulation signal to M/Ms for initiating cellular and humoral defensive activity. In support of this statement, ACE-M has been shown in mixed human leukocyte cultures assayed by ELISA techniques to induce production on a concentration gradient related basis IL-1 β and PG-E₂ ²⁴, and IL-1 β , IL-2, IL-6, TNF-alpha, GM-CSF, INF-alpha, INF-gama. ⁵⁷

One of the more general systems of humoral host defense and repair is induction of interferon production. ⁵⁸ INF-G activates genes that defend a cell from synthesizing aberrant moieties, such as dictated by viral nucleic acids. Non-genomic instruction conveyed by DNA and its complementary mRNA, may be dictated by integrated viral DNA or oncogenes. ⁵⁹ The synthetic assembly-line in the cytosol of all cells is audited by multiple cellular mechanisms that sense and activate enzymes that destroy errors and

non-genomic transcribed mRNA that functions through the pirating of the cell's synthetic organelles. A cell's internal audit system is activated by interferon release during a viral infection ⁶⁰ or malignancy by oncogene-dictated products. ⁶¹ Kausner, in his review of glycoprotein cell-editing, reported that assembly errors in glycosylation, that is, non-genomic nucleic acid-dictated synthesis that occurs in the ER and results in "abnormal" cell glycoproteins, can be corrected by the administration of INF-G. ⁴¹

There have been disappointing responses of important human tumors treated with recombinant DNA (rDNA) produced interferons as noted in a review of breast cancer treatment trials ⁶² renal cancer ⁶³ and non-osseous tumors. ⁶⁴ The above clinical study results stand in stark contrast to the benefits attained by endogenously synthesized cytokines whose production is induced by administration of ACE-M to suppress allergic based infections ^{3,4}, promote more rapid wound healing ^{4,5}, foster antiviral activity ^{2,48} and induce antitumor action. ^{6,7,8}

Endogenously produced interferons are signal molecules produced by complex leukocyte interactions with activities similar to interleukins. ACE-M works by inducing the production of interactive and interdependent cytokines active in host defense and healing. We propose an explanation for the discouraging reports of poor therapeutic efficacy in the treatment of malignancy with these potent, biologically active substances produced by expensive recombinant DNA technology. Endogenously produced interferons, acting as cell modulators, are released in ultra-micro quantities localized to the tissues surrounding wounds or tumors. (A viral or bacterial infection with a general systemic febrile reaction presents an exception that constitutes a desirable defense reaction against a microbe that has a narrow zone of temperature tolerance.) The conditions in diseased tissue that warrant the influx of humoral and cellular defensive action are orchestrated initially, and monitored continually by M/M cells that modulate interferon and cytokine production. In a large lesion, or with multiple lesions, the M/Ms direct a variety of cells surrounding a mass or at a lesion's margins to respond at different phases of inflammation or repair. ¹² Granulation tissue proliferation with epithelial cell growth to cover the surface may be active in one area of a large ulcer, while granulocytic leukocytes are stimulated to migrate to a focus in the lesion where a staphylococcal infection has occurred. These contrasting steps in the orderly temporal sequence of host response are coordinated by M/M cells distributed throughout viable tissues having capillary circulation.

In our laboratory we have termed the physiological phenomenon of distributive, continuous monitoring of micro-environmental conditions and

release of appropriately sequenced stimulatory and inhibitory signal cytokines to responsive target cells: as a system of site-, condition-, time-, specific response (SCTSR). It is this critical distributive monitoring by macrophages stimulated by ACE-M of the contrasting wound conditions that produces the unprecedented acceleration of healing, quality of healed tissue, plus a broad range of ameliorative activities that are fostered by this BRM.

The endogenously produced, self-antigen signature and ultra-micro-dosing, synthesized, maintained, terminated and limited to the field of tissues invaded, damaged or ready for repair is not possible with exogenously administered agents. ACE-M is a biological BRM that primarily stimulates M/Ms in situ to enhance normal SCTSR functions in diseased and healing tissue. ⁴ The authors' analysis of observed animal and human responses to ACE-M is that enhanced activation followed by appropriate suppression of sequential steps of multi-focal cellular responses, in different stages of inflammatory response or repair for various micro-environments of a large wound or lesion are not disrupted, shortened, or prolonged by administering the BRM ACE-M. The use of ACE-M enhances normal physiology, maintains distributive macrophage monitored activity and also takes advantage of the complex sorting and distribution function of the Golgi complex. This mechanism of action avoids inappropriate inhibition feed-back induced by excessive, exogenously administered cytokines. The above explanations are theoretical constructs developed to explain demonstrated practical therapeutic benefits from the use of ACE-M. For example, ACE-M applied to deep, large, experimental burns as a 0.5% hydrogel resulted in more rapid healing (40% faster or 30 days vrs. 50 days ave. healing time for controls and standard agents), reduction of infection, and less scarring of the skin and parenchymal tissues as compared to controls and standard therapeutic agents used in current practice. ⁵

There are other serious problems with rDNA engineered agents. Recent evidence has been provided indicating that administration of rDNA engineered interferon has resulted in antibody production in response to this foreign glycoprotein causing the development of host antinuclear antibodies and autoimmune thyroid disease ⁶⁵

Suppression of cytochrome P-450 (desmolmase complex) electron transport has been demonstrated as a result of therapy with rDNA produced interferon. ⁶⁶ The cytochrome system in the mitochondria is a major source of energy bond transfer from hydrogen bonds to phosphate bonds that constitutes the primary source for energy upon which cellular functions in mammalian cells are dependent. This same suppressive action against cytochrome P-450, acting in liver cells to detoxify plant toxins and organic

drugs, may be the basis for acquired drug toxicity for agents (theophylline, antipyrine, opiates, plant derived anti-tumor agents) metabolized by liver cells. ⁶⁷

Recombinant DNA engineered granulocyte/macrophage colony stimulating factor (G/M-CSF) used to stimulate a hypocellular bone marrow has resulted in the induction of an excessive proliferation of aggressive marrow histiocytic cells leading to death of patients in a manner similar to non-Hodgkin's lymphoma. ^{68,69,70}

In summary, we propose that ACE-M induces cellular production of endogenous cytokines under control of the host's genes (1) when, (2) where and (3) in the amount needed in response to molecular cues present in the cellular micro-environment or SCTSR. Toxic and other untoward events are minimal due to the physiological and exquisitely targeted delivery of endogenously produced, potent defensive cell products delivered in ultra-micro-molecular amounts at the time dictated by tissue conditions.

PHARMACOGNOSY

McAnalley, Eberendu and Moore determined the basis for disagreements over the presence of a medically beneficial principle in the gel of the aloe vera plant. ¹¹ A hydrolytic enzyme is released when the plant is injured or compressed to express gel from the leaf central core. Thus, the active agent disappears with time due to the activity of this enzyme. Considering the nature of this enzyme, conflicting investigators could both be correct even though one reported no benefit and another reported a medical benefit attributed to the use of aloe vera gel. The Carrington Laboratories research team, developed and patented a means to inactivate the mannosidase enzyme that cleaves the complex mannose polymer. ¹ Inactivation of the enzyme made it possible to extract, purify, and characterize the molecular structure of ACE-M. A composition-of-matter patent (U.S. No. 4,735,935) has been issued for ACE-M in over 60 countries, including the United States, Canada, and most European nations.

ACE-M has been given the trade name of Carrasyn and is described as acetylated beta-D-mannan (Figure 5). The polymers of linked mannose units have polydispersed molecular weights. Multiple tissue culture challenges to living cells, numerous species animal toxicity tests, and appropriate human testing have disclosed no significant ACE-M toxicity or serious side-effects. ^{71, 72, 73, 74, 75}

STEPS LEADING TO THE USE OF ACEMANNAN IN CLINICAL MEDICINE

Development of the basic science principles we have presented for ACE-M came in response to AIDS patient anecdotal reports of claimed efficacy associated with the personal use of an particular aloe gel beverage (Caraloe™). This formulation was specifically developed to process the plant extract in a way to protect this hydrolase sensitive macro-molecule. Once the medical claims alledged by HIV-1 patients were confirmed under physician observation, research was directed more aggressively toward finding the physiologically active molecule in the plant. Biochemical and physiological mechanisms to explain the nature for such ameliorative responses were sought. Investigations relating to *in vitro* pre-clinical and human pilot clinical uses of ACE-M will be briefly summarized. In view of multiple patient claims for benefit against the HIV-1 viral syndrome and the international focus on the AIDS epidemic, antiviral and immune system stimulation activity will be emphasized.

ACE-M blocked proliferation of Herpes II, feline rhinotracheitis, measles, and HIV-1 in virus-susceptible tissue culture target cells. ⁷⁶ It was demonstrated with mRNA probe, dot-blot technology that ACE-M addition to HIV-1 infected lymphoid cell lines inhibited viral mRNA transcription from HIV-1 integrated viral DNA. ⁷⁷ In human lymphoid cell lines the combination of ACE-M and AZT reduced to one-sixtieth (1/60) the dose of AZT required alone to be virucidal. Similar ACE-M synergism of Herpes II virus inhibition by acyclovir was also shown. ⁷⁸ In human mixed-leukocyte cultures, addition of ACE-M stimulated cytokine release through complex M/M lymphocyte interactions resulting in increased numbers and activation of cytotoxic T lymphocytes having antiviral and anti-tumor function. ⁷⁹

In animal models, antiviral activity of ACE-M was demonstrated in common chicken and murine virus infections. ⁴⁸ ACE-M blocked the infection of human paramyxovirus that transmits influenza in cell lines. ⁸⁰ Administration of a single oral dose of ACE-M (5 mg/kg) blocked infection of test animals by feline rhinotracheitis virus. ⁸¹ In this animal the symptoms induced by this virus are similar to the common-cold in humans.

A single intraperitoneal (IP) injection of ACE-M (3micro g/kg) totally destroyed implanted tumors (Norman Murine Sarcoma) in 35% of mice and prolonged the life in 100% of test animals. ACE-M induced elevations of tumor necrosis factor (TNF) and interleukin-2 (IL-2) in peritoneal monocytes collected from these experimental animals. ⁷

Wilson and McDaniel (unpublished data) noted that ACE-M injections caused clinical remissions and normalized laboratory tests in ten cats with feline leukemia and two dogs having canine lymphoma. To formally test this observation, weekly intra-peritoneal (IP) administration of ACE-M to 49 cats with feline leukemia yielded 71% clinical and laboratory improvement at 12 weeks post-therapy and 58% survived 5 months.⁸² Cats and dogs with a variety of malignant tumors were given IP injections of ACE-M.⁸³ Fibrosarcoma and lymphomas were the most responsive to reductions of tumor mass. Infiltrative, non-resectible tumors developed a peripheral fibrous capsule and could be resected after weekly injections of ACE-M (5mg/kg). Scrutchfield and McMullin (unpublished data) noted total elimination of equine sarcomas after injection of ACE-M into the skin lesions. ACE-M administered IP made fibrosarcomas in canine and feline subjects surgically resectable or eliminated the tumors.⁸⁴ In response to these reports, in 1991 the U.S. Department of Agriculture approved use of injectable ACE-M to treat sarcomas in cats and dogs.⁸⁵

The systemic and progressive demonstration of desirable responses to ACE-M in experimental models has been extended to human pilot studies. Addition of ACE-M (500 mg/day, orally) to the medication regimen of patients who had become unresponsive to conventional therapy eliminated clinical symptoms and rapidly healed bowel lesions in ten ulcerative colitis patients in acute flare.⁸⁶ This pilot project has progressed to a multiple centered FDA protocol study expected to be completed in 1994.

In an FDA individual physician clinical pilot study with 14 HIV-1 reactive patients, a 71% improvement in clinical and laboratory scoring was achieved after 90 days of daily treatment with 800 mg of ACE-M in capsules. Reductions or elimination of detectable HIV-1 virus in leukocyte co-cultures from selected, more compliant patients and reductions in P-24 core antigen (Abbott Diagnostics), were noted.⁸⁷

In a subsequent clinical pilot study with 15 HIV-1 reactive patients, a 69% improvement in clinical and laboratory scoring was observed at 90 days therapy.⁸⁸ Serial assays of CD-4 lymphocyte levels were as indicated in Table 1. P-24 core antigen levels were reduced or became undetectable in patients with pre-treatment detected levels of the antigen. Skin-test anergy to common antigens (candida, mumps, trichophyton, histoplasmin, tuberculin and tetanus) was converted to reactive. There was a reduction in severity or incidence, and in some cases elimination, of opportunistic infections. Weight gains and return to normal physical activity were recorded in the first 12 months in the favorably responding patients.

AIDS patients in the 1986 pilot study were offered gratis, a continued supply of oral acemannan and an annual evaluation. Four patients that elected to continue on oral acemannan were alive in excess of 84 months and were last evaluated in 1993. Since 1989 these patients have had no serious opportunistic infections, no hospitalizations, maintained stable or normal to mildly reduced, but stable CD-4 lymphocyte counts and have been gainfully employed. It was also noted that these AIDS patients maintained on oral acemannan, had sustained, significantly elevated CD 8 lymphocyte levels (See Table 2). Cytotoxic T-lymphocytes having antiviral activity are in this cell population and were shown to have enhanced activity induced by ACE-M.⁷⁹ The long-term survivors' serum HIV-1 mRNA levels, as determined by two-primer PCR methodology, was significantly lower than a nucleoside analogue treated comparison population.⁸⁹

In March 1994 three of the 1986 pilot patients were alive and continue to take the oral BRM regularly. They have been the most compliant patients for continued intake of oral acemannan as Caraloe™. Evidence of their CD4 and CD8 lymphocyte levels are provided in Table 2. All the patients have gained weight and shown restoration of reactions to skin test antigens. Two patients are full-time employed and the third does part-time work. These patients have not had a major opportunistic infection in 8 years or required hospitalization. Their response to an appropriate antibiotics given for early symptoms of an infection is rapid.

The twelve deceased study patients included; 2 overt suicides by drug overdose, an accidental overdose to cocaine, 3 depressed over economic and personal events who terminated all therapy including oral acemannan, 1 renal/bone marrow/liver failure from multiple medication toxicities for CMV retinitis and 5 from infections associated with the HIV-1 syndrome. Only two of these Last 5 patients maintained a level of 75% compliance as rated on an annual basis for the daily intake of oral ACE-M. Patients lived 12 to 24 months after terminating ACE-M intake.

Retrospective analysis was made in 1987 of the 29 AIDS patients treated in the two initial exploratory pilot studies. It was noted that although the average laboratory and clinical status had improved, there were responsive, moderately responsive, and non-responsive individuals as indicated by clinical assessments, and CD4 plus CD8 lymphocyte levels. Pre-treatment laboratory and clinical data compared to the global response to ACE-M was analyzed in an attempt to identify which patients might be expected to respond to the BRM. A hypothesis was developed that a P-24 core antigen level of greater than 300 pg/dL (a measure of virus load or expression) and

an absolute CD-4 lymphocyte count of less than 150 mm³ (an indicator of immune system damage) would prospectively identify the poorly responsive patient population. It was proposed that patients with P-24 core antigen levels of less than 300 pg/dL and a CD-4 absolute lymphocyte counts of over 150 mm³ would identify, prior to therapy, patients more likely to respond favorably to ACE-M.

In 1988, 26 symptomatic HIV-1 patients were evaluated (Watson and McDaniel Pilot Study) and a prediction was made using the above criteria regarding the expected response to ACE-M. In this "predictive" pilot of 26 patients, 16 patients were projected to respond favorably to ACE-M and 10 were predicted to do poorly ⁹⁰ (See Table 3).

CRITERIA FOR A FAVORABLE RESPONSE TO ACEMANNAN THERAPY

1. INCREASE IN CD-4 LYMPHOCYTES
2. DECREASE IN P-24 CORE ANTIGEN, IF DETECTED,
OR REMAIN NON-DETECTABLE
3. IMPROVEMENT IN GLOBAL CLINICAL SCORING

Achievement of all three assessment criteria was rated as a favorable response. Patients attaining two of the three study criteria were also included in correct predictions for calculating predictive accuracy. The overall ability to predict the response of patients to ACE-M was approximately 88% in that 23 of 26 patients responded as projected prior to treatment with ACE-M for the 90 day observation period.

The final evaluation of the three open-label, clinical pilot studies involving 55 patient treatment periods was that ACE-M warranted further, blinded-clinical investigation. In response, in 1989 a double-blind clinical study was conducted in Belgium with 47 symptomatic HIV-1 reactive patients.⁹¹ The patients were organized into four groups to receive azidothymidine (Zidovudine, AZT) alone, ACE-M alone, AZT and ACE-M in combination, and a double-placebo. ACE-M demonstrated no toxicity, stabilized CD-4 lymphocyte counts, and produced improved clinical scoring. There was a suggestion that the toxicity and emetic effect of AZT was reduced in the group receiving AZT and ACE-M. This small placebo-controlled study supported the findings of the three open-label pilot clinical studies done in the United States that indicated ACE-M has potential efficacy in treatment of HIV-1 reactive patients. In 1993 the patients in this study were evaluated. It was determined that in a comparison of the four groups in this study, the

longest survivors had received oral acemannan for the six months of this study (personal communication).

Subsequently, a study to test the efficacy of ACE-M in advanced and terminal AIDS patients was initiated in early 1991. The study involved cooperation between Carrington Laboratories, Inc. of Irving, Texas and the Canadian HIV Trials Network. The Canadian patients selected had suffered combined damage from the HIV-1 virus and long-term AZT toxicity. The patients were treated in a double-blind placebo controlled study with oral acemannan capsules. The study results indicated that advanced AIDS patients, also subjected to the continued toxicity of AZT administration, showed clinical and laboratory evidence of abatement for the rate of deterioration of CD4 lymphocyte counts ($p=.04$) by the addition of oral ACE-M to their treatment regimen. ⁹²

CONCLUSIONS

In his keynote address at the 1982 Prix d'Immunologie Behring-Metschnikoff Awards, E.L. Cooper, PhD. called for identification of the unifying molecule or scheme responsible for simultaneous activation of humoral and cellular host defenses. ⁹³ The authors of this communication propose that there is no single molecular signal. It is submitted that the infinite variability of cell-surface molecules built upon a biochemical core of polymeric mannose sugars (mannans) present in the cell wall of microorganisms and cell membrane of life forms on this planet provide the initial activation signal that activates monocyte/macrophages (M/M). The M/Ms release secondary signals or monokines, such as TNF, INF-G, IL-1, and IL-6 that bind to membrane receptors of target cells, primarily leukocytes, endothelium, fibroblasts, and liver parenchymal cells. Ultimately, the receptor binding results in gene activation, synthesis and release of humoral products, and activation of cellular components of host defense that are recognized as the inflammatory reaction. The inflammatory reaction is comprised of simultaneous, interactive, humoral and cellular responses.

It has been demonstrated that the acetylated mannan, ACE-M activates the humoral and cellular cascade of the inflammatory response. Mediated through the sensing capacity of M/Ms, ACE-M accelerates and fosters constructive modulation of the activities essential for host defense and healing. This non-toxic, complex mannan, as an initial high molecular weight polymer phagocytized by M/M cells, provides a signal that simulates invasion by an infectious agent. A massive activation of humoral and cell defense is followed by a secondary phase in which the normal physiology of defense and healing are augmented by hydrolysis of ACE-M into fragments

of products essential for host defense and repair. There is a strong suggestion that the signal provided by ACE-M has a role to play in preventive medicine if the polymer is administered in a timely manner. 94, 95

The pioneering work of Metschnikoff, who identified "The Cells' " role in host defense, Von Behring, who defined humoral host defense, and Coley, who first used "The Signal" provided by complex mannans for activation of the inflammatory response for tumor destruction, and the thousands who have contributed to defining biochemically and immunologically "The Systems," have provided the foundation for the addition of this cornerstone for the treatment of multiple diseases. That foundation stone is the BRM acemannan. Future physiologically active carbohydrate molecules are destined to follow. The authors predict that glycobiology, a unique class of molecules with low toxicity, working in concert with and enhancing normal cellular physiology and host defense, are destined to deliver medical benefits far beyond the expectations of prior science. The authors appreciatively stand upon and acknowledge the powerful foundation of labor, observation, imagination, interpretation, knowledge, commitment, and persistence provided by those who started their observations over a century ago. The information they have provided forms a harmonious matrix for support and recognition of the roles played by "The Cell, The Signal and The Systems" activated by acemannan.

This manuscript is dedicated to Terry Pulse, M.D. (1951-1991) and to the expired and living patients and sacrificed experimental animals that have contributed to making this manuscript possible.

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