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(54) Title: ENGINEERING THE LOCAL INFLAMMATORY RESPONSE AS A MEANS OF CONTROLLED RELEASE DRUG DELIVERY

(57) Abstract

A product adapted for implantation and suitable for controlled release of a biologically active substance, comprising: the biologically active substance and at least one cellular regulator, wherein the introduction of said product subcutaneously into a subject, induces or inhibits a local tissue response, and wherein said cellular regulator is present in sufficient amount to affect the local tissue response, such as to affect the kinetics of release of the biologically active substance from the product.

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ENGINEERING THE LOCAL INFLAMMATORY RESPONSE AS A MEANS OF CONTROLLED RELEASE DRUG DELIVERY

Field of the Invention

This invention relates generally to drug delivery systems and particularly to a product, consisting of a drug and cellular regulator, which, when introduced subcutaneously into a human or animal, affects the local tissue response, thereby affecting the release and absorption rate of the drug into general circulation.

Background of the Invention

There is a need for a reliable, controlled release drug delivery system in which a drug can be delivered to a subject over a prolonged period without repeated administration. Clinical therapies often require that a continuous dosage of a drug be administered, or that multiple drugs be administered in sequence at regular intervals in continuous dosages over extended periods of time.

There is a long history of controlled release drug delivery. Methods include long acting oral dosage forms, bolus injections, transdermal patches and sub-cutaneous implants.

Previous efforts to develop implantable drug delivery systems have used polymeric or nonpolymeric materials. The polymeric systems consist either of matrices of non-biodegradable polymers or matrices of

biodegradable polymers. Such polymer-based systems are combined with the drug to create either a matrix erosion system, in which the drug is evenly distributed in a polymer matrix and is released as the polymer breaks down in biological fluid after introduction into the subject, or a matrix diffusion system in which the drug is released by diffusion through the polymer matrix, or a matrix diffusion/erosion system in which the drug is released by diffusion through the polymer matrix, as well as by erosion as the surface of the polymer breaks down. Microspheres in aqueous or oil suspension, have also been used in which the drug is coated with a bioerodible polymer and injected subcutaneously. The microspheres erode and release the drug according to the size and number of microspheres. In addition, systems utilizing hydrogels and polymeric reservoirs have been devised. The nonpolymeric systems include compressing mixtures of the drug and an excipient into a pellet. Such nonpolymeric systems can also be made of a totally fused pellet or a partially fused pellet. In a totally fused pellet, a drug and an excipient are melted and recrystallized to form a crystalline matrix; in a partially fused pellet, a mixture of a drug and an excipient having a lower melting temperature than the drug is heated and cooled such that only the excipient melts and recrystallizes. Cholesterol matrix systems have also been developed.

The systems suffer from various inherent problems

including: foregoing general permeability to macromolecules; swelling of macromolecules, resulting in their being trapped in the erosion or diffusion systems; highly water soluble macromolecules exhibiting volatile dissolution kinetics in implants; proteins aggregating under certain pH conditions, turning into gel-like substances and thereby impeding release; large molecular weight molecules having problems being taken up by transport cells; surface erosion resulting in indefinite duration that may present a prolonged period of sub-effective drug release, and the requirement of removing the implant at the end of the effective release period.

Many of these problems have been overcome by relatively recent nonpolymer implant technology relating to totally fused and partially fused implants. See, for example, U.S. Patent Nos. 4,748,024 and 4,892,734 issued to Leonard, co-pending application Serial No. 07/565,273, "Multiple Drug Delivery System", filed August 9, 1990 and co-pending application Serial No. 07/163,328, "Partially Fused Peptide Pellet", filed March 2, 1988, the entire disclosures of which are incorporated herein by reference.

All of the foregoing approaches, however, suffer from a failure to recognize and take into account a critical factor involved in implantable long term delivery systems, that is, the effect on drug release of the local tissue response to the implanted systems. The recognition of this factor, as well as

an approach to account for and take advantage of it, forms the basis of the present invention.

Summary of the Invention

The invention provides a product, including a biologically active substance, at least one cellular regulator, and optionally an excipient, and a method whereby such product is introduced subcutaneously into a human or animal.

The cellular regulator is capable of controlling, by either stimulating or inhibiting, one or more of the processes that are associated with the local inflammatory response resulting from the subcutaneous implant, such as production of macrophages, proliferation of fibrous tissue encapsulating the implant, infiltration of the fibrous capsule by new blood vessels and lymphatics, and transport of lipophilic or hydrophilic molecules into or out of the encapsulated implant. By specifically regulating colony stimulation, angiogenesis, and tissue generation in this way, the cellular regulator aids in generating a "paraglandular" compartment surrounding the subcutaneous implant, which is capable of active and interactive processing, including the release of the biologically active substance from the "paraglandular" compartment. This invention thus exploits the naturally occurring local inflammatory response by artificially manipulating the presence, concentration, and order of appearance, of one or more cellular regulators, so as to affect the kinetics of

release of the biologically active substance from the encapsulated implant.

The biologically active substance preferably is a drug. The cellular regulator preferably is a cytokine. The optional excipient may be part of any one of a variety of drug delivery systems, including polymeric systems, non-polymeric systems, microspheres, hydrogels, polymeric reservoirs or cholesterol matrices, which serve as the embodiment for the implant.

Brief Description of the Drawings

Fig. 1 is a cross-sectional view of a "paraglandular" compartment after implanted drug pellet was removed, as viewed by scanning electron microscopy (SEM);

Fig. 2 is a cross-sectional view of the compartment lumen surrounding an implanted pellet, as viewed by SEM;

Fig. 3 is a cross-sectional view of the compartment wall showing blood vessels and relative tissue densities, as viewed by SEM;

Fig. 4 is a cross-sectional view of two blood vessels of capillary size in the middle region of the capsule wall, as viewed by SEM;

Fig. 5 is another view of the sample depicted in Fig. 4 with intact erythrocytes inside blood vessels, as viewed by SEM;

Fig. 6 is a thin section of a fibroblast with collagen fibrils of normal periodicity, as viewed by

transmission electron microscopy (TEM);

Fig. 7 is a thin section of fibrous wall, excised at 6 months, showing a few lipid-engorged foam cells among loose collagen fibrils, as viewed by TEM;

Fig. 8 is a thin section of fibrous wall, excised at 13 months, showing significantly denser populations of lipid-engorged foam cells and denser collagen fibrils than are present in Fig. 7 at 6 months, as viewed by TEM.

Detailed Description of the Invention

This invention pertains to a drug delivery system, including a drug and a cellular regulator, which, when introduced subcutaneously into a subject, affects the local tissue response, and thereby results in controlled release of the drug.

When a foreign body is subcutaneously introduced into a human or animal, a naturally occurring local inflammatory response occurs. This response is characterized by the stimulation of macrophages, proliferation of fibrous tissue encapsulating the foreign body, and infiltration of the fibrous capsule by new blood vessels and lymphatics. See, e.g., S.M. Wahl et al., "Role of Growth Factors in Inflammation and Repair," J. Cell. Biochem., 40:193-199 (1989); A. Roberts et al., "Transforming growth factor type ß: Rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro," Proc. Natl. Acad. Sci., USA, 83:4167-4171 (1986).

The specific regulation of this inflammatory

response is modulated by naturally occurring, locally acting, cellular regulators, which affect colony stimulation, angiogenesis, and tissue generation. See, e.g., K. Arai et al., "Cytokines: Coordinators of Immune and Inflammatory Responses," Annu. Rev. Biochem., 59:783-836 (1990). The composite effect of such interactions is to generate a "paraglandular" compartment surrounding the foreign body, capable of active and interactive processing.

It has been discovered that this paraglandular compartment is an important factor in determining the release kinetics of implanted delivery systems. This invention is designed to exploit the local inflammatory response by artificially manipulating both the presence and concentrations of various cellular regulators to favorably influence the controlled release of drug from an implanted delivery system. By modulating the active processing and equilibration of the various cell and tissue populations, the invention is designed to affect drug release and absorption from the foreign body implant. This invention thus represents a new approach to drug delivery systems.

The invention involves the implantation of a biologically active substance which is to be delivered to a human or an animal in a therapeutically effective amount, and at least one cellular regulator which is present in sufficient amount to affect the local inflammatory response which will result from implantation.

A. The Biologically Active Substance

The biologically active substance may be any substance having biological activity, including protein, polypeptide, polynucleotide, nucleoprotein, polysaccharide, glycoprotein, lipoprotein, and synthetic and biologically engineered analogues of such molecules.

Preferably, the biologically active substance is a drug. A drug is a substance used on or administered to humans or animals as an aid in diagnosis, treatment or prevention of disease or other abnormal conditions, for relief of pain or suffering, or to control, affect, maintain or improve a physiological or pathological condition.

Classes of drugs which are intended to be included within this invention include anti-AIDS substances, anti-cancer substances, antibiotics, anti-viral substances, enzyme inhibitors, neurotoxins, opioids, hypnotics, tranquilizers, anti-convulsants, muscle relaxants and anti-Parkinson substances, anti-spasmodics and muscle contractants, anti-hypertensives, analgesics, anti-pyretics and anti-inflammatory agents, local anesthetics, prostaglandins, anti-depressants, anti-psychotic substances, anti-emetics, imaging agents, specific targeting agents, neurotransmitters and proteins.

Anti-AIDS substances are substances used to treat or prevent Autoimmune Deficiency Syndrome (AIDS). Examples of such substances include CD4, 3'-azido-3'-deoxythymidine (AZT),

9-(2-hydroxyethoxymethyl)-guanine acyclovir (acyclovir), phosphonoformic acid, 1-adamantanamine, peptide T, and 2',3' dideoxycytidine.

Anti-cancer substances are substances used to treat or prevent cancer. Examples of such substances include methotrexate, cisplatin, prednisone, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, ethinyl estradiol, tamoxifen, testosterone propionate, fluoxymesterone, vinblastine (VLB), vincristine, vindesine, etoposide, teniposide, dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bieomycin, plicamycin (mithramycin), mitomycin (mitomycin C), -asparaginase, hydroxyurea, procarbazine (N-methylhydrazine, MIH), mitotane, aminoglutethimide, mechlorethamine, cyclophosphamide, melphalan (-sarcolysin), uracil mustard, chlorambucil, busulfan, carmustine (BCNU), lomusline (CCNU), semustine (methyl-CCNU), streptuzocin (steptozotocin), dacarbazine (DTIC: dimethyltriazenomidazolecarboxamide), methotrexate (amethopterin), fluorouracil (5-fluorouracil: 5-FU), cytarabine (cytosine arabinoxide), mercaptopurine (6-mercaptopurine: 6-MP), thioguanine (6-thioguanine: TG).

Antibiotics are art recognized and are substances which inhibit the growth of or kill microorganisms. Antibiotics can be produced synthetically or by microorganisms. Examples of antibiotics include pennicillin, tetracycline, minocycline, doxycycline,

vanomycin, bacitracin, kanamycin, neomycin, erythromicin and cephalosporins. Examples of cephalosporins include cephalothin (keflin, seffin), cephapirin (cefadyl), cefazolin (ancef, kefzol), cephalexin (keflex), cephradine (anspor, velosef), cefadroxil (duricef, ultracef), cefamandole (mandol), cefoxitin (mefoxin), cefaclor (ceclor), cefuroxime (zinacef), cefonicid (monocid), ceforanide (precef), cefotaxime (claforan), moxalactam (moxam), ceftizoxime (cefizox), ceftriaxone (rocephin), and cefoperazone (cefobid).

Anti-viral agents are substances capable of destroying or suppressing the replication of viruses. Examples of anti-viral agents include α-methyl-1-adamantane methylamine (ri mantadine), 1-ß-D-ribofuranosyl-1,2,4-triazole-3 carboxamide (ribavirin), 9-[2-hydroxy-ethoxy]methylguanine (Acyclovir), adamantanamine, 5-iodo-2'-deoxyuridine (Idoxuridine) and adenine arabinoside (Vidarabine).

Enzyme inhibitors are substances which inhibit an enzymatic reaction. Examples of enzyme inhibitors include edrophonium chloride, N-methylphysostigmine,(-)-, neostigmine bromide, physostigmine sulfate, tacrine HCL (THA), tacrine,1-hydroxy maleate, iodotubercidin, p-bromotetramisole,(-)-, 10-(α-diethylaminopropionyl)- phenothiazine hydrochloride (As-1397), calmidazolium chloride, hemicholinium-3, 3,5-dinitrocatechol (OR-486), diacylglycerol kinase inhibitor I (R59022),

diacylglycerol kinase inhibitor II (R59949), 3-phenylpropargylamine, N^6-monomethyl-L-arginine acetate, carbidopa, 3-hydroxybenzylhydrazine HCl (NSD-1015), hydralazine HCl (apresoline), clorgyline HCl, deprenyl HCl,L(-)-, deprenyl HCl,D(+)-, hydroxylamine HCl, iproniazid phosphate, 6-MeO-tetrahydro-9H-pyrido-indole, nialamide, pargyline HCl, quinacrine HCl, semicarbazide HCl, tranylcypromine HCl, N,N-diethylaminoethyl-2,2-diphenylvalerate hydrochloride, 3-isobutyl-1-methylxanthne, papaverine HCl, indomethacind, 2-cyclooctyl-2-hydroxyethylamine hydrochloride (CONH), (±)-2,3-dichloro-α-methylbenzylamine (DCMB),(LY-78335), 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride, p-aminoglutethimide,(±)-, p-aminoglutethimide tartrate,R(+)-, p-aminoglutethimide tartrate,S(-)-, 3-iodotyrosine,L-, α-methyltyrosine,L-, α-methyltyrosine,D L-, and allopurinol.

Neurotoxins are substances which have a toxic effect on the nervous system, e.g. nerve cells. Neurotoxins include adrenergic neurotoxins, cholinergic neurotoxins, dopaminergic neurotoxins, and other neurotoxins. Examples of adrenergic neurotoxins include N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride. Examples of cholinergic neurotoxins include acetylethylcholine mustard hydrochloride acetyl AF-64. Examples of dopaminergic neurotoxins

include 6-hydroxydopamine HBr, 1-methyl-4-(2-methylphenyl)-1,2,3,6-tetrahydro-pyridine hydrochloride, 1-methyl-4-phenyl-2,3- dihydropyridinium perchlorate, N-methyl-4-phenyl-1,2,5,6- tetrahydropyridine HCl, 1-methyl-4-phenylpyridinium iodide. Other neurotoxins include L-ß-methyl-α,ß-diaminopropionic acid hydrochloride, (±)-ß-methyl-α,ß-diaminopropionic acid hydrochloride, L-ß-oxalyl-α,ß-diaminopropionic acid, and quinolinic acid.

Opioids are substances having opiate like effects that are not derived from opium. Opioids include opioid agonists and opioid antagonists. Opioid agonists include codeine sulfate, fentanyl citrate, hydrocodone bitartrate, loperamide HCl, morphine sulfate, noscapine, norcodeine, normorphine, thebaine. Opioid antagonists include nor-binaltorphimine HCl, buprenorphine, ß-chlornaltrexamine 2HCl, ß-funaltrexamione HCl, nalbuphine HCl, nalorphine HCl, naloxone HCl, naloxonazine, naltrexone HCl, and naltrindole HCl(NTI).

Hypnotics are substances which produce a hypnotic effect. Hypnotics include pentobarbital sodium, phenobarbital, secobarbital, thiopental and mixtures, thereof, heterocyclic hypnotics, dioxopiperidines, glutarimides, diethyl isovaleramide, α-bromoisovaleryl urea, urethanes and disulfanes.

Tranquilizers are substances which provide a tranquilizing effect. Examples of tranquilizers include chloropromazine, promazine, fluphenzaine,

reserpine, deserpidine, and meprobamate.

Anti-convulsants are substances which have an effect of preventing, reducing, or eliminating convulsions. Examples of such agents include primidone, phenytoin, valproate, Chk and ethosuximide.

Muscle relaxants and anti-Parkinson agents are agents which relax muscles or reduce or eliminate symptoms associated with Parkinson's disease. Examples of such agents include mephenesin, methocarbomal, cyclobenzaprine hydrochloride, trihexylphenidyl hydrochloride, levodopa/carbidopa, and biperiden.

Anti-spasmodics and muscle contractants are substances capable of preventing or relieving muscle spasms or contractions. Examples of such agents include atropine, scopolamine, oxyphenonium, and papaverine.

Anti-hypertensives are substances capable of counteracting high blood pressure. Examples of such substances include α-methyldapa and the pivaloyloxyethyl ester of α-methyldapa.

Analgesics are substances capable of preventing, reducing, or relieving pain. Examples of analgesics include morphine sulfate, codeine sulfate, meperidine, and nalorphine.

Anti-pyretics are substances capable of relieving or reducing fever and anti-inflammatory agents are substances capable of counteracting or suppressing inflammation. Examples of such agents include aspirin (salicylic acid), indomethacin, sodium indomethacin

trihydrate, salicylamide, naproxen, colchicine, fenoprofen, sulindac, diflunisal, diclofenac, indoprofen and sodium salicylamide.

Local anesthetics are substances which have an anesthetic effect in a localized region. Examples of such anesthetics include procaine, lidocain, tetracaine and dibucaine.

Prostaglandins are art recognized and are a class of naturally occurring chemically related, long-chain hydroxy fatty acids that have a variety of biological effects. Examples of such agents include E2 and E1.

Anti-depressants are substances capable of preventing or relieving depression. Examples of anti-depressants include imipramine, amitriptyline, nortriptyline, protriptyline, desipramine, amoxapine, doxepin, maprotiline, tranylcypromine, phenelzine, and isocarboxazide.

Anti-psychotic substances are substances which modify psychotic behavior. Examples of such agents include phenothiazines, butyrophenones and thioxanthenes.

Anti-emetics are substances which prevent or alleviate nausea or vomiting. An example of such a substance includes dramamine.

Imaging agents are agents capable of imaging a desired site, e.g. tumor, in vivo. Examples of imaging agents include substances having a label which is detectable in vivo, e.g. antibodies attached to fluorescent labels. The term antibody includes whole antibodies or fragments thereof.

Specific targeting agents include agents capable of delivering a therapeutic agent to a desired site, e.g. tumor, and providing a therapeutic effect. Examples of targeting agents include agents which can carry toxins or other agents which provide beneficial effects. The targeting agent can be an antibody linked to a toxin, e.g. ricin A or an antibody linked to a drug.

Neurotransmitters are substances which are released from a neuron on excitation and travel to either inhibit or excite a target cell. Examples of neurotransmitters include dopamine, serotonin, γ-aminobutyric acid, norepinephrine, histamine, acetylcholine, and epinephrine.

The term protein is art-recognized and for purposes of this invention also encompasses peptides. The proteins or peptides may be any bioactive protein or peptide, naturally occurring or synthetic. Examples of proteins include antibodies, enzymes, steroids, growth hormone and growth hormone-releasing hormone, gonadotropin-releasing hormone, and its agonist and antagonist analogues, somatostatin and its analogues, gonadotropins such as luteinizing hormone and follicle-stimulating hormone, peptide-T, thyrocalcitonin, parathyroid hormone, glucagon, vasopressin, oxytocin, angiotensin I and II, bradykinin, kallidin, adrenocorticotropic hormone, thyroid stimulating hormone, insulin, glucagon and the numerous analogues and congeners of the foregoing molecules.

The examples discussed above may be listed in the salt or non-salt form but for purposes of this invention both forms are intended to be encompassed. Further, if a particular salt-form of a drug is listed, other art recognized biologically accepted salts can be used in place of the listed salt-form. Examples of acceptable salts include hydrochloride, hydrobromide, sulfate, laurelate, palmatate, phosphate, nitrate, borate, acetate, maleate, tartrate, oleate, salisilate, salts of metals, means or organic cations, e.g. quarternary ammonium.

This invention is also intended to encompass derivatives or equivalents of the above discussed drugs. A derivative is a drug which is structurally similar to the foregoing list of drugs and is capable of achieving the same or substantially the same function or activity. An equivalent is an agent capable of achieving the same or substantially the same intended function or activity.

The biologically active substance is present in sufficient amount to achieve a therapeutic effect for at least three months of delivery. In certain preferred embodiments the biologically active substance is present in sufficient amount to achieve a therapeutic effect for at least three months of systemic delivery. A therapeutically effective dose is that amount necessary to prevent, treat, or reduce the symptoms associated with the particular condition or disease being treated.

B. The Cellular Regulator

The cellular regulator is any substance that affects the local tissue response, including but not limited to a cytokine or an oncogene product homologous to a cytokine, or any compound which may inhibit or otherwise affect such regulators, such as a steroidal or non-steroidal anti-inflammatory. See, e.g., K. Arai et al., "Cytokines: Coordinators of Immune and Inflammatory Responses," Annu. Rev. Biochem., 59:783-836 (1990); F.R. Balkwill and F. Burke, "The cytokine network," Immunology Today, 16:299-304, (September, 1989). Cytokines are involved in controlling the proliferation and differentation of mammalian cells and cellular interactions in the immune and inflammatory responses.

By affecting the local tissue response, it is meant that the cellular regulator is present in sufficient amount to accelerate, decelerate, increase, or decrease the local inflammatory response to the implant. Functionally, the cellular regulator is present in sufficient amount to increase or decrease the release of biologically active substance relative to an implant with no cellular regulator. This effect may result for example from the cellular regulator stimulating or retarding macrophage production, proliferation of fibrous tissue encapsulating the implant, infiltration of the fibrous capsule by new blood vessels and lymphatics, transport of lipophilic molecules across the encapsulated implant, transport of hydrophilic molecules across the encapsulated

implant, or preventing or suppressing chemotaxis.

The cellular regulator is in addition to the biologically active substance. The cellular regulator is present in sufficient concentration to affect the local tissue response, but is not present in sufficient concentration to achieve a therapeutic effect for the particular condition or disease being treated.

The cellular regulator preferably is an interleukin, interleukin inhibitor or interleukin receptor, including interleukin 1 through interleukin 10; an interferon, including alpha, beta and gamma; a hematopoietic factor, including erythropoietin, granulocyte colony stimulating factor, macrophage colony stimulating factor and granulocyte-macrophage colony stimulating factor; a tumor necrosis factor, including alpha and beta; a transforming growth factor (beta), including beta-1, beta-2, beta-3, inhibin, and activin; a chemotactic factor, including neutrophil-activating protein, monocyte chemoattractant protein, macrophage-inflammatory protein, SIS (small inducible secreted), platelet factor, platelet basic protein, and melanoma growth stimulating activity; a growth factor, including epidermal growth factor, transforming growth factor (alpha), fibroblast growth factor, platelet-derived growth factor, platelet-derived endothelial cell growth factor, insulin-like growth factor, nerve growth factor and bone growth/cartilage-inducing factor (alpha and beta); a steroidal or non-steroidal

anti-inflammatory.

The invention embodies a system in which one or more than one cellular regulators are introduced. Various combinations of cellular regulators, as well as various times of introduction of different cellular regulators, can be devised to either stimulate or inhibit various aspects of the local inflammatory response, so as to finely tune the rate of release of the biologically active substance and maximize the therapeutic efficacy of the particular biologically active substance involved in the patient's treatment.

C. Delivery Method

The invention embodies delivering the biologically active substance and the cellular regulator into the subject by injection or implantation. If the injection method is to be used, a phased effect on the local inflammatory response can be achieved by varying the timing and order of the injections. If the implantation method is to be used, the biologically active substance and the cellular regulator may be present in the same physical unit or in separate physical units. More than one cellular regulator may also be used, either in the same physical unit or in separate physical units. Varying the timing and order of implantations of the physical units can be used to achieve a phased effect on the local inflammatory response. In addition, the biologically active substance and cellular regulator may be introduced alone or in combination with an excipient.

The excipient may be part of a polymeric system consisting of matrices of biodegradable or non-biodegradable polymers; a nonpolymeric system consisting of compressed mixtures, totally fused pellets or partially fused pellets; microspheres; hydrogels; polymeric reservoirs; or cholesterol matrices.

A polymeric system consists of matrices of polymers combined with a biologically active substance. Such systems include (i) matrix erosion systems, in which the biologically active substance is evenly distributed in a polymer matrix and is released as the polymer breaks down in biological fluid after introduction into the subject, (ii) matrix diffusion systems in which the biologically active substance is released by diffusion through the polymer matrix, and (iii) matrix diffusion/erosion systems in which the biologically active substance is released by diffusion through the polymer matrix, as well as by erosion as the surface of the polymer breaks down. Biodegradable polymers that have been used in such systems include hydroxycarboxylic acids, especially lactic acid and glycolic acid. Cholesterol and ethylene vinyl acetate copolymers have also been used. See, for example, U.S. Patent No. 4,591,496 issued to Cohen et al., which describes a polymeric system consisting of mixing a drug and a polymer, e.g. ethylene-vinyl acetate copolymer powders, below the glass transition temperature of the polymer, and compressing the mixture at a temperature above the transition point.

Nonpolymeric systems can be fabricated by compressing mixtures of the biologically active substance and a nonactive biocompatible binder into a pellet. The rate of release and the uniformity of release depend both on the relative amounts of the drug and binder and on the homogeneity of the mixture prior to compression. Nonpolymeric systems also include totally fused pellets in which the biologically active substance is melted together with a nonpolymeric carrier and then recrystallized by cooling, to form the fused pellet. See, for example, U.S. Patent Nos. 4,748,024 and 4,892,734, issued to Leonard. In addition, a partially fused pellet can be fabricated by mixing a biologically active substance and a nonpolymeric carrier having a lower melting temperature than the biologically active substance, and heating and then cooling the mixture, such that only the carrier melts and recrystallizes, capturing the unmelted drug. Such totally and partially fused pellets are characterized by nondiffusional, erosion-based, drug release. See, for example, co-pending applications Serial No. 07/565,273, "Multiple Drug Delivery System," filed August 9, 1990 and Serial No. 07/163,328, "Partially Fused Peptide Pellet," filed March 2, 1988.

Microspheres are systems in aqueous or oil suspensions, in which the drug is coated with a bioerodible polymer and injected subcutaneously. The microspheres erode and release the drug according to the size and number of microspheres.

A hydrogel is designed to release a biologically active substance contained therein, when the composition is placed in an aqueous environment. See, for example, U.S. Patent No. 4,526,938 issued to Churchill et al., which describes a composition comprising a polypeptide and a copolymer, in which the hydrophobic component is biodegradable and the hydrophilic component may or may not be biodegradable, and in which the composition is capable of absorbing water to form a hydrogel when placed in water or an aqueous physiological type environment, from which the polypeptide is then released over an extended period of time.

A polymeric reservoir is a tubular device possessing interconnected porous walls. The interconnected porous reservoir walls provide a continuous path for the migrating biologically active substance which then diffuses from the reservoir at a rate governed by the tortuosity of the diffusion path. See, for example, U.S. Patent No. 4,702,917 issued to Schindler, in which the polymeric reservoir is fabricated from a polylactone, including polycaprolactone or its copolymers, or polyvalerolactone and its copolymers, containing an additive, such as a polyether which is selectively removed by treatment with an appropriate solvent to form the interconnected pores therein.

A cholesterol matrix delivery system comprises a cholesterol matrix permeable to passage by diffusion of the biologically active agent contained therein.

See, for example, U.S. Patent No. 4,452,775 issued to Kent, in which the matrix consists of cholesterol powder and cholesterol prills optionally in combination with a binding agent and a lubricating agent, and in which the biologically active substance is dispersed throughout the matrix.

The entire disclosures of U.S. Patent Nos. 4,452,775, 4,702,917, 4,526,938, 4,591,496 are expressly incorporated herein by reference.

The presence of a cellular regulator, as described in this invention, is designed to overcome the various drawbacks that each of these previously described systems exhibit on their own. The stimulation or inhibition of the local inflammatory response upon introduction of a cellular regulator into these systems will result in an enhanced "paraglandular" compartment and a means for controlled release of the drug.

The cellular regulator may be present as a core within a pellet, or the cellular regulator may be coated onto the surface of a pellet so as to have an initial stimulatory effect on the local inflammatory response, or the cellular regulator may be impregnated throughout a pellet to provide a continuous effect on the local inflammatory response, or the cellular regulator may be layered in a pellet to provide a time-variant effect on the local inflammatory response.

While the inventor does not wish to be bound by any theory of the invention, it is believed that

chemotaxis of foam cells (engorged lipophages) from the foreign implant, through the fibrous tissue, and toward the vasculature and lymphatics, occurs. Since the implant appears to remain coated with macrophages throughout, it is further hypothesized that a dynamic equilibrium or flux rate of new macrophages and migrating lipophages exists and is regulated.

Analysis of the local tissue response to a bioerodible subcutaneous drug implant has been carried out using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). These studies support the conclusion that an integral microanatomy devoted to the active processing of a drug implant exists. In these studies, norethindrone (NET) pellets, ANNUELLE™ (Endocon, Inc. Walpole, MA), composed of NET and cholesterol (85:15% respectively), were implanted into human volunteers and subsequently removed at various time points between 3 and 10.5 months post-implantation.

Figure 1 is an SEM of a cross-sectional view of a "paraglandular" compartment 10, excised after 7 months of implantation of a drug pellet in a subject, with the implanted drug pellet removed. The wall 12, lumen 14, and bed 16 of the compartment 10 are apparent. Capillary openings 18 are present at the bed 16. Connective tissue 20 is loose at the periphery of the compartment 10, and much denser towards the bed 16. Figure 2 is an SEM of a cross-sectional view of the compartment lumen 14, excised after 7 months, surrounding an implanted drug pellet 22. Figure 3 is

an SEM of the compartment wall 12, excised after 13.5 months, showing blood vessels 24 and relative connective tissue densities. Looser connective tissue 20 is present at the outer compartment wall 12. Figure 4 is an SEM of two blood vessels 24 of capillary size, excised after 13 months, in the middle region of the capsule wall 12. Connective tissue 20 surrounds the blood vessels 24. Figure 5 is an SEM of another view of the sample depicted in Fig. 4, showing intact erythrocytes 26 inside blood vessels 24. Connective tissue 20 surrounds the blood vessels 24. Figure 6 is a TEM of a thin section of a fibroblast 28, excised after 3 months, with collagen fibrils 30 of normal periodicity in the fibrous portion of the compartment. Figure 7 is a TEM of a thin section of fibrous wall 32, excised after 6 months, showing a few lipid engorged foam cells 34, i.e., lipid-laden macrophages, among loose collagen fibrils 30. Figure 8 is a TEM of a thin section of fibrous wall 32, excised after 13 months, showing significantly denser populations of lipid engorged foam cells 34 and denser collagen fibrils 30 than are present in Fig. 7 after 6 months of drug pellet implantation. A comparison of Figures 7 and 8 supports the theory that the concentration of foam cells increases with time post-implantation of the drug pellet. The above pictures are consistent with the foam cells either entering the blood and lymphatic vessels, or extruding the lipid at the vessel surface.

Mass spectrometric data supports the theory that the foam cells absorb the NET as it is released from the pellet surface. In the same studies in which the micrographs were taken, NET levels in the capsule tissue at 3 to 10.5 months post-implantation, were, respectively, 0.05% and 8.4%. Such an increase correlates qualitatively with the increase in foam cell population in the capsule wall for the same period.

The above data suggests that the local inflammatory response plays an essential role in the active processing of drug delivery systems. This invention utilizes the theory that the implant, in inducing a local inflammatory response, results in the recruitment of macrophages and the concomitant release of various cytokines. The implant becomes coated with macrophages, and absorption of the drug from the implant becomes, inter alia, a function of foam cell transport to the local vasculature and lymphatics. Some of the cytokines stimulate fibroblast production and the establishment of angiogenesis for the capsule. The resultant serum concentrations of released drug are a function of this active processing of the drug implant. Thus, by regulating the rate and extent of this active processing, by manipulating the various elements that contribute to this processing, one can regulate the rate and extent of drug release from the implant.

One preferred embodiment would be the use of a jacketed implant which is the subject of the

co-pending application Serial No. 07/565,273, "Multiple Drug Delivery System" (the disclosure of which is incorporated herein by reference). In this embodiment, an angiogenesis factor would be combined in matrix with a bio-erodible polymer, such as poly-dl lactide or glycolide, as a sheath around a core drug implant. The sheath would be formed so as to gradually erode, releasing the angiogenesis factor, and thus preparing the tissue environment for delivery of the core drug.

Example 1

One important compound of recombinant origin is growth hormone of either human or bovine species (hGH:bGH).

It is desirable to provide sustained release of a few hundred micrograms of GH per day in vivo. Such sustained release has not been achieved, because the protein is not well absorbed and is rapidly degraded in the subcutaneous environment by proteases or tumor necrosis factor (TNF), both directly or indirectly, as a result of macrophage stimulation in situ. Due to this effect, much more compound than is clinically required (one or more milligrams/day) is currently attempted for release from compressed implants for less than 30 days.

While the macrophage is directly or indirectly the source of the hostile factors mentioned above, it is also directly or indirectly the source of the growth factors which are associated with sufficient

neovascular development (angiogenesis) necessary to optimize the absorption of the active protein.

It would, therefore, be desirable to suppress the macrophage function while supplying sufficient growth factor necessary for angiogenesis by releasing GH into the subcutaneous environment. Because GH is nonlipophilic, it suffers additional difficulties relative to absorption in the lipid environment.

Utilizing the methods of partial melting elaborated in a co-pending application, matrices of protein and lipophilic compounds such as steroids can be acheived by selecting combinations of excipients and active ingredients that allow the melting and recrystallization of the excipient only. This does not preclude the material which undergoes a phase change from also being an active ingredient.

A method of obtaining the desired results described above is as follows:

1. 30 milligrams of bGH is intimately mixed together with 0.3 milligrams of corticosterone or similar crystalline, steroidal anti-inflammatory compound. This mixture is then packed into an enclosed form and heated to the melting point of the corticoid (145 degrees C.) and allowed to cool. The resulting aggregate is then ground into fine grains of approximately 100 microns @.

2. The granular powder of number one is introduced into an intimate mixture of 5

milligrams of cholesterol palmitate and 0.7 milligrams of epidermal growth factor, transforming growth factor beta or more specific angiogenic cytokine.

3. The resulting mixture is packed into a Teflon tube and partially fused into a rod-shaped implant according to the methods described in U.S. Patent Application Ser. No. 07/163,328 (filed March 2, 1988 and entitled "Partially Fused Peptide Pellet," the disclosure of which is incorporated herein by reference), by heating to the melting point of the cholesterol palmitate (79 degrees C.), which is below the melting points of all other components of the mixture.

4. The rod is allowed to cool and recrystallize at room temperature.

5. The resulting pellet is implanted into the subcutaneous tissue of a cow by means of a Harman Injector; U.S. Patent No. 4,820,267.

The corticoid is soluble within the cholesterol palmitate matrix and thus diffuses from the rod carrying the GH with the result of both suppressing the local inflammatory response (thus macrophage) and providing a lipid buffer for the protein. As the corticoid leaches from the rod, surface erosion of the cholesterol palmitate will release angiogenic

factor -- representing about 8% of the non-GH/corticoid component of the matrix, thus stimulating neovascularization at the local implant site.

Those skilled in the art will recognize or will be able to ascertain with no more than routine experimentation numerous equivalents to the specific products and processes described herein. Such equivalents are considered to be within the scope of the invention and are intended to be covered by the following claims in which I claim:

CLAIMS

1. A product adapted for implantation and suitable for controlled release of a biologically active substance, comprising: the biologically active substance and at least one cellular regulator, wherein the introduction of said product subcutaneously into a subject, induces or inhibits a local tissue response, and wherein said cellular regulator is present in sufficient amount to affect the local tissue response, such as to affect the kinetics of release of the biologically active substance from the product.

2. The product of claim 1 wherein said cellular regulator is selected from the group consisting of an interleukin, interferon, hematopoietic factor, tumor necrosis factor, transforming growth factor, chemotactic factor, growth factor and anti-inflammatory.

3. The product of claim 1 wherein said cellular regulator is an interleukin.

4. The product of claim 1 wherein said cellular regulator is an interferon.

5. The product of claim 1 wherein said cellular regulator is a hematopoietic factor.

6. The product of claim 1 wherein said cellular regulator is a tumor necrosis factor.

7. The product of claim 1 wherein said cellular regulator is a beta transforming growth factor.

8. The product of claim 1 wherein said cellular regulator is a chemotactic factor.

9. The product of claim 1 wherein said cellular regulator is a growth factor.

10. The product of claim 1 wherein said cellular regulator is an anti-inflammatory.

11. The product of claim 1 further comprising an excipient.

12. The product of claim 11 wherein said excipient is part of a polymeric system, comprising matrices of the excipient, non-biodegradable polymers, which are combined with the biologically active substance.

13. The product of claim 11 wherein said excipient is part of a polymeric system, comprising matrices of the excipient, biodegradable polymers, which are combined with the biologically active substance.

14. The product of claim 11 wherein said excipient is part of a nonpolymeric system comprising compressed mixtures of the biologically active

substance and the excipient, which is a nonactive biocompatible binder.

15. The product of claim 11 wherein said excipient is part of a nonpolymeric system comprising a totally fused pellet of the biologically active substance and the excipient, which is a nonpolymeric carrier.

16. The product of claim 11 wherein said excipient is part of a nonpolymeric system comprising a partially fused pellet of the biologically active substance and the excipient, which is a nonpolymeric carrier.

17. The product of claim 11 wherein said excipient is part of a microsphere system in which the biologically active substance is coated with the excipient, which is a bioerodible polymer.

18. The product of claim 11 wherein said excipient is part of a hydrogel system comprising the biologically active substance and the excipient, a copolymer, which system is capable of absorbing water.

19. The product of claim 11 wherein said excipient is part of a polymeric reservoir system in which the interconnected porous reservoir walls are fabricated from the excipient, which is a polylactone.

20. The product of claim 11 wherein said excipient is part of a cholesterol matrix system in which the excipient, a matrix containing cholesterol powder and cholesterol prills, is interspersed with the biologically active substance.

21. The product of claims 12-19 wherein the cellular regulator is selected from the group consisting of an interleukin, interferon, hematopoietic factor, tumor necrosis factor, transforming growth factor, chemotactic factor and growth factor.

22. In a method for controlled release of a biologically active substance, which includes subcutaneous introduction of the biologically active substance into a subject, the improvement comprising:

administering at least one cellular regulator present in sufficient amount to affect the local tissue response resulting from the introduction of the biologically active substance, and thereby to affect the release kinetics of the biologically active substance.

23. A method for controlled release of a biologically active substance, comprising:

introducing the biologically active substance subcutaneously into a subject such that a local tissue response is induced, and

introducing at least one cellular regulator

in sufficient amount to affect the local tissue response and thereby to affect the release kinetics of the biologically active substance.

24. The method of claims 22 or 23 wherein the cellular regulator is introduced into the subject by implantation.

25. The method of claims 22 or 23 wherein the cellular regulator is introduced into the subject by injection.

26. The method of claims 22 or 23 wherein the biologically active substance and the cellular regulator are present in a single physical unit.

27. The method of claims 22 or 23 wherein the biologically active substance and the cellular regulator are contained in different physical units.

28. The method of claims 22 or 23 wherein more than one cellular regulator is introduced and the different cellular regulators are introduced at different times so as to either stimulate or inhibit various aspects of the tissue response.

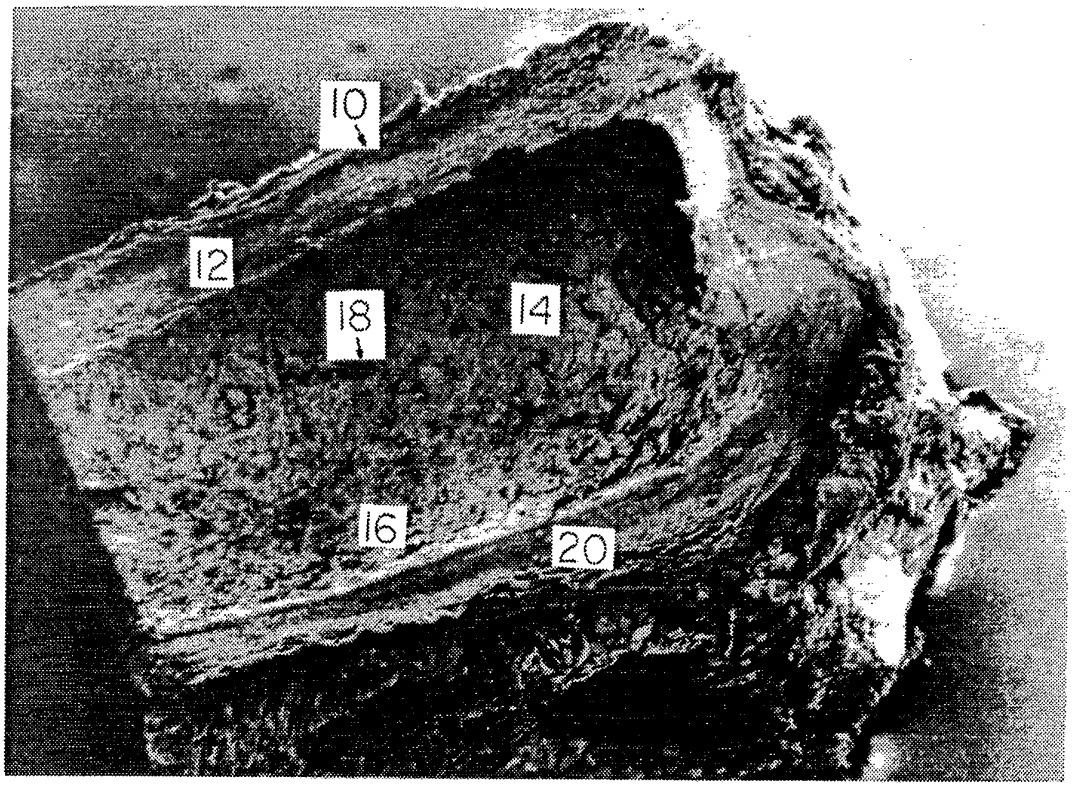

FIG. 1

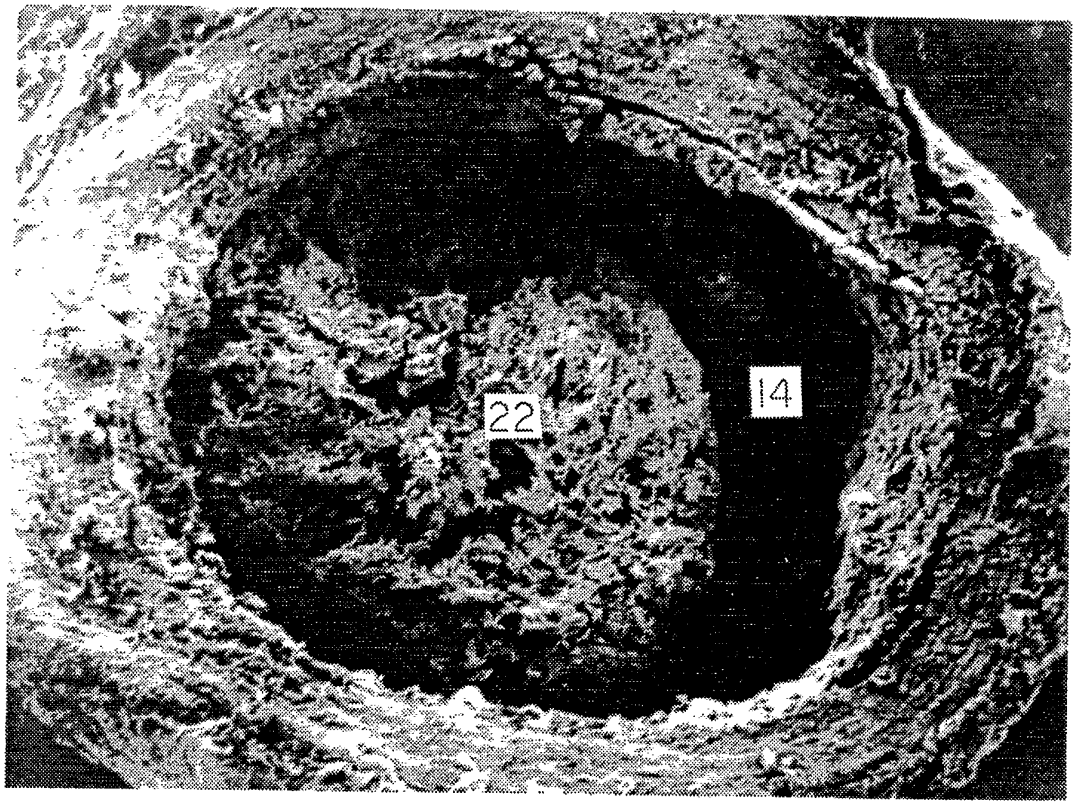

FIG. 2

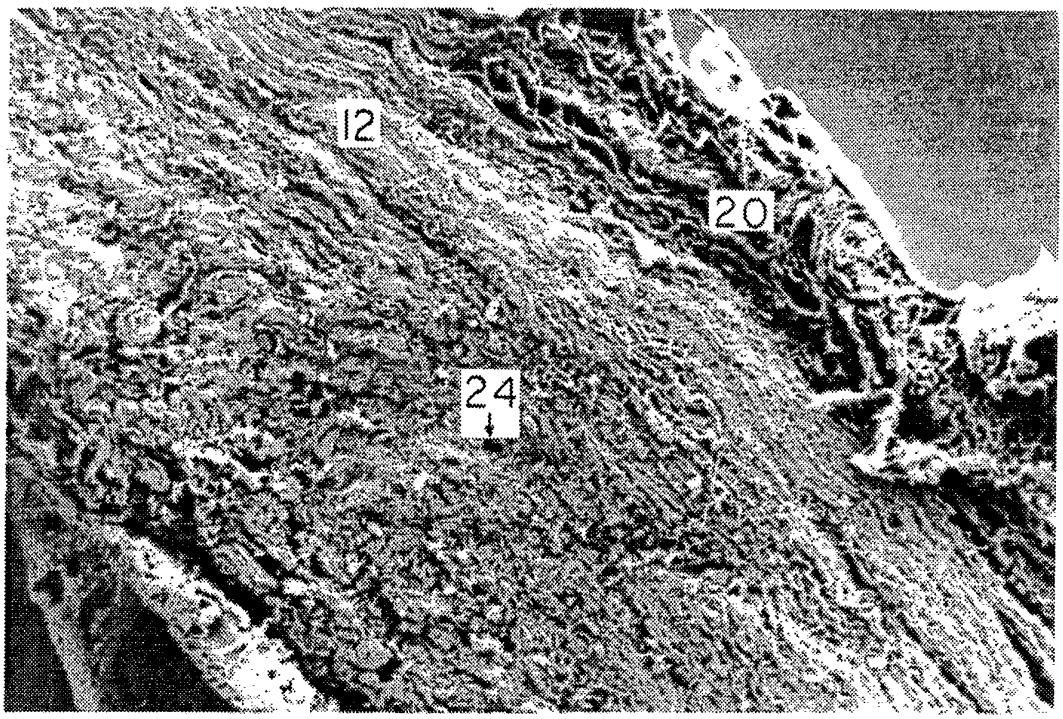

FIG. 3

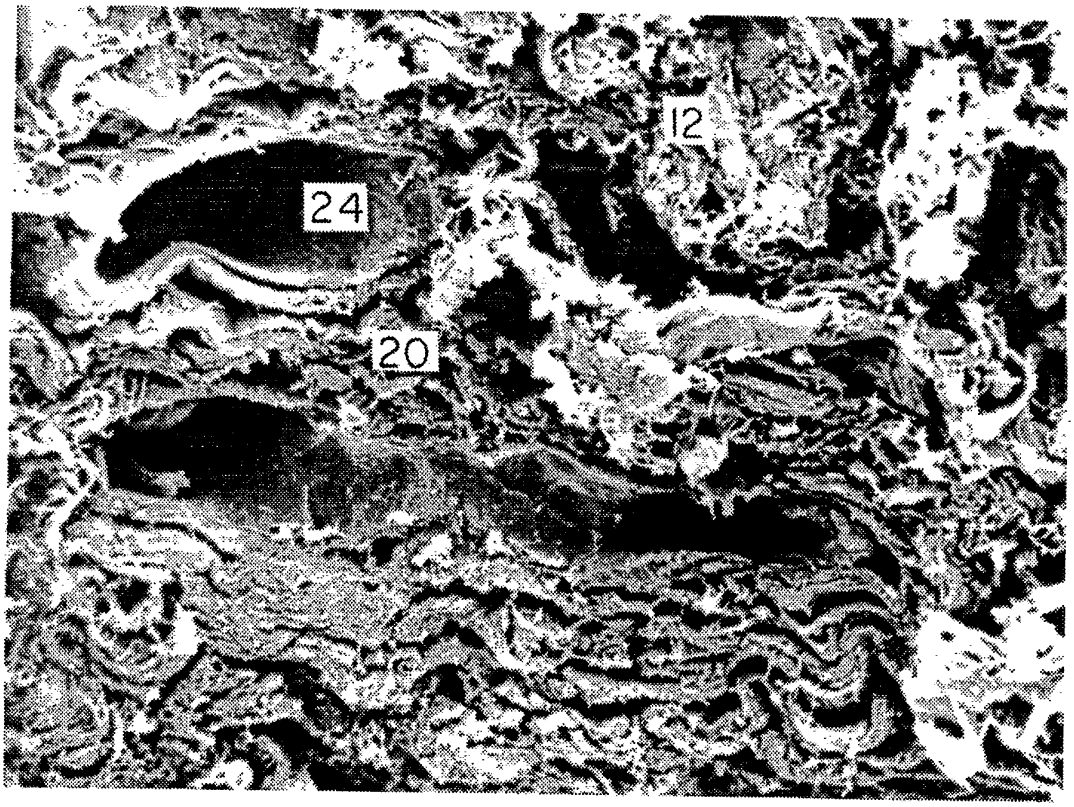

FIG. 4

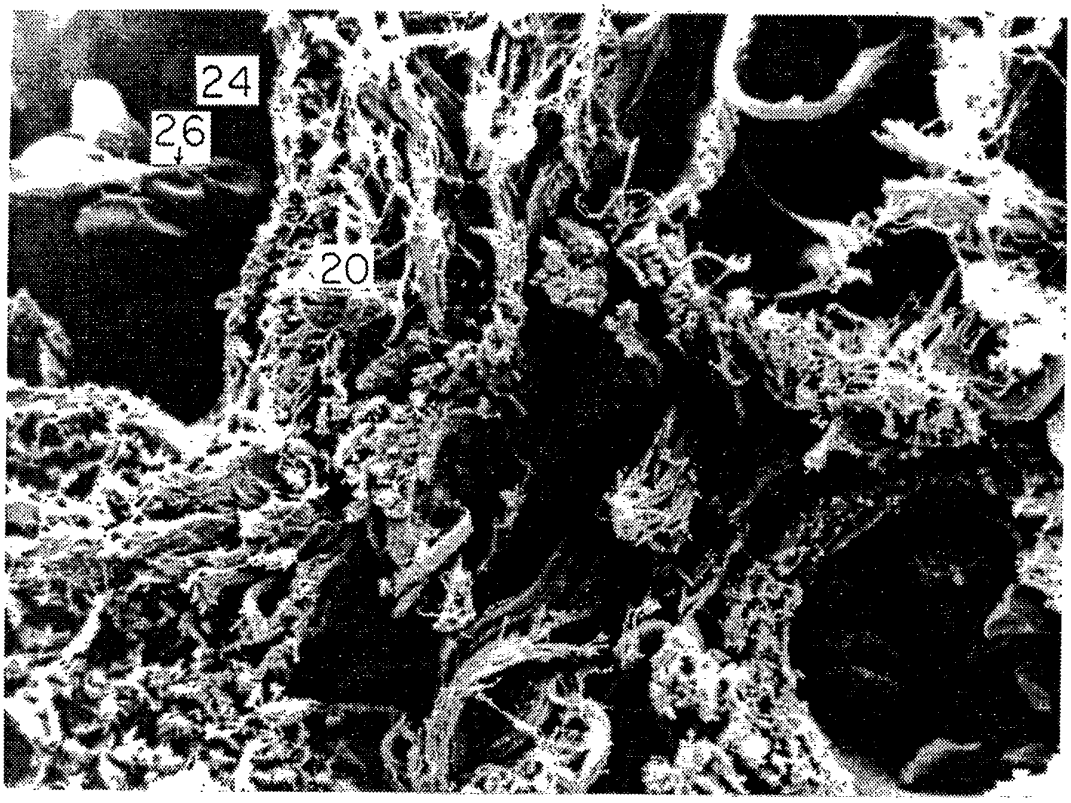

FIG. 5

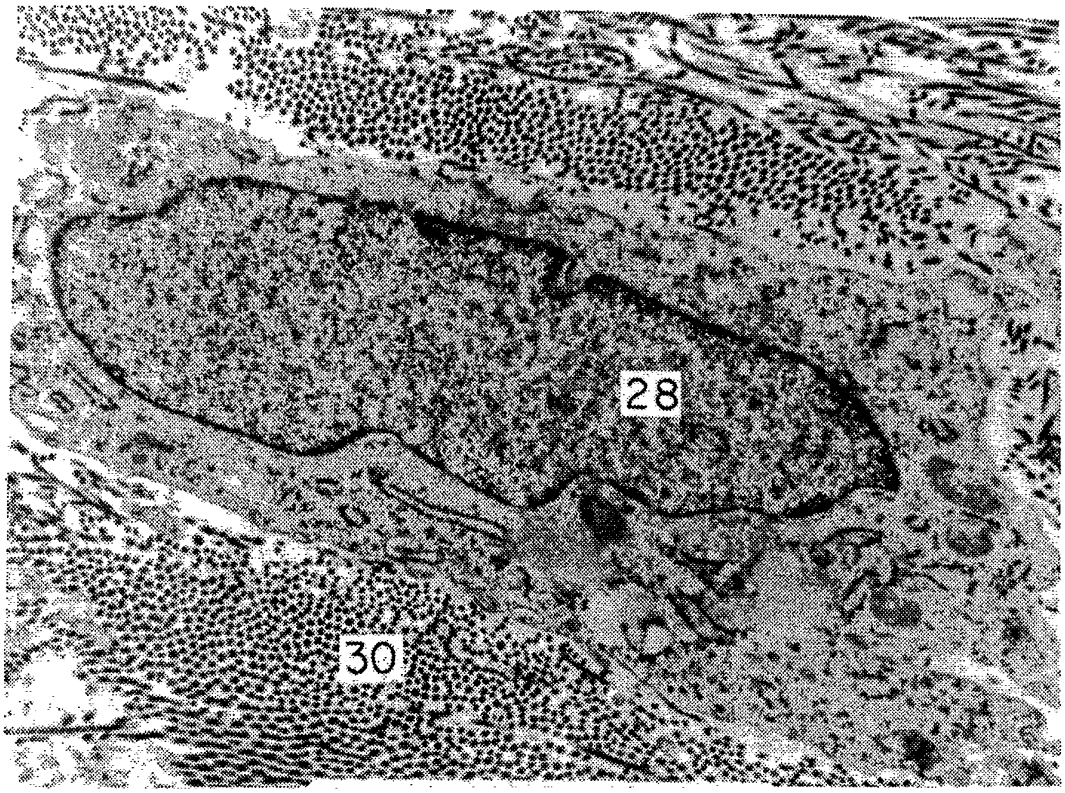

FIG. 6

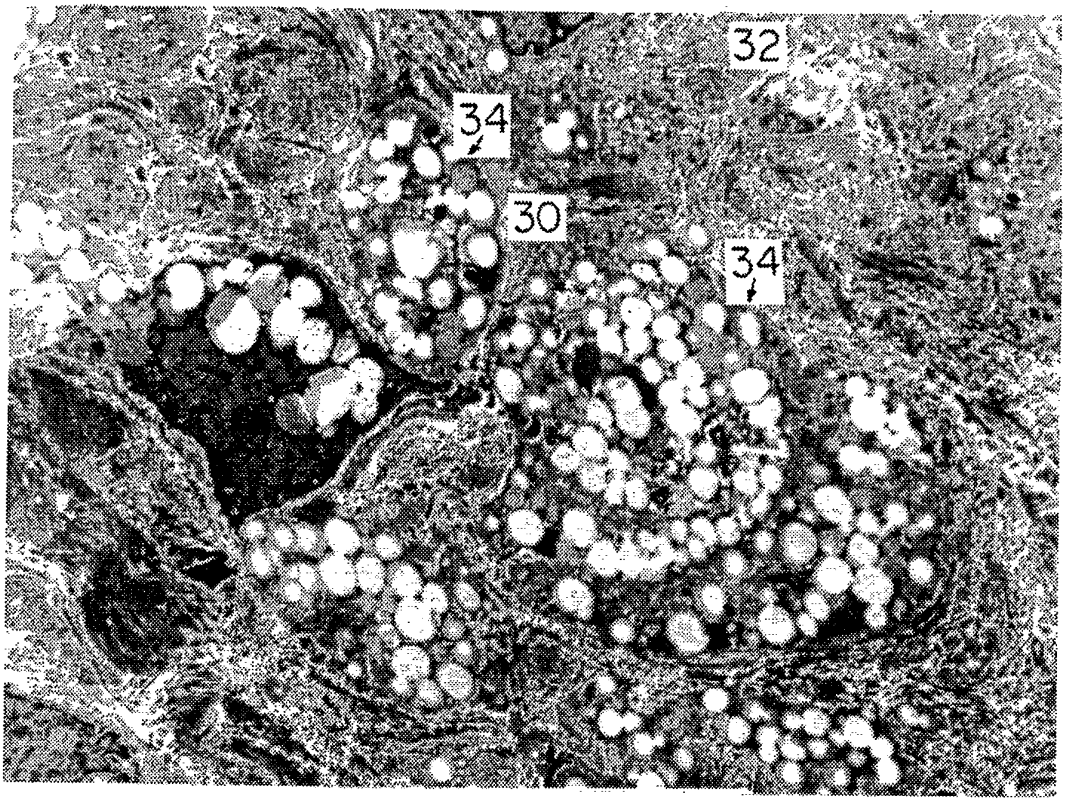

FIG. 7

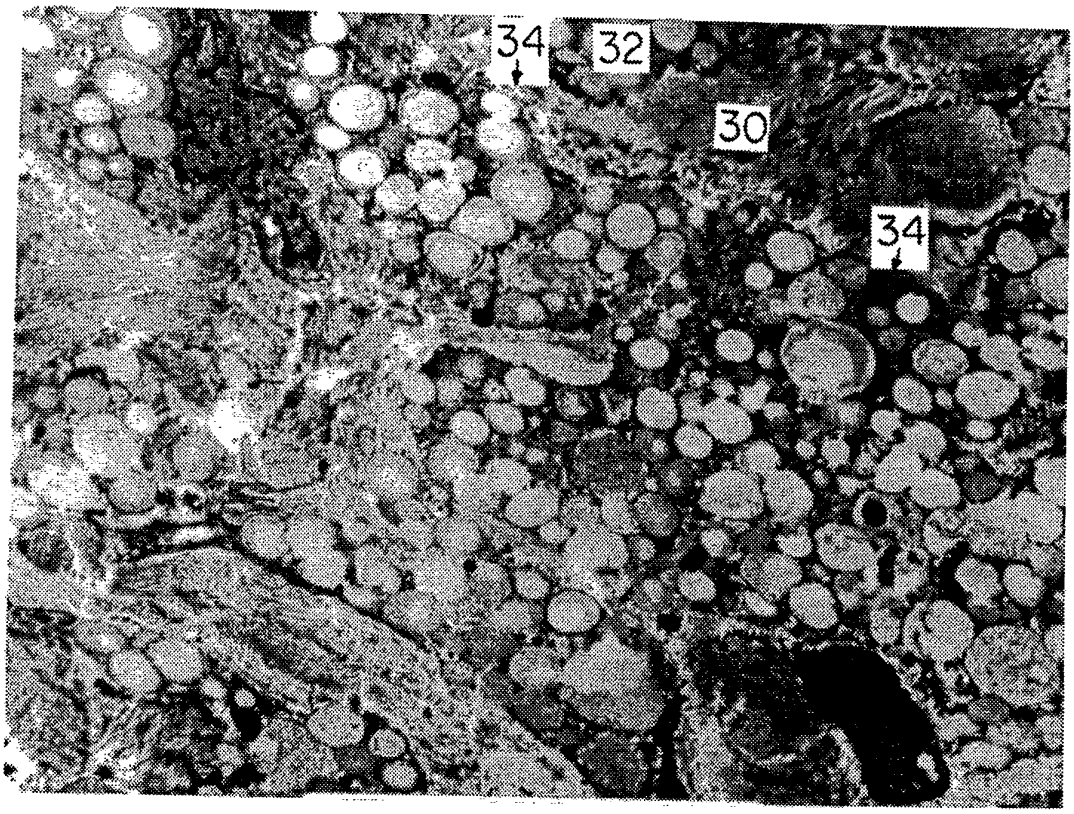

FIG. 8

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/04059

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 9/22 A 61 K 45/06

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	WO,A,9202211 (ENDOCON, INC.) 20 February 1992, see page 5, paragraph 2; page 19, paragraph 1 - page 20, paragraph 1; claims 1-7,21-23 (cited in the application)	1-5,11, 15,22-24,26, 27
X	WO,A,9010437 (ENDOCON, INC.) 20 September 1990, see page 1, paragraph 1; pages 25-27, example 1; page 27, last paragraph - page 28; claims 1-6,21 (cited in the application)	1,2,10, 11,16, 20,21, 22-24, 26
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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
10-08-1992	04. 09. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Dagmar Franz

Form PCT/ISA/210 (second sheet) (January 1985)

Mme Dagmar FRANZ

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

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Y	US,A,4748024 (R.J. LEONARD) 31 May 1988, see column 9, example 2	14,15
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9204059 SA 60583

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 27/08/92
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9204059 SA 60583

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The members are as contained in the European Patent Office EDP file on 27/08/92
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