



Office de la Propriété
Intellectuelle
du Canada

Un organisme
d'Industrie Canada

Canadian
Intellectual Property
Office

An agency of
Industry Canada

CA 2249448 C 2003/10/28

(11)(21) **2 249 448**

(12) **BREVET CANADIEN
CANADIAN PATENT**

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 1997/03/19
(87) Date publication PCT/PCT Publication Date: 1997/10/02
(45) Date de délivrance/Issue Date: 2003/10/28
(85) Entrée phase nationale/National Entry: 1998/09/21
(86) N° demande PCT/PCT Application No.: US 1997/004328
(87) N° publication PCT/PCT Publication No.: 1997/035595
(30) Priorités/Priorities: 1996/03/26 (60/014,097) US;
1997/03/10 (08/814,187) US

(51) Cl.Int.⁶/Int.Cl.⁶ A61K 35/54
(72) Inventeurs/Inventors:
FITZPATRICK-MCELLIGOTT, SANDRA G., US;
HUNCHAR, JEFFREY G., US;
LEE, YOUNG-ZOON, US;
BECK, LEE R., US
(73) Propriétaire/Owner:
ARKION LIFE SCIENCES, US
(74) Agent: DIMOCK STRATTON CLARIZIO LLP

(54) Titre : COMPOSITION ANTI-INFLAMMATOIRE A BASE D'OEUFS, TECHNIQUE D'ISOLATION ET USAGE
(54) Title: EGG ANTI-INFLAMMATORY COMPOSITION, METHOD OF ISOLATION AND USE

(57) **Abrégé/Abstract:**

The invention is directed to an anti-inflammatory composition obtained from the eggs of an avian that has been hyperimmunized with one or more antigens, and, in particular, bacterial antigens. The invention is further directed to an egg or egg product comprising effective amounts of the anti-inflammatory composition. The invention is also directed to a method for isolating the anti-inflammatory composition. The invention is finally directed to methods of treating inflammation using the anti-inflammatory composition and/or egg or egg products comprising effective amounts of the anti-inflammatory composition.



PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 35/54	A1	(11) International Publication Number: WO 97/35595 (43) International Publication Date: 2 October 1997 (02.10.97)
(21) International Application Number: PCT/US97/04328 (22) International Filing Date: 19 March 1997 (19.03.97) (30) Priority Data: 60/014,097 26 March 1996 (26.03.96) US 08/814,187 10 March 1997 (10.03.97) US (71) Applicant: DCV BIOLOGICS L.P. [US/US]; 3521 Silverside Road, Wilmington, DE 19810 (US). (72) Inventors: FITZPATRICK-McELLIGOTT, Sandra, G.; 27 Long Point Lane, Media, PA 19063 (US). HUNCHAR, Jeffrey, G.; 922 Kirby Drive, West Chester, PA 19380 (US). LEE, Young-Zoon; 9043 Winthrop Drive, Cincinnati, OH 45249 (US). BECK, Lee, R.; 1670 North Liberty-Keuter Road, Lebanon, OH 45036 (US). (74) Agents: KRIKELIS, Basil, S. et al.; E.I. du Pont de Nemours and Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: EGG ANTI-INFLAMMATORY COMPOSITION, METHOD OF ISOLATION AND USE (57) Abstract The invention is directed to an anti-inflammatory composition obtained from the eggs of an avian that has been hyperimmunized with one or more antigens, and, in particular, bacterial antigens. The invention is further directed to an egg or egg product comprising effective amounts of the anti-inflammatory composition. The invention is also directed to a method for isolating the anti-inflammatory composition. The invention is finally directed to methods of treating inflammation using the anti-inflammatory composition and/or egg or egg products comprising effective amounts of the anti-inflammatory composition.		

EGG ANTI-INFLAMMATORY COMPOSITION AND METHOD OF TREATING AND PREVENTING INFLAMMATION

FIELD OF THE INVENTION

The invention generally relates to a composition produced in a natural foodstuff and for its use in treating and preventing inflammation. The invention more specifically relates to an egg anti-inflammatory composition, methods for its production in partially purified forms, and methods for its use in the treatment and prevention of inflammation, inflammatory responses such as those found in arthritis and auto-immune diseases, and prevention thereof.

BACKGROUND OF THE INVENTION

Inflammation, as defined in Dorland's Medical Dictionary, is "a localized protective response, elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off both the injurious agent and the injured tissue." It is characterized by fenestration of the microvasculature, leakage of the elements of blood into the interstitial spaces, and migration of leukocytes into the inflamed tissue. On a macroscopic level, this is usually accompanied by the familiar clinical signs of erythema, edema, hyperalgesia (tenderness), and pain.

During this complex response, chemical mediators such as histamine, 5-hydroxytryptamine, various chemotactic compositions, bradykinin, leukotrienes, and prostaglandins are liberated locally. Phagocytic cells migrate into the area, and cellular lysosomal membranes may be ruptured, releasing lytic enzymes. All of these events can contribute to the inflammatory response.

Inflammation in patients with rheumatoid arthritis probably involves the combination of an antigen with an antibody complement causing the local release of chemotactic and chemoactivating compositions that attract leukocytes. The leukocytes phagocytose the complexes of antigen-antibody and complement, and also release the many enzymes contained in their lysosomes. These lysosomal enzymes then cause injury to cartilage and other tissues, and this furthers the degree of inflammation. Cell-mediated immune reactions may also be involved.

Prostaglandins, which are key intracellular regulators of cellular function, are also released during this process.

The Arthus reaction is an inflammatory response brought about by the formation of immune complexes at subcutaneous sites where an antigen complexes with antibody to that antigen. Neutrophils characteristically attach to the Fc portion of the immunoglobulin complex that forms at the subcutaneous injection site where they release digestive enzymes, causing visible acute inflammation. Thus the reaction is primarily neutrophil-mediated and agents that effect the development of the reaction do so via an effect on these cells.

There are several pathways whereby an agent might interfere with neutrophil migration from the blood vessels to an inflammatory site. One likely pathway is the inhibition of margination, the reversible adhesion or "sticking" of inflammatory cells to the endothelial cell lining of the blood vessel wall. This adhesion is precisely controlled by the quality and quantity of adhesion and integrin molecules that act as "Velcro®" for white blood cells and the lining of blood vessels. In the normal state about 50% of neutrophils are reversibly adhered, but during an acute inflammatory response, adhesion becomes much stronger and is a key step in the process of neutrophil migration. While prostaglandins are unlikely to be directly involved in the chemotactic response, another product of the metabolism of arachidonic acid, leukotriene, is a very potent chemotactic substance.

The inflammatory response is any response characterized by inflammation as defined above. It is well known, to those skilled in the medical arts, that the inflammatory response causes much of the physical discomfort (i.e., pain and loss of function) that has come to be associated with different diseases and injuries.

Arthritis

Arthritis manifests itself in a variety of forms. Some of the more common forms include rheumatoid arthritis, osteoarthritis and generalized rheumatism.

Rheumatoid arthritis is an autoimmune disease characterized by pain, swelling and stiffness in the joints. Rheumatoid arthritis is a disease which afflicts women in particular. Rheumatoid arthritis is an extremely disabling disease and usually strikes adults between the ages of 30 and 40 years, while the occurrence of clinical illness is greatest among those aged 40 - 60 years. Although drug therapy is somewhat effective, as many as 7% of rheumatoid arthritis sufferers are disabled to some extent as quickly as 5 years after disease onset, and within 10 years, as many as 50% are too disabled to work (*Medical Sciences Bulletin*, December 1994).

Osteoarthritis produces similar symptoms to rheumatoid arthritis. In particular, although osteoarthritis begins as a degeneration of articular cartilage whereas rheumatoid arthritis begins as inflammation in the synovium, each process approaches the other as the disease progresses. In osteoarthritis, as cartilage deteriorates and joint congruence is altered, a reactive synovitis often develops. Conversely, as rheumatoid arthritis erodes cartilage, secondary osteoarthritis changes in bone and cartilage develop. At the end stages of both osteoarthritis and rheumatoid arthritis, the involved joints appear the same.

Some other forms of arthritis include Ankylosing Seronegative Spondyloarthropathy (ankylosing spondylitis) and reactive arthritis. These conditions are often referred to as the “B-27 associated diseases,” and are difficult to differentiate from rheumatoid arthritis. In some cases ankylosing spondylitis, Reiters syndrome or psoriatic arthritis are present coincidingly with Rheumatoid Arthritis in the same patient. In many cases, these patients are treated with the same disease modifying drugs as those suffering from progressive rheumatoid arthritis.

Onset of arthritis generally occurs after the age of 30 in those who are susceptible to such disease. However, some forms of arthritis may be initiated by different causes, such as slow virus infections. Because there is great overlap, many physicians consider these forms as “generalized rheumatism” and approach management of the diseases in the same way. Some diseases which fall into this category include Chronic Fatigue Syndrome, fibromyalgia (fibrositis)

and gout. In fact, for some patients, evidence is accumulating for superimposition of rheumatoid arthritis and fibromyalgia. (Harris, Edward D. and W. B. Saunders, *Rheumatoid Arthritis*, 1997).

Autoimmune Diseases

As stated above, rheumatoid arthritis is an autoimmune disease, and as such, its etiology is much the same as the etiology of any other autoimmune disease. Generally, the body normally recognizes the difference between its own by-products and foreign invaders (i.e. bacteria, viruses, fungi and protozoans, to name a few). When an immune cell (T or B lymphocyte) reacts to a “self-protein” during its development, that cell is deemed defective and usually destroyed or inactivated. Sometimes, however, a “self-reactive” immune cell will escape destruction. At a certain later time, that cell can be activated and trigger an immune response. Activation is thought to occur after infection with a common bacteria or virus which contains a polypeptide having a stretch of amino acids which match a stretch on the defective self-protein. Several bacteria, such as *Streptococcus*, *Mycoplasma*, and *borrelia*, have been implicated in the initiation of the disease, as well as certain viruses, namely retroviruses. In addition to Rheumatoid Arthritis, autoimmunity often results in such diseases as juvenile diabetes, multiple sclerosis, Graves’ disease, Meneri’s disease, myasthenia gravis, lupus erythematosus and psoriasis. (*Medical Sciences Bulletin*, September, 1994).

Autoimmunity effects specific organs. For example, some autoimmune diseases of liver bile ducts, and kidneys are: primary biliary cirrhosis, necrotizing glomerulonephritis, “idiopathic” crescentic glomerulonephritis, virus-induced liver and kidney disease, chronic hepatitis, autoimmune and drug-induced hepatitis (Gershwin, Manns, and Mackay 1992). Immune destruction of the islets of Langerhans results in diabetes mellitus (Hagopian and Lernmark 1992) and insulin autoantibodies have been described (Palmer 1987).

There is a large category of systemic vasculitides diseases in which autoimmune mechanisms have been suggested as the cause of the pathogenesis. Some of the diseases are: leukocytoclastic angiitis, polyarteritis nodosa, Goodpasture’s syndrome, Kawasaki disease, Wegener’s granulomatosis, Churg-Struass syndrome, giant-cell arteritis, Takayasu arteritis, immune-

complex-mediated, lupus, rheumatoid, and cryoglobulinemic vasculitis, Henoch Schönlein purpura (Kallenberg, 1996; Jennette, Jones, Falk, 1992).

There is also a body of evidence that autoimmunity may play a role in many forms of heart disease including: postpericardiotomy and postmyocardial infarction syndromes, myocarditis, and idiopathic dilated cardiomyopathy. Autoimmunity may be responsible for the progression of acute disease of heart muscle to degenerative (Rose, Neumann, Burek, Herskowitz 1992).

Symptomatic involvement of skeletal muscle is common in many autoimmune diseases such as polymyositis or inflammatory myopathy (which may include rheumatoid arthritis, polymyalgia rheumatica, myasthenia gravis, myasthenic myopathy, neurogenic atrophy, motor neuron disease, fibromyalgia, fibrositis, muscular dystrophy, endocrine, metabolic, and carcinomatous myopathy). (Hollingsworth, Dawkins, Thomas 1992).

Other diseases with autoimmune origins may be uveitis, Vogt-Koyanagi-Harada syndrome, (Detrick and Hooks 1992), and Sjögren's syndrome, scleroderma, ankylosing spondylitis, dermatomyositis, psoriasis, psoriatic arthritis, Reiter's syndrome (NIH 1994).

Also evidence of autoantibodies has been found in Alzheimer's disease (Singh et al., 1992), dementia complex (Mastroianni et al., 1991) and autistic children (Singh et al., 1993).

Several neurologic diseases such as Sydenham's Chorea, chronic obsessive-compulsive disorders (OCD), attention deficit hyperactivity disorder (ADHD), Tourette's Syndrome (TS) and some cases of schizophrenia may have an auto-immune component and may be associated with anti-neuronal antibodies (Medical Sciences Bulletin, Sept. 1994).

This summary is not all inclusive and those in the art are familiar with other autoimmune diseases, such as, for example Guillain-Barré syndrome (idiopathic polyneuritis).

Treatment of Inflammatory Disorders

In order to treat inflammatory related disorders, it is a common medical practice to administer pharmacological agents that reduce the physical discomfort of the inflammatory response. Agents having these properties are classified as anti-inflammatory. Anti-inflammatory drugs are used for the treatment of a wide spectrum of disorders, and the same drugs are often used to treat different diseases. Treatment with anti-inflammatory drugs is not for the disease, but most often for the symptom (i.e., inflammation).

The anti-inflammatory, analgesic, and anti-pyretic drugs are a heterogeneous group of compounds, often chemically unrelated, which nevertheless share certain therapeutic actions and side-effects. Corticosteroids represent the most widely-used class of compounds for the treatment of inflammation. Proteolytic enzymes represent another class of compounds that are thought to have anti-inflammatory effects. Hormones that directly or indirectly cause the adrenal cortex to produce and secrete steroids represent another class of anti-inflammatory compounds.

Unfortunately, the natural and synthetic corticosteroid preparations cause a number of severe side effects, including elevation of blood pressure, salt and water retention, kidney damage and increased potassium and calcium excretion. Moreover, corticosteroids may mask the signs of infection and enhance dissemination of infectious microorganisms. These hormones are considered unsafe for use in pregnant women, and long-term corticosteroid treatment has been associated with gastric hyperactivity and/or peptic ulcers. Treatment with corticosteroids may also aggravate diabetes mellitus, requiring higher doses of insulin, and may produce psychotic disorders. Hormonal anti-inflammatory agents which indirectly increase the production of endogenous corticosteroids have the same potential for adverse side-effects.

Presently, another common treatment for inflammation, and in particular rheumatoid arthritis, other arthritis and other autoimmune diseases, is drug therapy. In general, patients are initially treated with “first-line” agents, usually non-steroidal anti-inflammatory drugs (NSAIDs) which primarily relieve the symptoms. The patients are later treated with “second-line” or disease-modifying agents (DMARDs) such as methotrexate, gold compounds, penicillamine,

sulfasalazine, and antimalarial drugs. However, all of the above drugs have serious side effects, especially when administered in elevated doses. For example, aspirin, an NSAID, may produce indigestion and stomach pain; phenylbutazone may produce stomach ulcers and phenacetin may lead to kidney disease. Methotrexate may cause oral ulceration and gastrointestinal (GI) side effects.

A number of non-steroidal, anti-inflammatory agents (NSAID's), have been described. Among these, the most widely used are the salicylates. Acetylsalicylic acid, or aspirin, is the most widely prescribed analgesic-antipyretic and anti-inflammatory agent. Examples of steroidal and non-steroidal anti-inflammatory agents are listed in the Physician's Desk Reference.

Accordingly, in spite of the large number of pharmaceutical drugs that are currently available for treatment of the various forms of arthritis and autoimmune disease, there is still a need for a simple, effective treatment that can alleviate as well as prevent the onset of such diseases, while reducing the complication of side effects.

If a natural food product could be obtained, it would provide an easily administratable, readily available, and safe therapeutic composition for the treatment and prevention of inflammatory diseases such as arthritis and autoimmune diseases.

Related Art:

Various genera of the class Aves, such as chickens (*Gallus domesticus*), turkeys, and ducks, produce antibodies in blood and in eggs against antigens and immunogens related to avian diseases, as well as against other antigens and immunogens. For example, LeBacq-Verheyden et al. (Immunology 27:683 (1974)) and Leslie, G.A.. et al. (J. Med. 130:1337 (1969)), have quantitatively analyzed immunoglobulins of the chicken. Polson, A., et al. (Immunological Communications 9:495-514 (1980)) immunized hens against several proteins and natural mixtures of proteins, and detected IgY antibodies in the yolks of the eggs. Fertel, R., et al. (Biochemical and Biophysical Research Communications 102:1028-1033 (1981)) immunized hens against prostaglandins and detected antibodies in the egg yolk. Jensenius et al. (Journal of

Immunological Methods 46:63-68 (1981)) provide a method of isolating egg yolk IgG for use in immunodiagnostics. Polson et al. (Immunological Communications 9:475-493 (1980)) describe antibodies isolated from the yolk of hens that were immunized with a variety of plant viruses.

U.S. Patent No. 4,357,272 discloses the isolation of antibodies from the yolks of eggs derived from hyperimmunized hens. The hyperimmunization was elicited by repetitive injections of antigens derived from plant viruses, human IgG, tetanus antitoxin, snake antivenins, and Serameba. U.S. Patent No. 4,550,019 discloses the isolation from egg yolks of antibodies raised in the hen by hyperimmunization with immunogens having a molecular or particle weight of at least 30,000. The immunogens used to hyperimmunize the chickens were selected from among plant viruses, human immunoglobulins, tetanus toxin, and snake venoms.

U.S. Patent No. 4,748,018 discloses a method of heterologous passive immunization of a mammal that comprises parenterally administering purified antibody obtained from the eggs of an avian that has been immunized against the corresponding antigen. It teaches that mammals with a history of consumption of the antibody containing egg material are tolerized to the antigen and then can be effectively immunized without causing serum or sickness or anaphylactic reactions.

U.S. Patent No. 5,772,999 assigned to DCV-Biologics, discloses a method of preventing, countering or reducing chronic gastrointestinal disorders or Non-Steroidal Anti-Inflammatory Drug-induced (NSAID-induced) gastrointestinal damage in a subject by administering hyperimmunized egg and/or milk or fractions thereof to the subject.

Eggs from chickens immunized against specific bacterial antigens were disclosed as providing anti-atherosclerotic effects in mammals in U.S. Patent No. 5,215,746.

Eggs containing antibody to *S. mutans* were obtained from chickens immunized with *S. mutans* (Otake et al., J. Dental Research 70:162-166(1991)) and inhibited dental caries development.

None of these references, however, disclose or suggest that hyperimmune eggs when administered to animals, have the capability to prevent or reduce inflammation, treat or prevent arthritis or treat or prevent autoimmune diseases. Nor do these references disclose or suggest a method providing a reasonable expectation that hyperimmunization of an avian could produce an avian which lays eggs having such a capability. Finally, these references do not suggest or disclose the presence of an anti-inflammatory composition in an egg from an avian.

SUMMARY OF THE INVENTION

The invention is based on the inventors' discovery that there is anti-inflammatory activity in egg and egg products, and particularly in egg products obtained from hyperimmunized avians, which when administered to a subject animal, in particular, mammals, prevents or reduces inflammation, for example arthritis and/or autoimmune diseases, in the subject animal.

In particular, the invention is directed to a partially purified anti-inflammatory composition for the treatment and prevention of arthritis and/or an autoimmune disease, the composition comprising an egg product obtained from the eggs of an avian which has been hyperimmunized with an antigenic or genetic vaccine. The anti-inflammatory composition was partially purified from fractions isolated from both egg yolk and egg white.

The invention is also directed to a hyperimmunization process which produces supranormal levels of an anti-inflammatory composition in an avian egg. It is the inventors' belief that the level of the anti-inflammatory composition can be increased in both egg yolk and egg white by the process of hyperimmunization.

The invention is also directed to the use of such egg compositions for the treatment of at least one of the following disorders in a subject animal: arthritis and/or an autoimmune disease. The use comprises administering to the subject animal a partially pure anti-inflammatory composition or a composition of the present invention comprising the partially pure anti-inflammatory composition of the present invention. This aspect encompasses administering whole egg itself and/or fractions thereof.

The invention is based on the inventors' discovery that there is anti-inflammatory activity in egg and egg products, and particularly in egg products obtained from hyperimmunized avians, which when administered to a subject animal, in particular, mammals, treats or prevents arthritis and/or autoimmune diseases in the subject animal.

The invention is additionally directed to a method wherein the egg product is administered to the subject animal in combination with a drug selected from the group consisting of non-steroidal, anti-inflammatory drugs and disease-modifying, anti-arthritic drugs.

The invention is finally directed to a composition for reducing antibodies against autoimmune diseases in a subject animal, the composition comprising an effective amount of an egg product derived from an avian which has been hyperimmunized with an antigenic or genetic vaccine.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1, is a flow chart of the purification of the less than 3,000 molecular weight anti-inflammatory composition from powdered egg yolk.

Figure 2, is a flow chart of the purification of the less than 3,000 molecular weight anti-inflammatory composition from powdered egg white.

Figure 3, is an absorbance depiction of the separation of the partially purified anti-inflammatory composition by Sepharose™ Column.

Figure 4, is a chromatogram of the HPLC separation of partially purified anti-inflammatory composition from hyperimmune egg yolk.

Figure 5, is a graph showing the effect of hyperimmune egg on the incidence of arthritis using the Rat Type II Collagen Model.

Figure 6, is a graph showing the effect of the hyperimmune egg, its fractions and the partially purified anti-inflammatory composition on the severity of arthritis in the Rat Type II Collagen Model.

Definitions:

The term "egg" or "egg product" means any whole egg (table, hyperimmunized or otherwise) or any product or fraction derived therefrom.

The term "anti-inflammatory egg or egg fraction" means egg or egg fractions containing the anti-inflammatory composition disclosed herein.

The term "anti-inflammatory composition" means the composition disclosed herein which counteracts or suppresses the inflammatory process.

The term "partially pure egg anti-inflammatory composition" means an anti-inflammatory composition at least of the purity described in Example 4 and the exemplary materials and figures.

The term "combinatorial derived immunogens" refers to a process of generating molecular diversity among immunogens by way of combinatorial synthesis.

The term "bioengineered immunogens" refers to immunogens which are obtained through the process of gene cloning technologies and genetic rearrangements which allow the insertion of encoding nucleotides which can give rise to molecules having antigenic or immunogenic properties.

The term "genetic vaccine" refers to a nucleic acid vaccine which is generally produced by recombinant technologies and which may elicit an immune response.

The term “hyperimmunization” means exposure to one or more antigens such that an immune response is elevated and maintained above the natural unexposed state.

The term "hyperimmune egg" or “hyperimmune egg product” each means whole egg or any product or fraction derived therefrom, obtained from egg-producing animals maintained in a hyperimmune state.

The term “supranormal levels” means levels in excess of those found in eggs of egg-producing animals not maintained in a hyperimmune state.

The term "table egg" or “table egg product” means a whole egg or any product or fraction derived therefrom, obtained from egg-producing animals which are not maintained in a hyperimmune state.

The term “immunogen” means a substance that is able to induce a humoral antibody and/or cell-mediated immune response rather than immunological tolerance. The term signifies the ability to stimulate an immune response as well as react with the products of it, e.g., antibody.

The term "inflammation" is used in its art-recognized sense as a localized protective response elicited by injury or destruction of tissues which serves to destroy, dilute or wall off both the injurious agent and the injured tissue, characterized in the inappropriate, uncontrolled form by the classical sequence of pain, heat, redness, swelling, and loss of function, and histologically involving a complex series of events, including dilation of the arterioles, capillaries, and venules with increased permeability and blood flow, exudation of fluids including plasma proteins, and leukocyte migration into the inflammatory focus.

The term “arthritis” means any of a variety of disorders marked by inflammation and degeneration of connective tissue structures, especially the joints and related structures. It may be attended by pain, stiffness, or limitation of motion of these parts. Some forms of arthritis

include rheumatoid arthritis, osteoarthritis, ankylosing seronegative spondyloarthropathy, reactive arthritis, chronic fatigue syndrome, fibromyalgia (fibrositis) and gout.

The term “autoimmune disease” is applied the standard medical definition as found in standard medical dictionaries such as Dorland’s and Taber’s. A description of a variety of autoimmune diseases can be found in the Background section of this document. The following disorders are considered autoimmune diseases: rheumatoid arthritis, juvenile diabetes, multiple sclerosis, Graves’ disease, Meneri’s disease, myasthenia gravis, lupus erythematosus, psoriasis, systemic scleroderma, rheumatic fever and Sjorgren syndrome.

The term "treatment" means that the onset of the symptoms (including pain) of the disorder and/or pathogenic origin of the disorder be delayed or completely prevented, or, if present, the symptoms be ameliorated or completely eliminated. For example, the hyperimmune egg product treats arthritis and/or autoimmune disease not only by suppressing the symptoms of the disorder in humans and other mammals, but also by acting as a prophylactic agent to counteract the presence of the disorder in the recipient.

The term “prevention” means that the progression of the disease is reduced and/or eliminated or that the onset of the disease is eliminated.

The term "administer" means any method of providing a subject with a substance, including orally, intranasally, parenterally (intravenously, intramuscularly, or subcutaneously) rectally, topically or intraocularly.

The term “animal” means the animal kingdom definition.

The term “target animal” refers to an animal which functions as the egg or egg product producing animal.

The term "subject animal" refers to the animal which is administered the egg or egg product produced by the target animal.

DETAILED DESCRIPTION OF THE INVENTION

The invention generally relates to a composition and method for treatment and prevention of inflammation in general, and particularly arthritis and autoimmune diseases. The composition is preferably a natural food product which comprises hyperimmune egg or egg product. The food product, when administered by the method of the invention, not only provides relief from the pain and other symptoms caused by arthritis and autoimmune diseases, but can delay, and even prevent, the onset of such diseases. The preferred antigen mixture injected into the avians to produce the hyperimmune egg product does not contain specific antigens which are known to cause inflammation or autoimmune diseases. Therefore, it is surprising that administration of hyperimmune egg or egg product obtained from avians immunized against a mixed antigen vaccine is effective in reducing the symptoms of and preventing inflammation and autoimmune diseases when administered to a subject.

The Invention

The product and method of the invention relate particularly to the use of hyperimmune egg, which is a natural food product, in the treatment and prevention of inflammatory diseases such as autoimmune diseases and arthritis. Being natural, this food product can be used to treat and prevent such diseases without the fear of side effects, except, of course, for allergic reactions in those intolerant to eggs.

In a preferred embodiment, the invention comprises a hyperimmune egg or egg product which is effective in treating and preventing arthritis and/or an autoimmune disease in a subject animal. The hyperimmune egg is obtained from an egg-producing animal, and more preferably, an avian, which has been hyperimmunized with at least one immunogen. The hyperimmune egg product preferably administered orally to the subject animal. The hyperimmune egg or egg product can be further separated into more potent fractions which can subsequently be administered to a subject animal in a variety of forms.

The hyperimmune egg or egg product, including the partially purified anti-inflammatory composition of the invention is effective in treating and preventing inflammation, and in particular, all forms of arthritis, including, but not limited to, rheumatoid arthritis, osteoarthritis, ankylosing seronegative spondyloarthropathy, reactive arthritis, chronic fatigue syndrome, fibromyalgia (fibrositis) and gout. The egg product of the invention is equally effective in treating autoimmune diseases, such as rheumatoid arthritis, juvenile diabetes, multiple sclerosis, Graves' disease, Meneri's disease, myasthenia gravis, lupus erythematosus, psoriasis, systemic schleroderma, rheumatic fever and Sjogren syndrome among others.

In an alternate embodiment, the invention also comprises an anti-inflammatory composition obtained from avian eggs, its partial purification, and the administration of the partially purified composition to a subject for the treatment of inflammation. The invention further comprises the same anti-inflammatory composition partially purified from the egg of an avian that has been hyperimmunized with one or more immunogens, and, in particular, bacterial antigens, or their synthetic equivalent. The anti-inflammatory composition is present in hyperimmunized eggs at supranormal levels which provide anti-inflammatory activity in subject animals.

Characteristics of the partially purified anti-inflammatory composition:

The partially pure anti-inflammatory composition has the following characteristics:

- 1) has anti-inflammatory activity in a subject animal;
- 2) is present in both the egg white and egg yolk of avian eggs;
- 3) when isolated from egg yolk, has greater anti-inflammatory activity than when isolated from egg white;
- 4) has a molecular weight of less than approximately 3000 daltons;

5) is non-proteinaceous and non-steroidal;

6) is heat stable; and

7) is orally active and is not degraded by digestive enzymes.

The approximately 3,000 dalton molecular weight is deduced from the isolation and purification of the composition wherein the isolation and purification process uses an ultra-filtration membrane that does not allow the passage of molecular species greater than approximately 3,000 Dalton therethrough. The partially purified anti-inflammatory composition is determined to be non-proteinaceous and non-steroidal because it is small in size and is not degraded by enzymes which degrade proteins. Moreover, the composition is orally active and is not degraded by digestive enzymes. The small stable form of the composition (as differentiated from proteins which are much larger) facilitates its absorption from the digestive tract. Finally, the partially purified anti-inflammatory composition is heat-stable.

The partially purified egg anti-inflammatory composition can be isolated from whole egg, egg yolk and egg white. The anti-inflammatory composition partially purified from egg yolk shows higher anti-inflammatory activity than the anti-inflammatory composition partially purified from egg white.

It is the inventors' finding that supranormal levels of the partially purified anti-inflammatory composition can be isolated from whole egg, egg yolk and egg-white from an egg-producing animal which has been immunized.

Hyperimmunization of the Egg-producing Animal:

As just described, it is the inventors' finding that the anti-inflammatory composition is present in hyperimmune eggs at supranormal levels which provide anti-inflammatory activity in subject animals.

Hyperimmune Egg or Egg Product

The hyperimmunized egg or egg product can be produced by any egg-producing animal. It is preferred that the animal be a member of the class Aves or, in other words, an avian. Within the class Aves, domesticated fowl are preferred, but other members of this class, such as turkeys, ducks, and geese, are a suitable source of hyperimmune egg product.

When such egg-producing animals are brought to the specific state of immunization by means of, for example, periodic booster administration of immunogens, the animals will produce eggs that, when consumed by a subject, will have beneficial properties including supranormal levels of the anti-inflammatory composition which are effective in the treatment and prevention of inflammatory related diseases as well as arthritis and autoimmune diseases in that subject.

The induction of immune sensitivity alone is insufficient to cause the appearance of supranormal levels of the egg anti-inflammatory composition in eggs, as is shown by the fact that "table eggs", do not contain these supranormal levels, even though the avians have been sensitized against various immunogens during normal immunization against avian diseases and during normal exposure to environmental factors. It is the inventors' finding that it is only in the specific hyperimmune states that the eggs have the desired supranormal levels of the anti-inflammatory composition.

This special state of hyperimmunization, in which the egg will contain higher levels of the anti-inflammatory composition, is preferably achieved by administering an initial immunization, followed by periodic boosters with sufficiently high doses of specific immunogens or mixtures of immunogens. The preferred dosage of booster should be equal to or greater than 50% of the dosage necessary to produce primary immunization of the avian. Thus, there is a threshold booster dosage below which the properties are not produced in the avian's egg, even though the avian is in what normally would be called an immune state. Having knowledge of the requirement for developing and maintaining a hyperimmune state, it is within the skill of the art to vary the amount of immunogen administered, depending on the egg-producing animal, genera and/or strain employed, in order to maintain the animal in the hyperimmune state.

The hyperimmune state is preferably produced by any immunogen or combination of immunogens. Hyperimmunization is preferably achieved by multiple exposures to multiple immunogens, multiple exposure to single immunogens, or single exposures to libraries of immunogens. Nearly any immunogen can be used to induce the hyperimmune state, including, but not limited to, bacterial, viral, protozoan, allergan, fungal or cellular substances.

In addition to immunizations with naturally occurring immunogens, immunization may also be accomplished using immunogens which are synthetically derived by combinatorial chemistries. The basic strategy is to assemble multiple combinations of chemical building blocks for producing a population of molecules with diversity. Several methods have recently been developed for solid and solution phase combinatorial synthesis of libraries of oligomers (Fodor, S. et al., Science 251:767 (1991); Houghton, R. et al., Nature 354:82 (1991)) as well as small organic molecules (Bunin, B. & Ellman, J., J. Am. Chem. Soc. 114:10997 (1992)). Rapid multiple peptide and oligomer synthesis can serve as a source for combinatorial derived immunogens. Furthermore, an alternative strategy would allow the addition of organic building blocks in combinatorial fashion to a backbone molecule for improved immunogenicity.

Alternative modes of hyperimmunizing egg producing animals can be used in place of anti-immunogenic vaccines and include the use of genetic vaccines. In particular, any DNA construct (generally consisting of a promoter region and an antigen encoding sequence) will trigger an immune response. Genetic vaccines consist of antigen-coding vectors, fragments of naked DNA, plasmid DNA, DNA-RNA antigens, DNA-protein conjugates, DNA-liposome conjugates, DNA expression libraries, and viral and bacterial DNA delivered to produce an immune response.

Methods of DNA delivery include, but are not limited to, particle bombardment, direct injection, liposomes, jet injection (Fynan, E.F. et al., Proc. Natl. Acad. Sci. USA 90:11478-11482 (1993)). When applying these delivery methods, much smaller quantities may be necessary and generally result in more persistent immunogen production. The nucleic acids that code for known or unknown immunogens, promoter regions (notably CMV cauliflower mosaic virus) and SV40

bacterial origin can be replicated in bacteria to produce plasmid DNA for use in DNA injections. Although several routes of parenteral administration of the DNA are effective in chickens, the preferred method is intramuscular injection to the breast muscle. Vaccine trials are carried out in egg laying avians, preferably chickens. Repeated immunizations are given at one to two week intervals for up to six months.

It is preferred that the amounts of DNA used are generally in the order of 50-300 µg of DNA in saline for direct injection. For particle bombardment, 4-100 µg of DNA co-precipitated onto gold beads by the addition of 2.5 M CaCl_2 are preferred. Repeated immunizations can be given intradermally by this method of accelerating DNA coated particles into the live animal.

Hyperimmunization Procedure

The following list of steps is an example of a preferred procedure used to bring an egg-producing animal to a heightened state of immunity from which the resultant hyperimmunized egg or egg product can be administered to a subject:

2. Selecting one or more immunogens.
3. Eliciting an immune response in the egg-producing animal by primary immunization.
4. Administering booster vaccines of antigens of appropriate dosage to induce and maintain the hyperimmune state.
5. Testing the hyperimmunized eggs for anti-inflammatory activity levels.
6. Collecting and processing the eggs.

Below is a more detailed description of the steps of this procedure.

Step 1: Any immunogen or combination of immunogens may be employed as a vaccine. The immunogens can be bacterial, viral, protozoan, fungal, cellular, or any other substances to which the immune system of an egg-producing animal will respond. The critical point in this step is that the immunogen(s) must be capable of inducing immune and hyperimmune states in the egg-producing animal. Although only a single immunogen may function as the vaccine for the

method of the invention, one preferred vaccine is a mixture of polyvalent bacterial and viral antigens selected from the following antigen families: the enteric bacilli and bacteroides, pneumococci, pseudomonas, streptococci, bacilli, staphylococci, neisseria, clostridia, mycobacteria, actinomycetes chlamydiae, and mycoplasma. Viral antigens are preferably selected from the following antigen families: adenoviruses, picornaviruses and herpes viruses, although other viral antigen families will work.

In a preferred embodiment, a polyvalent vaccine referred to as Series 100 (S-100) vaccine is used. The bacteria included in the S-100 vaccine are listed in table 1 of Example 1. This vaccine has been previously described in U.S. Patent Nos. 5,106,618 and 5,215,746, both assigned to Stolle Research and Development Corporation. Another preferred vaccine for use is the EB-100E vaccine, the details of which are also described in Example 1.

Step 2: The vaccine can be either killed or live-attenuated vaccine and can be administered by any method that elicits an immune response. It is preferred that immunization be accomplished by administering the immunogens through intramuscular injection. The preferred muscle for injection in an avian is the breast muscle. Dosage is preferably 0.05 - 5 milligrams of the immunogenic vaccine. Other methods of administration that can be used include intravenous injection, intraperitoneal injection, intradermal, rectal suppository, aerosol, oral, topical or ocular administration. When DNA techniques are used for the hyperimmunization process, much smaller quantities are required, generally 300 micrograms.

It can be determined whether the vaccine has elicited an immune response in the egg-producing animal through a number of methods known to those having skill in the art of immunology. Examples of these include enzyme-linked immunosorbent assays (ELISA), tests for the presence of antibodies to the stimulating antigens, and tests designed to evaluate the ability of immune cells from the host to respond to the antigen. The minimum dosage of antigen necessary to induce an immune response depends on the vaccination procedure used, including the type of adjuvants and formulation of antigen(s) used as well as the type of egg-producing animal used as the host.

Step 3: The hyperimmune state is induced and maintained in the target animal by repeated booster administration of an appropriate dosage at fixed time intervals. The time intervals are preferably 2 - 8 week intervals over a period of 6 - 12 months. However, it is essential that the booster administrations do not lead to immune tolerance. Such processes are well known in the art.

It is possible to use other hyperimmunization maintenance procedures or combination of procedures, such as, for example, intramuscular injection for primary immunization and intravenous injection for booster injections. Further procedures include simultaneously administering microencapsulated and liquid antigen, or intramuscular injection for primary immunization, and booster dosages by oral administration or parenteral administration by microencapsulation means. Several combinations of primary and hyperimmunization as known to those skilled in the art.

Step 4: It is necessary to test the eggs for anti-inflammatory activity levels. This can be accomplished by any clinical and pre-clinical evaluation that tests the effects of either the hyperimmune egg, or products derived therefrom, on inflammation. Chemical-induced inflammation of the rat is a preferred standard assay for anti-inflammatory drugs. (See Example 6).

Step 5: This step involves the collection and processing of the egg(s) containing the anti-inflammatory composition. The egg can be collected by conventional methods. Processing the egg can also be accomplished in a variety of ways described later in this document. The egg can also be further processed to purify the anti-inflammatory composition as described below.

Partial Purification Process:

The egg anti-inflammatory composition can be partially purified from whole egg, egg yolk or egg white. An example of a preferred partial purification process is as follows:

1. Preparation of a water-soluble fraction from an egg;

2. Ultrafiltration of the water-soluble fractions;
3. Separation of fractions by ion or anion exchange chromatography; and
4. Bioassay of the separated fractions for anti-inflammatory activity.

The following is a more detailed description of this process:

Step 1: The anti-inflammatory composition can be partially purified from whole egg, egg yolk or egg white. In a preferred embodiment, the composition is purified from egg yolk. The lipid portion is removed from the whole egg or egg yolk by methods well-known to those having skill in the art. For example, in the case of spray-dried egg yolk powder, defatting can be accomplished with solvents (propane, butane or hexane or with binary solvents), supercritical CO₂, enzymes and the like, and in the case of liquid egg yolk, defatting can be accomplished by the caprylic acid separation method (CAPS) disclosed by Lee (U.S. Pat. No. 5,367,054). No fat removal is necessary for egg white, and thus the liquid or powdered form of the egg white can be either heated or dissolved directly by conventional methods and as described in the examples listed below. The whole egg, egg yolk or egg white is then preferably processed into either liquid or powder form, and is further processed to obtain water soluble fractions. (See Examples)

Step 2: The resulting water soluble fractions, from whole egg, egg yolk or egg white, are subjected to ultrafiltration using ultrafiltration systems equipped with a 3,000 molecular weight cut-off membrane. The ultrafiltration process separates molecules having a molecular weight of more than approximately 3,000 daltons from those having a molecular weight of less than approximately 3000 daltons. Once filtered, the resulting ultra-filtrates contain molecules of less than approximately 3,000 dalton molecular weight are then lyophilized, weighed, and prepared for bioassay testing and further separation.

Step 3: Fractions from the less than 3,000 dalton ultra-filtrate can be separated by, for example, Reverse Phase High Pressure Liquid Chromatography to further purify the partially pure anti-inflammatory composition. As an alternate or additional strategy, the egg anti-inflammatory composition in the ultra-filtrate can be further characterized by anion exchange

DEAE-Sepharose chromatography. In a specific disclosed embodiment, the preferred ultrafiltration is by Amicon RA 1000 (3K MWCO) and DEAE ion-exchange chromatography. It is understood, however, that equivalent techniques and materials could be used to isolate the composition, given the information herein, and the knowledge available to the person of ordinary skill in the art.

Step 4: The anti-inflammatory activity of the composition can be tested by a standard bioassay which determines anti-inflammatory activity. Some examples include inhibition of leukocyte migration, rat paw edema test, adjuvant-induced arthritis, collagen induced arthritis, and intra-vital microscopy among others. Comparisons of egg anti-inflammatory composition with known anti-inflammatory drugs such as aspirin and indomethacin can also be done. And finally, clinical tests for rheumatoid arthritis, degenerative joint disease and injury induced arthritis can also be used to determine anti-inflammatory activity.

The anti-inflammatory action of the partially pure egg anti-inflammatory composition is measured by bio-assays. A preferred bio-assay is the rat type II collagen assay as set forth in Example 6.

Alternative bio-assays include the pleural leukocyte migration inhibition assay as described in Vinegar et al., "Some Quantitative Characteristics of Carrageenan Induced Pleurisy in the Rat," Proc. Soc. Exp. Bio. Med. 143:711-714 (1973); Ammendola, G. et al., "Leukocyte Migration and Lysozomal Enzymes Release in Rat Carrageenan Pleurisy," Agents and Actions 5:250-255 (1975); Vinegar, R. et al., "Quantitative Studies of the Pathway to Acute Carrageenan Inflammation," Fed. Proc. 35:2447-2456 (1976)

The leukocyte migration inhibition assay is another useful assay which is generally performed as follows: Samples containing the anti-inflammatory composition are administered to the artificially inflamed adult female rats with 1% carrageenan solution and then the anti-inflammatory effect of the samples at each dosage is determined (using Automated Image

Analysis Technique) by the reduction in the number of leukocytes in the pleural exudates of the treated rats as compared to those of control rats.

Alternatively, the anti-inflammatory action of the substantially pure anti-inflammatory composition can be tested on edema caused by injecting carrageenan into rat footpads (Winter, C.A., Risley, G.A., Nuss, A.W., "Carrageenan-Induced Edema in the Hind Paw of the Rat as an Assay for Anti-Inflammatory Drugs," Proc. Soc. Exper. Biol. Med. 3:544 (1967)).

A variety of other tests may be used. (See Wetnick, A.S., and Sabin, C., "The Effects of Clonixin and Bethaurethasone on Adjuvant-Induced Arthritis and Experimental Allergic Encephalomyelitis in Rats." Jap. J. Pharm. 22:741 (1972)).

Processing and Administration:

Once the egg-producing animals have been sufficiently hyperimmunized, it is preferred that the eggs from these animals are collected and processed to produce a hyperimmune egg product. Subsequently, the hyperimmune egg product can be administered to the subject.

The egg and/or the egg product thereof, including the partially purified anti-inflammatory composition of the present invention, are administered to a subject animal by any means that treats or prevents inflammation in inflammatory diseases including arthritis or autoimmune disease in the subject animal. It is preferred that administration occur by directly feeding the egg or any derivative of the egg to the subject animal. It is important to note that whole egg, egg yolk, and egg white are natural food ingredients which are non-toxic and safe.

It is generally known in the art that the yolk and /or white factions contain the agent or agents responsible for the beneficial properties observed and referred to above. Those having ordinary skill in the art would clearly recognize that further separation could provide more potent fractions or elimination of undesirable components and purification, such as in the case of the partially purified anti-inflammatory composition, will allow for other modes of administration such as administering egg product parenterally, subcutaneously, intravenously, intramuscularly,

intraperitoneally, intranasally, orally or topically. In addition, such further separation will provide for the ability to make encapsulated products and pharmaceutical compositions with said egg or fraction thereof.

When it comes to treatment and prevention of a particular disorder, whether it be inflammation in general or a form of arthritis or an autoimmune disease, the hyperimmune egg product or any active fraction thereof, including the partially purified anti-inflammatory composition, is preferably administered to the subject in an amount that is immunologically effective in treating and preventing the particular disorder. Duration and intensity of the treatment will depend upon the particular condition, whether it is present, and, if so, the advancement of the condition in the subject. The hyperimmune egg product or any active fraction thereof, including the partially purified anti-inflammatory composition, are also provided in any amount that treats and/or prevents the condition and the symptoms of the condition. For example, in some cases, daily amounts ranging from less than one to several whole, hyperimmune eggs (or hyperimmune egg products containing the equivalent of less than one to several whole, hyperimmune eggs) can be administered to the subject depending on the particular circumstance of the condition. More potent fractions can be separated and concentrated by methods described herein as well as other known methods in the art, from several hundred eggs.

In one embodiment, the egg product of the invention as well as the partially purified anti-inflammatory composition was found to be effective in treating rheumatoid arthritis in rats using a collagen-induced arthritis animal model (see Example 2). This animal model is well recognized by those in the art as one which parallels the rheumatoid arthritis effect in humans. In addition to treatment of arthritis, it was determined that the egg product, when administered to rats prior to induction of arthritis, delayed, and in some cases, prevented the onset of the arthritic symptoms. Thus, the egg product of the invention is effective in not only treating the symptoms of the disease, but also delaying and/or preventing the onset or progression of the disease.

In an alternative embodiment, the egg product was tested in humans and showed positive effects in treating various forms of arthritis in several humans suffering from such symptoms (see

Examples 2A-2C). The humans who were treated by the egg product demonstrated a clinical reduction in such symptoms as pain in addition to a general reduction in swelling and stiffness.

Indicative of an effect on autoimmune disease is the surprising reduction of Type II collagen antibodies by the egg product as well as the partially purified anti-inflammatory composition of the invention in this disease model, which was also seen in rats (see Example 6) It is contemplated that the egg product of the invention is effective in reducing antibodies involved with other autoimmune diseases such as juvenile diabetes, multiple sclerosis, Graves' disease, Meneri's disease, myasthenia gravis, lupus erythematosus, psoriasis, systemic scleroderma, rheumatic fever and Sjogren syndrome among others.

As described in the background of the invention, patients having arthritis are presently treated with non-steroidal anti-inflammatory drugs (NSAIDs) and/or disease modifying anti-arthritis drugs (DMARDs) in order to relieve the symptoms of the arthritic condition. As shown in the examples (See Example 8), patients were administered the egg product of the invention concurrently with the present drug therapy. The egg product worked synergistically with the drug therapy to reduce the symptoms of the particular disorder. The egg product can therefore be administered concurrently with alternative treatment for arthritis and autoimmune diseases to produce a synergistically better effect.

In addition, as is well known in the art, NSAIDs and DMARDs can cause severe gastrointestinal damage. U.S. patent number 5,772,999 assigned to DCV, Inc., to which reference may be made, discloses that hyperimmune egg product is effective in treating NSAID-induced gastrointestinal damage. Therefore, an alternative embodiment is contemplated for cases where the subject is taking or is preparing to take NSAIDs for the treatment of an arthritic condition. In such a case, the subject can also be administered the egg product of the invention to, not only treat the arthritic condition, but also protect the gastrointestinal system from NSAID-induced damage. In such treatments, a lower dose of hyperimmune egg may be sufficient. Also, the subject animal suffering from the disorder can be administered egg product prior to beginning its regimen of NSAIDs and DMARDs, in order to better prepare the gastrointestinal system.

Preparations of the anti-inflammatory composition for parenteral administration include sterile aqueous or nonaqueous solutions, suspensions or emulsions. Examples of nonaqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils such as olive oil and injectable organic esters such as ethyl oleate.

With particular reference to the partially pure anti-inflammatory composition, oral administration is preferably accomplished through solid dosage forms which include capsules, tablets, pills, powders and granules, among others. In solid dosage forms, the anti-inflammatory composition is admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluent. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents, pH sensitive polymers, or any other slow-releasing encapsulants which are typically used as encapsulating compositions in the food and drug industry. Tablets and pills can additionally be prepared with an enteric coating.

Liquid dosage forms of the anti-inflammatory composition for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs, containing inert diluents commonly used in the pharmaceutical art. Besides inert diluents, compositions can also include wetting agents, emulsifying, and suspending, and sweetening agents.

The hyperimmune egg or egg product, including the partially purified anti-inflammatory composition of the invention is effective in treating and preventing inflammation, and in particular, all forms of arthritis, including, but not limited to, rheumatoid arthritis, osteoarthritis, ankylosing seronegative spondyloarthropathy, reactive arthritis, chronic fatigue syndrome, fibromyalgia (fibrositis) and gout. The egg product of the invention is equally effective in treating autoimmune diseases, such as rheumatoid arthritis, juvenile diabetes, multiple sclerosis, Graves' disease, Meneri's disease, myasthenia gravis, lupus erythematosus, psoriasis, systemic scleroderma, rheumatic fever and Sjogren syndrome among others. (See Background section of this document)

In an alternative embodiment, the egg or any fraction thereof, including the partially purified anti-inflammatory composition, is integrated into a nutritional supplement. One preferred method for preparing the egg or any fraction thereof to be incorporated into a nutritional supplement involves drying the egg into an egg powder. Although various methods are known for drying eggs, spray drying is a preferred method. The process of spray drying eggs is well known in the art. Such a dried egg powder can be incorporated into drinks in the form of , for example, protein powders, powder building drinks, protein supplements and any other nutritional, athlete-associated products. In addition, the egg powder can be used in bake mixes, powder bars, candies, cookies, etc. Other examples of egg processing include, making an omelet, soft or hard-boiling the egg, baking the egg, or, if desired, the egg can be eaten raw or processed as liquid egg.

Administration dosage and frequency will depend on the age and general health condition of the patient, taking into consideration the possibility of side effects. Administration will also be dependent on concurrent treatment with other drugs and patients' tolerances of the administered drug.

Examples of inflammatory conditions that may be treated by administration of the egg, egg product, and/or the anti-inflammatory composition of the present invention include acute and subacute bursitis, acute non-specific tendinitis, systemic lupus erythematosus, systemic dermatomyositis, acute rheumatic carditis, pemphigus, bullous dermatitis, herpetiformis, severe erythema, multiform exfoliative dermatitis, cirrhosis, seasonal perennial rhinitis, bronchial asthma, ectopic dermatitis, serum sickness, keratitis, ophthalmic iritis, diffuse ureitis, chondritis, optic neuritis, sympathetic ophthalmia, symptomatic sarcoidosis, Loeffler's syndrome, berylliosis, hemolytic anemia, mastitis, mastoiditis, contact dermatitis, allergic conjunctivitis, psoriatic arthritis, ankylosing spondylitis, acute gouty arthritis, herpes zoster rheumatoid arthritis, osteoarthritis, any other degenerative joint diseases, and any other related auto immune diseases. Further, the isolated and purified egg product may be used to treat individuals who are exposed to potentially inflammatory agents such as allergens.

Effective amounts:

With regard to administration to a subject of the hyperimmunized egg or egg product, it has been determined, and is detailed in the following examples, that the preferred dose range of hyperimmunized egg or egg product to be given to a subject is between 100 mg to 10 grams per kilogram of subject weight.

With regard to the partially purified anti-inflammatory composition itself, it has been determined that the preferred dose range of the partially purified composition, purified and isolated from whole egg, egg yolk and egg white of a hyperimmunized egg, is between 1 microgram and 400 milligrams of the anti-inflammatory composition.

The dosage of active ingredients may be varied: however it is necessary that the amount of the active ingredient shall be such that a suitable dosage form is obtained. It will be recognized that the selected dosage form depends upon the desired therapeutic effect, on the route of the administration and on the duration of the treatment.

It is of significant importance to point out that the egg product of this invention has been shown to be safe, non-toxic, ideal for long term use and has no side effects other than on human allergies to eggs. The egg product can be orally administered either alone or in combination with drug therapy, for long term use for arthritic and autoimmune diseases.

The advantageous properties of this invention can be observed by reference to the following examples which illustrate the invention.

EXAMPLES**Example 1**Preparation of S-100 Vaccine

The multivalent vaccine known as "Series 100" or "S-100," disclosed in U.S. Pat. No. 5,215,746 and containing the bacteria shown in Table 1 (obtained from the American Type Culture

Collection), was reconstituted with 15 ml of medium and incubated overnight at 37C. Once good growth was obtained, approximately one-half of the bacterial suspension was used to inoculate one liter of broth which was then incubated at 37C. The remaining suspension was transferred to sterile glycol tubes and stored at -20C for up to six months.

After good growth was visible in the culture, the bacteria were harvested by centrifugation. The bacterial pellet was resuspended in sterile physiological saline solution and the bacterial sample was centrifuged three times to wash the cells. After the third wash, the pellet obtained was resuspended in a small amount of double distilled water.

The medium-free bacterial suspension was heat-killed by placing the suspension in a glass flask in an 80C water bath overnight. The viability of the broth culture was tested with a small amount of heat-killed bacteria. Broth was inoculated with heat-killed bacteria, incubated at 37C for five days and checked daily for growth, as the bacteria have to be killed for use in the vaccine.

The heat-killed bacteria were lyophilized until dry. The dry bacteria were then mixed with sterile saline solution to a concentration of 2.2×10^8 bacterial cells/ml saline (1.0 optical density reading at 660 nm).

TABLE 1

Antigens in the S-100 Vaccine

Name	Media	Name	Media
Staphylococcus simulans	BHI	Pseudomonas aeruginosa	BHI
Staphylococcus epidermidis	BHI	Klebsiella pneumoniae	BHI
Streptococcus pyogenes, A Type 1	APT	Salmonella typhimurium	BHI
Streptococcus pyogenes, A Type 3	APT	Haemophilus influenzae	BHI
Streptococcus pyogenes, A Type 5	APT	Streptococcus mitis	APT
Streptococcus pyogenes, A Type 8	APT	Proteus vulgaris	BHI
Streptococcus pyogenes, A Type 12	APT	Shigella dysenteriae	BHI

Streptococcus pyogenes, A Type 14	APT	Diplococcus pneumoniae	APT
Streptococcus pyogenes, A Type 18	APT	Propionibacter acnes	Broth
Streptococcus pyogenes, A Type 22	APT	Streptococcus sanguis	APT
Aerobacter aerogenes	BHI	Streptococcus salivarius	APT
Escherichia coli	BHI	Streptococcus mutans	BHI
Salmonella enteritidis	BHI	Streptococcus aelactiae	APT

EB-100E Vaccine

The EB-100E vaccine is known by the trade name of Scournmune®-CRT, manufactured by Schering-Plough Animal Health, of Kenilworth, New Jersey, USA. The vaccine consists of *Clostridium perfringens*, type C, *Escherichia coli*, porcine rotavirus, and transmissible gastroenteritis.

Immunization Procedure For Hyperimmune Egg Product

A killed preparation of pathogens was prepared as described above. For the first vaccination, the bacteria were mixed with complete Freund's adjuvant, and 5.6 mg of bacterial material were injected into the breast muscle of a chicken. For the remaining vaccines, the bacterial preparation was mixed with the incomplete Freund's adjuvant and injected into the chickens at two-week intervals for six months.

Eggs were collected from the hyperimmunized chickens and then sprayed dried into a powder form. During the spray drying procedure, inlet temperatures did not exceed 320 Degrees F, exhaust temperatures were maintained in accordance with producing powder in the range of 3.0 to 4.0 percent finished moisture, and pump pressure was maintained around 2500 to 4000 P.S.I. Lower temperatures ranging from 100 – 160 F were used, and samples were monitored for moisture content during the drying process to obtain a final product having any consistency desired.

Example2

Preferred Method for Preparing a Partially Purified Anti-Inflammatory Composition
From Egg

The following examples describe a method (suitable for large scale purification) for obtaining the anti-inflammatory composition from avian eggs in a partially pure, low molecular weight, non-aggregated form. Whole eggs, hyperimmunized and control table eggs, were cracked and the egg white was separated from the yolk and both were spray-dried. Hyperimmunized eggs were obtained as described in Example 1. Egg white powder was processed separately to obtain the aqueous fraction for ultra-filtration.

All of the purification steps were performed so as to minimize possible contamination with bacteria or pyrogens. Sterile water was used to prepare solutions and all glassware was de-pyrogenated. In addition, the solution was sterile filtered.

Example 2a

Preparation of Egg; Anti-Inflammatory Composition From Egg Yolk Powder

Solvent Extraction

The dried egg yolk as prepared in Example 1, was subjected to liquid solvent extraction with either propane, or butane to separate the lipids from the aqueous yolk fraction containing the anti-inflammatory composition. Briefly, 500 grams of dry egg yolk powder was placed in a column, to which was added 4 liters of liquid propane solvent. The solvent supernatant and extracted lipid were removed. Six additional solvent extractions were performed for a total of six lipid extractions.

Ultra-filtration

Four hundred grams of dry de-fatted egg yolk was diluted with 4 liters of sterile distilled water and homogenized with a Virtis (handishear). The yolk mixture was either centrifuged at 24 RPM or allowed to stand refrigerated until the non-dissolved yolk particles precipitated. The resulting aqueous fraction was ultrafiltered using an Amicon RA1000 ultra-filtration system equipped with 3,000 dalton cut-off spiral-wound membrane. The pump speed was maintained at 20 psi inlet

pressure and 15 psi outlet pressure. The <3,000 daltons molecular weight permeates were sterile filtered using a 0.45 µm Sterile disposable Nalgene filter and lyophilized or frozen, for storage, bioassay testing or further purification. Figure 1 shows a flow chart of the purification process.

Molecular species below 3,000 daltons from egg yolk contain the anti-inflammatory composition in a low molecular weight, non-aggregated form. From 400 grams of starting material, the yield of anti-inflammatory composition was approximately 12 grams or 3% of the total. Bioassay testing of this 3KDa fraction for anti-inflammatory activity showed high levels of activity (example 5).

Example 2b

Preparation of Egg Anti-inflammatory Activity from Egg White Powder

Four hundred grams of egg white, isolated from both hyperimmunized egg as described in Example 1 and control table egg, was diluted with four liter of deionized water. The mixture was homogenized and filtered through a 40 µm filter and ultrafiltered through a 3KDa MW CO ultra-filtration system (Figure 2). From 400 g of egg white powder, 8.6 g or 2.15 % of anti-inflammatory composition was recovered.

This example shows the preparation of the active anti-inflammatory composition from egg white material of both hyperimmune and control table eggs.

Example 3

DEAE Ion-Exchange Chromatography

The egg anti-inflammatory composition was also further characterized by anion exchange DEAE chromatography.

3,000 Dalton ultra-filtration permeates from both delipidated egg yolk and egg white were prepared in the same manner as described in Example 2. A DEAE Sepharose column (2.5 x 40.0 cm) was previously equilibrated at room temperature with 1,000 ml of 20 mM ammonium acetate buffer in sterile double distilled pyrogen free water pH 8.0. 3,000 Dalton permeate of

delipidated egg yolk (15 grams) or egg white (18 grams) was dissolved in 200 ml of sterile pyrogen free double distilled water and applied to the DEAE Sepharose column (pharmacia Biotech., Fast flow) with a flow rate of 100 ml/h. Components of 3,000 Dalton permeate were eluted with following buffers to separated egg anti-inflammatory composition and other molecules. With the same flow rate DEAE Sepharose was washed with 200 ml of 20 mM ammonium acetate, pH 8.0 and 200 ml of 20 mM ammonium acetate, pH 6.5, to remove unbound materials. 200 ml of 150 mM ammonium acetate, pH 5.0, then was applied to elute the weakly bound molecules. More strong bound molecules were eluted with 200 ml of 150 mM ammonium acetate, pH 4.0. DEAE Sepharose column was finally regenerated with 400 ml of 1.5 N NaCl in 20 mM ammonium acetate, pH 6.5 (Figure 3).

Figure 3 shows a typical chromatogram of DEAE Sepharose separation of 3Kda permeate from delipidated egg yolk and egg white. Letters indicated the elution profile with buffers. Fractions were collected by every 15 ml eluent and their molecular absorbances at 280 nm and 220 nm were measured. Four fractions were pooled and the water was removed by lyophilization to dryness. Table 2 is the summary of the separation.

TABLE 2

DEAE separation of 3,000 Dalton permeates of delipidated egg yolk and egg white.

Fraction	Elute #	Vol(ml)	Weight (g)	Recovery (%)
Egg yolk				
I	1-22	294	11.79	78.6
II	23-40	252	0.40	2.7
III	41-56	224	0.44	2.9
IV	57-70	196	0.19	1.3

Egg white

I	2-26	350	15.33	85.2
II	27-46	280	0.23	1.3
III	47-60	196	0.32	1.8
IV	61-73	182	0.44	2.4

Example 4High Pressure LiquidChromatography (HPLC) Analysis of 3,000 Dalton Permeates and their DEAE Sepharose Fractions

3,000 Dalton permeates of 3,000 Dalton permeates of egg yolks were analyzed with reverse High Performance Liquid Chromatography (HPLC) (Shimadzu Unit, SPD-M10A VP Diode array detector, LC-6AD liquid chromatography, SCL-10A VP Degassor, LPM-600 Low pressure mixing, and CTO-10A VP Column oven). All of the fractions were prepared as 5.0 mg/ml in super-pure water followed by filtration through a 0.2 μ m filter. 20 μ l of each sample was applied on a Water Symmetry C18 column (3.9 x 150 mm) at 29°C and with a flow rate of 1.0 ml/min. A linear gradient of 0% - 60% acetonitril in dH₂O that contained 0.1% TFA was used as the mobile phase.

Figure 4 shows a Max plot chromatography of the separation of 3,000 Dalton permeate from delipidated PL-100 egg yolk. By using the same analytic condition, 3,000 Dalton permeate prepared from delipidated Table egg yolk was also obtained (chromatography has not shown).

Table 3 summaries the result of HPLC analysis of 3,000 Dalton permeates. Peaks that are greater than 0.5% of peak area were collected. The similarities of peaks were compared within +/- 0.5% variability in their retention times. Common peaks mean that they are present in both PL-100 egg yolk and Table egg yolk while PL-100 peaks indicates that they are only present in PL-100 egg. Comparing two fractions, 3,000 Dalton permeate of Pl-100 egg yolk shows 15 more peaks than that of Table egg yolk, suggesting that hyperimmunization on chicken could produce more biological components in its egg.

TABLE 3

3KDapermeate	Common peaks	PL-100 peaks	Table peaks	Total
PL-100	18	15	0	33
Table	18	0	5	23

Preparative HPLC

To obtain large quantities of the anti-inflammatory factor after ultrafiltration, the preferred step is to separate the partially purified, less than approximately 3,000 daltons molecular weight, composition on a preparative HPLC column. Accordingly, a 5.08 X 20 cm column was packed with Zorbax C8 packing material and 4 grams of the less than 3,000 daltons MW ultrafiltrate was purified during each run. Using a mobile phase of 80/20 H₂O/methanol with 0.05% TFA, at a flow rate of 90 ml per minute, the permeate was separated into the partially purified composition collected and lyophilize. Next the collected peak was re-tested on an analytical C8 column. With the method described, large quantities of the ultra-pure peak were obtained by preparative HPLC and analyzed for bioassay activity, elemental composition and chemical structure. Mass Spectroscopy, Elemental analysis, Infra-red spectroscopy and Nuclear Magnetic Resonance, NMR are being used to determine the structure of the factor.

Example 5**Anti-Arthritic Properties of Hyperimmune Egg Product in a Collagen-Induced Arthritis Model In Rats**

Collagen induced arthritis in rat is a popular experimental animal model of Rheumatoid arthritis and has been used by several laboratories and research institutions since 1977. Since its initial discovery, the animal model of collagen-induced arthritis has demonstrated many parallels to human rheumatoid arthritis. For example, Stuart and coworkers have demonstrated that several of the clinical signs and histological changes in the joints of arthritic rats resemble those in patients with Rheumatoid Arthritis (Stuart et al 1983). Of particular interest is the fact that many

Rheumatoid Arthritis patients exhibit autoimmunity to Type II collagen. Historically, this animal model has proven to be a useful predictive model of clinical efficacy of new modalities with immunosuppressive properties and potential immune modifying substances in terms of their anti-arthritic action.

The arthritis in mice and rats is generally induced by immunization with heterologous type II collagen, which initiates a combined humoral and cellular immune response targeted to joint tissues. Current therapies are inadequate or have side effects that limit their prolonged use.

The present example looks at the effect of the egg product and the partially purified anti-inflammatory composition of the invention orally administered to rats prior to and during arthritis induction with collagen II. The suppression of the incidence of arthritis was examined in a dose dependent manner when compared to a control group.

Method

30 Sprague-Dawley female rats-VAF+ (Charles Rivers, Wilmington, MA), weighing 100-125 gm, were randomized to 3 groups (10 animals/group). The experiment was repeated three times so that the final groups included 30 rats/treatment regimen with total of 90 rats for this study. Spray dried hyperimmune egg product (as prepared in Example 1) was diluted for oral gavage. 10% and 0.2% solutions of egg product for oral gavage were made every other day. The second day solution was stored at 4°C until use. Egg Product (3.5ml of the respective solutions) was orally gavaged into rats for 7 days prior to initiation of the type II collagen induced arthritis in rats and for 14 days after induction.

The method of inducing and evaluating Arthritis in rats was according to Trentham, et al. 1977. Briefly, Sprague-Dawley rats were immunized on the lower back by intra-dermal inoculation of 400 pepsin-treated native type II chick collagen (CII) (Genzyme, Boston, MA), solubilized at 1 mg/ml in 0.1 M acetic acid and emulsified 1:1 with Incomplete Freund's Adjuvant. Three groups: Water gavage (Control), 50X hyperimmune egg product (High Dose) (3.5ml of a 10% solution of hyperimmune egg product) and 1X hyperimmune egg product (Low Dose) (3.5ml of a

0.2% solution of hyperimmune egg product) with 10 rats per group were used. Starting on day 10 following intradermal collagen II immunization and daily thereafter until Day 21, the rats were clinically evaluated (blinded) for rat paw periarticular erythema (0 – 4+) and paw swelling (0 - 4+) for each limb.

The group arthritis index (AI) is a summation of paw scores based on the degree of incidence of arthritis and severity of arthritis as derived from the mean AI. On Day 21, all rats were sacrificed and bled. Serum were evaluated for antibody titer to type II collagen. Enzyme Linked Immunoassays (ELISA's) were used to measure specific antibody titer to Collagen Type II (Trentham, et al. 1983). The animals were then sacrificed with metafane overdose.

Results

The DCV egg product showed definitive antiarthritic properties in a dose-dependent fashion in the Type II collagen rat model.

Consumption of a high dose of the DCV egg product clearly demonstrated anti-inflammatory properties in this model by influencing both the cellular and humoral arm of the immune system.

The compound appeared to be nontoxic at both the low and high doses. The high dose group showed not only a statistically significant decrease in the incidence of arthritis, but also a delay in onset and a decrease in severity of arthritis as compared to the water control.

Administration of egg [DCV] product resulted in anti-arthritic properties in a dose-dependent fashion. The high dose of egg product produced the most effective reduction in the incidence and severity of arthritis symptoms (Table 4). Scores in Table 4 indicate a reduction in the number of swollen joints and a decrease in the severity of the disease with the high dose of egg product. Moreover, both the low and high dose of egg product caused a delay in the onset of the arthritic condition after collagen II injection (Table 5).

Table 4
Egg Product in Collagen-Induced Animal Model of Arthritis
Group

	Control	Control	High Dose
Expt. 1	80%*	78%	57%^
Expt. 2	80%	100%	63%
Expt. 3	90%	80%	66%

*** Incidence of arthritis**

^ This level of control is historically as good as steroids, NSAIDS, methotrexate/minocycline

Figure 5 shows that the high dose of egg product, given prior to immunization with Type II collagen, prevented the development of arthritic symptoms in more animals than those not given egg. Also, within eleven days of treatment with the egg product and thereafter for the entire duration of the study, a significant decrease in the incidence of arthritis was observed as compared to group that did not receive any egg.

Anti-arthritic effects were observed in the Type II collagen induced arthritis model with S-100 and PL-100 whole eggs. Upon separation of the egg, the yolk showed antiarthritic activity in the animal model but not the egg white. However, delipidating the same egg yolk completely abrogated the antiarthritic activity. This delipidated egg yolk, was further purified by 3000 molecular weight cut off size exclusion column, and tested in the rat Type II collagen model. The partially pure fraction showed high antiarthritic activity which was comparable to that shown by the whole PL-100 egg.

Another important observation made was that the rats fed with PL-100 eggs had a statistically significant reduction in the antibodies to Type II Collagen in their serum than in animals in the

control group. This would indicate that the PL-100 egg had an immunosuppressive effect in the animals and thereby aided in alleviating their arthritic symptoms in the joints.

TABLE 5

Average Day to Maximum Incidence of Arthritis

	Control	Low Dose	High Dose
Expt 1	14	19	20*
Expt 2	14	18	17
Expt 3	12	15	15

* Delay in onset of maximum arthritis after immunization with Collagen II.

At day 21 the arthritic index which reflects the degree and severity of arthritis was significantly reduced in animals which received the high dose of hyperimmune egg product as compared to the control group (2.46 ± 0.55 vs 4.167 ± 0.51 ; $p > 0.003$, Student t-test). The effect of the low dose of egg product did not differ significantly from the controls. In addition, the incidence of arthritis was significantly suppressed in the high-dose group compared to control (30% vs. 70%; $p < 0.03$). This decrease in the percent incidence of arthritis seen with the high dose of hyperimmune egg product (Table 5) is as good as has been reported for steroids, NSAIDS (non-steroidal anti-inflammatory drugs) methotrexate, and minocycline.

This example also shows that the high dose of egg product, given prior to inducing arthritis, prevented the development of arthritis in more animals than those not given egg. These results are demonstrated in Figure 6.

Figure 6 shows the prevention of arthritis by the high dose of egg product. A lower percentage of animals, orally gavaged with egg product, developed arthritis symptoms when compared to control animals, which did not receive egg. Animals were orally gavaged for 21 days, seven days prior to immunization (induction of arthritis) and fourteen days after immunization.

Conclusion

The data clearly shows a definitive anti-arthritic effect in rats for the egg product in a dose-dependent manner in the type II collagen induced rat arthritis model. Statistically significant decreases in the incidence, delay in onset, and a decrease in the severity of arthritis were observed in the animals receiving a high dose of hyperimmune egg product. This data shows the preventative effects of the high dose of the egg product on the incidence of arthritis. Most importantly, at both the high and low dose the hyperimmune egg product is nontoxic.

Example 6

Reduction of Collagen II Antibodies

Rats from Example 5 were bled on Day 21 and serum samples were collected. A standard ELISA assay was used to measure titers against Collagen II autoimmune antibodies. These results show that the animals fed either high or low doses of egg product had significantly lowered antibody titers to collagen II when compared to controls (Table 6).

TABLE 6

	Control	Low Dose	High Dose
Antibody titers to Collagen II (-log ₂)	9.3 + 0.03	8.5 + 0.28	8.3 + 0.36
Statistical Significance	---	p < 0.004	p < 0.005

The decrease in autoimmune antibody titers to Collagen II in the serum of animals gavaged with egg product when compared to control animals (no egg product) is highly significant. Because all groups of animals received the same level of collagen II to induce antibodies to Collagen II and the resulting arthritic symptoms, it was unexpected that the groups receiving egg product would have lower titers of collagen II antibodies on Day 21. This decrease in antibody titers to collagen II indicates a positive influence in both the cellular and humoral immune response for the hyperimmune egg product.

Example7Anti-inflammatory effect of Hyperimmunized Egg

This example shows the anti-inflammatory effect of hyperimmunized egg, (which is prepared as described in Example 1), on carrageenan induced skin edema in dogs on a diet including hyperimmunized egg. These results demonstrate that hyperimmune egg reduces inflammation. The effect on inflammation was comparable to that obtained with the non-steroidal anti-inflammatory drug ibuprofen at 10 mg /kg, ED₅₀, dose in this test.

Twenty White Eagle Beagles (four groups of five dogs each) were fed a basal diet (350 grams) of commercially available lamb and rice dog food. As illustrated in table 7, two groups of five dogs (groups 1 & 2) received only the basal diet during the conditioning period of approximately 100 days. In addition, dogs from group 2 were treated with the non-steroidal anti-inflammatory drug, ibuprofen, prior to inflammatory challenge with carrageenan. During this same period, the remaining two groups (3 & 4) were fed the basal diet along with hyperimmune egg (HIE). Group 3 received 3.5 grams of hyperimmune egg, while group 4 received 35 grams of hyperimmune egg added to the diet. At the end of the conditioning diet treatment, the dogs were challenged with an intra-dermal injection of 2% carrageenan and an inflammatory response was elicited.

The challenge process was as follows. One dog from each group was randomly selected for challenge and testing each day. This procedure was repeated daily for five days until all dogs from all groups were tested. Fifteen minutes prior to the challenge procedure, dogs from group 2 were given 10mg/kg ibuprofen orally. All dogs were anesthetized by intravenous injection and shaved on the left lateral side (approximately 6 X 8 inches). Two lines of 3 marks were made on the shaved area and numbered 1 - 6. An intra-dermal injection of 0.1 ml saline was given at mark 1 as a negative control. From mark 2 - 6, a 0.1 ml intra-dermal injection of a 2% solution of carrageenan was given. injections were administered by the same person for the duration of the study. Intra-dermal injections of this concentration of carrageenan was previously determined to cause a measurable swelling in 100% of the animals which could be reduced by 10 mg/kg ibuprofen. At the completion of the last injection of carrageenan, measurements were made of each swelling using a micrometer and the numbers were recorded. The anesthetized animals were

allowed to recover and six hours later the measurements were repeated and recorded. The mean size of the inflammation response was determined for each group.

TABLE 7

Groups	Treatment	Number of Animals Per Group	Mean Inflammatory Response	Mean Difference from Control	Significance P value
1 Control	Basal Diet	5	4.41	-	-
2 Drug	Ibuprofen (10mg/kg) + Basal Diet	5	2.25	2.2	P<0.01
3 Test	HIE egg 3.5 grams + Basal Diet	5	2.8	1.6	P<0.05
4 Test	HIE egg 35 grams + Basal Diet	5	2.38	2.0	P<0.05

These results showed a significant reduction in swelling in the animals fed with hyperimmune egg (3.5 grams plus basal diet and 35 grams plus a basal diet) indicating a preventative effect of the hyperimmune egg on inflammation when fed orally. Moreover these results show that the anti-inflammatory activity of the hyperimmune egg was comparable to the effect seen with the anti-inflammatory- drug ibuprofen. Differences at the $p<0.05$ level of significance were seen between the treated and control group.

Example 8

Effect of Egg Product on Arthritic Human Patients

A nutritional drink supplement was obtained which comprised a high protein, high carbohydrate powder containing 30% of recommended daily allowance of vitamins, and approximately 4.5 grams of hyperimmune powdered egg (approximately equal to 0.4 eggs). The drink supplement was provided by DCV, Inc., Wilmington, DE.

Three patients were administered the nutritional drink supplement for a period of two months. Clinical evaluations regarding tolerance to the product and clinical response, as evaluated by each patient's estimation of clinical status and a detailed clinical examination was performed prior to the study at one month, two months and three months (one month after ceasing the product). Clinical chemistry, hematology and urinalysis profiles were performed. Described below are the results of the treatments of each of these patients:

Example 8A

Patient #1

Patient #1 was a 29-year-old female, 5'3" 170 lbs with a previous history of rheumatoid arthritis (juvenile). On first examination the patient presented with joint pains, swelling, and tenderness of fingers, wrists, toes, feet, and knees. She complained of functional limitations such as inability to do knee bends or run, and had difficulty with kneeling. Her hematology tests had normal erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) readings. Patient's cholesterol was at 199mg/dl. She was being treated with Minocin 300mg/week, Loestrin Fe 1.5/30 and used multivitamins. The diagnosis was rheumatoid arthritis with uveitis.

After one month of consuming the Nutritional Drink Supplement on a daily basis, the patient's hand redness and swelling decreased, and the pain and swelling in her feet disappeared. Patient reported that she felt "less achy" and "felt 75% better". She reported that she had increased energy. Her ESR and CRP readings remained normal but her cholesterol dropped to 186 mg/dl. Patient continued her medications in conjunction with the egg-based drink.

During the second month, the patient felt that her hands were worse and she presented with swelling of fingers and wrists and reported numbness in hands at night. Her legs felt "better". Physician's global assessment was that patient was slightly worse. She still experienced pain when carrying shopping bags or upon opening boxes. Her ESR and CRP readings remained normal and her cholesterol remained at 189mg/dl. She continued medications and as scheduled, by the protocol, discontinued use of product.

After one month without the product, swelling and tenderness returned to hands and feet. Her function limitations remained as she first presented. Patient still felt 75% “better”. Her ESR and CRP readings remained normal as her cholesterol dropped to 180mg/dl.

Scoring of the arthritic symptoms was determined from the patient’s own assessment of the symptoms. In particular, the patient was asked to indicate the amount of swelling, pain and/or tenderness of the joints. Based upon this patient assessment, numbers were generated showing the degree of the symptoms. The numbers range from a high of 36 (greatest amount of swelling, pain and tenderness) to a low of 0 (no swelling, pain or tenderness).

Scores generated for Patient #1’s arthritic condition were as follows:

At presentation	14
One month on egg-based drink =	2
Two months on egg-based drink =	6
One month after discontinuing drink=	8

Example 8B

Patient #2

Patient #2 was an 88-year-old female 5’3” 104 lbs. with a previous history of rheumatoid arthritis and chronic sinusitis. She presented with pain in the lower extremities and shoulders. Physical examinations revealed swelling, pain, and tenderness of her fingers and ankles. Functionally she was only able to walk in a limited manner and had difficulty in bending her arms. Her hematology tests indicated elevated ESR (36 mm/hr), CRP (1.64mg/dl) and a total cholesterol at 220 mg/dl. She was being treated with: Methotrexate 5mg/week, Orudis KT 2/day, Ca 1500mg, Fosomax 10mg, Prednisone 5mg q2d (every other day), and took multivitamins. Again, the patient continued to take these medications in addition to the egg-based drink.

One month after starting the egg-based drink, she still complained of swelling of hands, knee pain, and inability to sleep. The patient reported that she was “50% better”, “feeling generally better” and “walking better.” She was still in pain, although her pain attacks were less frequent. Hematology results were ESR (30mm/hr) and CRP (1.64mg/dl), and an increase in cholesterol to 232 mg/dl was observed.

After two months of consumption of product, patient reported that “she felt 98% better”. Her functional limitations however, remained the same. Hematology results indicated a drop in her ESR (21mm/hr) and a return to normal in the CRP reading.(<0.5 mg/dl). Her total cholesterol remained at 234 mg/dl. Patient continued her medications but, as scheduled, discontinued the egg-based drink.

One month after discontinuing product patient complained of severe joint pains in her fingers and legs and exhibited swelling of wrists. She felt she was “50% worse”. Her ESR (27mm/hr) levels and cholesterol (257 mg/dl) started to climb, but her CRP (<0.5 mg/dl) levels remained normal.

Scoring of the arthritic symptoms was determined from the patient’s own assessment of the symptoms. In particular, the patient was asked to indicate the amount of swelling, pain and/or tenderness of the joints. Based upon this patient assessment, numbers were generated showing the degree of the symptoms. The numbers range from a high of 36 (greatest amount of swelling, pain and tenderness) to a low of 0 (no swelling, pain or tenderness).

Scores generated for Patient #2’s arthritic condition were as follows:

At presentation=	10
One month on egg-based drink =	7
Two months on egg-based drink =	2
One month after discontinuing drink=	10

Example 8CPatient #3

Patient #3 was a 79-year-old male 5'10" 152 lbs with a history of osteoarthritis and atherosclerotic cardiovascular disease. Symptoms were pain in the lower back, shoulders, knees and feet. Medications included: Nitroderm patch, Digoxin 0.125, aspirin 80mg and Feldene 20mg. Function limitations were: difficulty in moving arms and exhaustion. Hematology results were normal ESR and CRP and cholesterol readings at 217 mg/dl.

After one month of drinking the egg-product, along with the above-mentioned medication, the patient felt "slightly better (10-20%)". He still reported shoulder pain on right and left side, and reported that he was unstable on feet and experienced buckling of his right knee. Function limitations remained as previously reported. His ESR and CRP readings were normal and his cholesterol level was 180 mg/dl.

After two months of consuming product patient continued to feel "slightly better". Symptoms included right knee pain and difficulty in walking. Cholesterol readings were not obtained but ESR and CRP remained at normal levels.

After one month without product, patient felt slightly better overall but slightly worse than the previous month. Symptoms included buckling of right leg, shoulder pain and lower back pain. Joint assessment indicated pain in shoulders, and lower back. Functional limitations were difficulty in moving arms and feeling "exhausted". ESR and CRP readings were normal and cholesterol remained at 180 mg/dl.

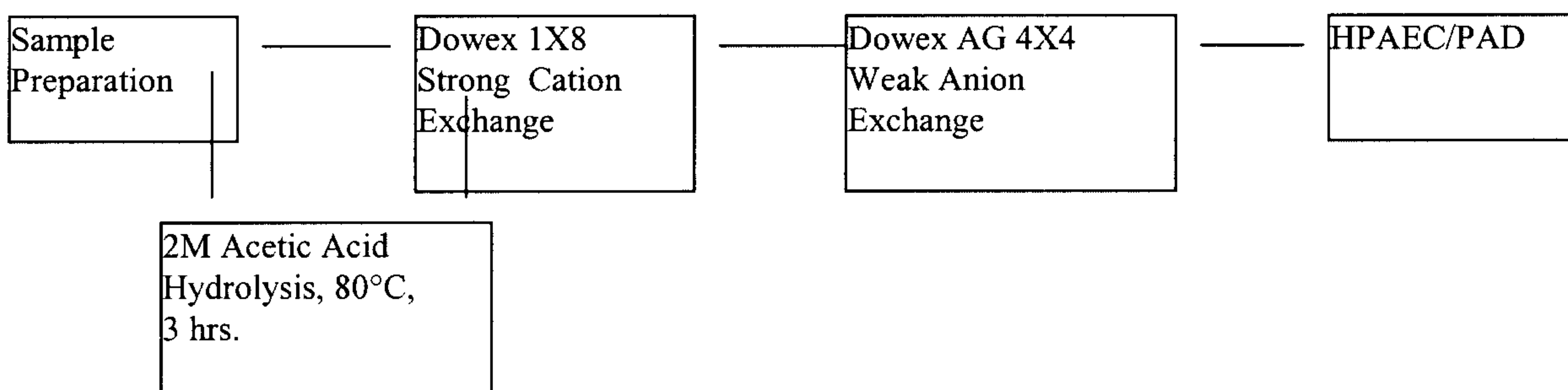
Scoring of the arthritic symptoms was determined from the patient's own assessment of the symptoms. In particular, the patient was asked to indicate the amount of swelling, pain and/or tenderness of the joints. Based upon this patient assessment, numbers were generated showing the degree of the symptoms. The numbers range from a high of 36 (greatest amount of swelling, pain and tenderness) to a low of 0 (no swelling, pain or tenderness).

Scores generated for Patient #3's arthritic condition were as follows:

At presentation=	11.5
One month on egg-based drink =	2
Two months on egg-based drink =	3
One month after discontinuing drink =	8.5

Other case histories showed similar improvements and demonstrated the effectiveness of the present invention in eliminating pain and inflammation in arthritic joints without side effects.

The invention has been described with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention as described above and as defined in the appended claims.

Example 9Sialic Acid AnalysisSialic Acid Release, Purification, And Analysis Flow ChartRelease And Purification Of Sialic Acids From Glycoproteins

Sialic acid release with relatively low O-acetyl loss was achieved with 1ml of 2M acetic acid at 80°C for 3 hours in a sealed, crimp-top vial. KDN is added as a pre-hydrolysis internal standard.

Following acid hydrolysis, the reaction mixture was cooled and the vial centrifuged to remove any insoluble material. Transfer the supernatant to a micron (0.5 ml), centricon (2 ml), or centriplus (15 ml) 10,000 molecular weight cut-off centrifugation device. The acetic acid in the filtrate was removed by vacuum centrifugation and the sample was redissolved in 0.5 ml of water.

The sample was applied to a 1 ml column of Dowex® 50 AG 1X8 (hydrogen form) in water to remove cationic contaminants. The flow through and 4-5 ml of water washings were collected in a thin walled centrifuge tube containing 40 µl of 1 M formic acid. The acidified washings were taken to dryness by vacuum centrifugation to remove the weak acids generated by the column.

The sample was redissolved in 0.5 ml of 10 mM sodium formate, pH 5.50 and applied to 1 ml column of Dowex® AG 3X4 (formate form) weak anion exchange resin that was buffered in 10mM sodium formate pH 5.50. The column was immediately washed with 5ml of 10mM formic acid. The flow through and washings were discarded. The sialic acids were eluted into a

thin walled centrifuge tube with 10ml of 1 M formic acid and the eluate was taken to dryness and then resuspend in water.

Note: All steps in this procedure can be performed at room temperature if preserving total O-acetylation is the only concern. If prevention of O-acetyl migration is necessary than all steps should be performed at 4°C with 1 M pyridine acetate buffer, pH 5.5 in place of 1 M formic acid.

Column Instructions

Dowex 50 AG 1X8, hydrogen form: 1 ml of resin was spooned into a small, disposable, plastic-fritted column. Column was primed with 5ml of 1 M HCl followed by washing with 5 ml aliquots of water until the pH rose above -4.

Dowex AG 3X4 (or 4X4), formate form: 1 ml of resin was spooned into a small, disposable, plastic-fritted column, and converted to hydroxide form by washing column with 5 ml of 1 M NaOH. Hydroxide form was then converted to formate form by washing column with 3-5 ml of 1 M formic acid or until pH of the flow through was -1.5. The column was washed with double DI water. The column was then buffered with 10mM sodium formate, pH 5.5.

Analysis Of Sialic Acids By Dionex HPAEC/PAD On The Carbopac PA-10 Column

Sialic acid samples were analyzed by HPAEC/PAD using the Dionex GP40 gradient pump, ED40 electrochemical detector and CarboPac PA-10 guard and analytical columns. Sample injection was made by an AS3500 autosampler. Sialic acids were eluted using 100mM NaOH (eluent C) and 100mM NaOH, 1M NaAc (eluent D) by gradient method. The separation gradient for this method was:

Time (min)	A%	B%	C%	D%	INJECT	TITL1
Initial	-	-	93.0	7.0		
0	-	-	93.0	7.0	X	X
10	-	-	70.0	30.0		
11	-	-	70.0	30.0		
12	-	-	93.0	7.0		
27	-	-	93.0	7.0		

This method required 27 minutes at a flow rate of 1ml/min. Sialic acids were quantitated using KDN as the internal standard. Sample peak areas were normalized to adjust for KDN signal loss due to hydrolysis or ion exchange cleanup.

Conclusion

Table 8

Sialic Acid Analysis Of PL-100 Egg and its Components

Egg Fractions	Free + Bound Sialic acid content per gm of Egg	% Sialic Acid
Table Egg Yolk	1.9 mg	.19%
Table Egg White	1 mg	.10%
3k fraction of PL-100 Egg Yolk	7.7 mg	.77%
3k fraction of PL-100 Egg White	0.75 mg	.075%

The above table shows the free and bound sialic acid content in various fractions of egg as analyzed by the Standard Dionex protocol and PAD detection. This procedure performed along with preliminary clean up over Dowex resins, results in quantitative recovery and eliminates other contaminating peaks from that position of the chromatogram.

Isolation of the 3k fraction of the egg yolk does not enrich the bound and free sialic acid content appreciably.

The biological activity of the 3k fraction of the egg yolk in the rat Type II Collagen assay cannot be attributed to the sialic acid content as it is very low (2.7mg/dose).

WHAT IS CLAIMED IS:

1. An anti-inflammatory composition, in highly purified form, wherein said composition is characterized by:
 - (1) being present in egg white and egg yolk of an egg produced by an egg-producing animal;
 - (2) being heat stable at 100°C for at least 30 minutes;
 - (3) having a negative ionic charge at neutral pH;
 - (4) being acid and base resistant in conditions of pH 4.0 - pH 9.0;
 - (5) being orally active; and
 - (6) having a molecular weight of less than 3,000 daltons.
2. The use of an effective amount of the anti-inflammatory composition of claim 1 for preventing, countering, or reducing inflammation in a subject, wherein the composition is in a form suitable for administration to the subject.
3. The use of claim 2 wherein the effective amount of the anti-inflammatory composition ranges from 5 micrograms to 300 milligrams.
4. The use of the composition of claim 1 to inhibit leukocyte migration in a subject, wherein the composition is a form suitable for administration to the subject at a dose sufficient to inhibit said leukocyte migration.
5. A method for highly purifying an anti-inflammatory composition from an egg of an egg-producing animal comprising:
 - (1) isolating a water soluble fraction from the whole egg, egg yolk or egg white;
 - (2) separating a less than 3,000 dalton permeate from the water soluble fraction;
 - (3) fractionating said less than 3,000 dalton permeate to recover a

biologically active fraction.

6. The method of claim 5 wherein said fractionating step further comprises reverse phase high pressure liquid chromatography of said less than 3,000 dalton permeate.
7. The method of claim 5 further comprising de-fatting the whole egg or the egg yolk prior to step (1).
8. The method of claim 5 further comprising lyophilizing said less than 3,000 dalton permeate of step (2).
9. The method of claim 5 wherein the separating step further comprises ultrafiltering the water soluble fraction through a filter with a molecular weight cut-off of 3,000 daltons.
10. The method of claim 5 wherein the egg-producing animal is maintained in a hyperimmunized state.
11. The method of claim 10 wherein the hyperimmunized state is induced by an antigenic, genetic, or bioengineered vaccine.
12. The method of claim 11 wherein the genetic vaccine comprises at least one antigen coding DNA construct selected from the group consisting essentially of fragments of naked DNA, plasmid DNA, viral DNA, DNA expression libraries, DNA-RNA antigens, DNA-protein conjugates and DNA liposome conjugates, and combinations thereof.
13. The method of claim 11 wherein the antigenic vaccine comprises at least one antigen selected from the group consisting of bacterial, viral, protozoan, fungal and cellular antigens.
14. An anti-inflammatory composition, in highly purified form, produced by a process

comprising:

- (1) isolating a water soluble fraction from the whole egg, egg yolk or egg white;
- (2) separating a less than 3,000 dalton permeate from the water soluble fraction;
- (3) fractionating said less than 3,000 dalton permeate to recover a biologically active fraction.

15. The anti-inflammatory composition of claim 14 wherein the egg-producing animal is in a hyperimmunized state.

16. The anti-inflammatory composition of claim 14 wherein the hyperimmunized state is induced by an antigenic, genetic, or bioengineered vaccine.

17. The anti-inflammatory composition of claim 16 wherein the genetic vaccine comprises at least one antigen coding DNA construct selected from the group consisting essentially of fragments of naked DNA, plasmid DNA, viral DNA, DNA expression libraries, DNA-RNA antigens, DNA-protein conjugates and DNA liposome conjugates, and combinations thereof.

18. The anti-inflammatory composition of claim 16 wherein the antigenic vaccine comprises at least one antigen selected from the group consisting of bacterial, viral, protozoan, fungal and cellular antigens and combinations thereof.

19. The use of an effective amount of the anti-inflammatory composition of claim 14 to prevent, counter, or reduce inflammation in a subject, wherein the composition is in a form suitable for administration to the subject.

20. The use of claim 19 wherein the effective amount of the anti-inflammatory composition ranges from 5 micrograms to 300 milligrams.

21. The use of the egg anti-inflammatory composition of claim 14 to inhibit leukocyte

migration in a subject, wherein the composition is in a form suitable for administration to the subject in an amount sufficient to inhibit the leukocyte migration..

22. An anti-inflammatory egg or egg fraction prepared by hyperimmunizing an egg-producing animal.

23. The anti-inflammatory egg or egg fraction of claim 22 wherein the egg-producing animal is hyperimmunized with an antigenic, genetic or bioengineered vaccine.

24. The anti-inflammatory egg or egg fraction of claim 23 wherein the genetic vaccine comprises at least one antigen coding DNA construct selected from the group consisting essentially of fragments of naked DNA, plasmid DNA, viral DNA, bacterial DNA, DNA expression libraries, DNA-RNA antigens, DNA protein conjugates and DNA liposome conjugates, and combinations thereof.

25. The anti-inflammatory egg or egg fraction of claim 23 wherein the antigenic vaccine comprises at least one antigen selected from the group consisting of bacterial, viral protozoan, fungal, and cellular antigens.

26. The anti-inflammatory egg or egg fraction of claim 22 comprising at least one anti-inflammatory composition, said anti-inflammatory composition being present at supranormal levels, said levels being effective for treating inflammation in a subject.

27. The anti-inflammatory egg or egg fraction of claim 26 wherein the anti-inflammatory composition is characterized by:

- (1) being present in egg white and egg yolk of said anti-inflammatory egg;
- (2) being heat stable at 100°C for at least 30 minutes;
- (3) having a negative ionic charge at neutral pH;
- (4) being acid and base resistant in conditions of pH 4.0 - pH 9.0;
- (5) being orally active; and

(6) having a molecular weight of less than 3,000 daltons.

28. The anti-inflammatory egg or egg fraction of claim 26 wherein the anti-inflammatory composition is highly purified from the anti-inflammatory egg or egg-product by a process comprising:

- (1) isolating a water soluble fraction from the whole egg, egg yolk or egg white;
- (2) separating a less than 3,000 dalton permeate from the water soluble fraction;
- (3) fractionating said less than 3,000 dalton permeate to recover a biologically active fraction.

29. The use of the anti-inflammatory egg or egg product of claim 22 to prevent, counter, or reduce inflammation in a subject having a weight, wherein the egg or egg product is in a form suitable for administration to the subject.

30. The use of claim 29, wherein the effective amount of the anti-inflammatory egg or egg product ranges from 1 to 40 grams per kilograms of subject weight.

31. The use of an effective amount of the anti-inflammatory egg or egg product of claim 22 to inhibit leukocyte migration in a subject, wherein the egg or egg product is in a form suitable for administration to the subject.

32. An anti-inflammatory composition, in substantially purified form, produced by a process comprising:

- (1) isolating a water-soluble fraction from eggs produced by an egg producing animal and;
- (2) separating a less than 30,000 dalton permeate from the water soluble fraction to recover a biologically active permeate.

33. The anti-inflammatory composition of claim 32 further comprising subjecting the less than 30,000 dalton filtrate to ion exchange chromatography after step (4).

34. The anti-inflammatory composition of claim 33 wherein the separating step further comprises ultrafiltering the water soluble fraction through a filter with a molecular weight cut-off of 30,000 daltons.

35. The anti-inflammatory composition of claim 32 further comprising removing endotoxins from said less than 30,000 dalton permeate.

36. The anti-inflammatory composition of claim 35 wherein the egg-producing animal is maintained in a hyperimmunized state.

37. The anti-inflammatory composition of claim 36 wherein the egg-producing animal is hyperimmunized with an antigenic, genetic, or bioengineered vaccine.

38. The anti-inflammatory composition of claim 37 wherein the antigenic vaccine comprises at least one antigen selected from the group consisting of bacterial, viral, protozoan, final, and cellular antigens.

39. The anti-inflammatory composition of claim 37 wherein the genetic vaccine comprises at least one antigen coding DNA construct selected from the group consisting of fragments of naked DNA, plasmid DNA, viral DNA, bacterial DNA, DNA expression libraries, DNA-RNA antigens, DNA-protein conjugates and DNA liposome conjugates, and combinations thereof.

40. The anti-inflammatory composition of claim 32 further comprising defatting the eggs prior to step (1).

FIG. 1

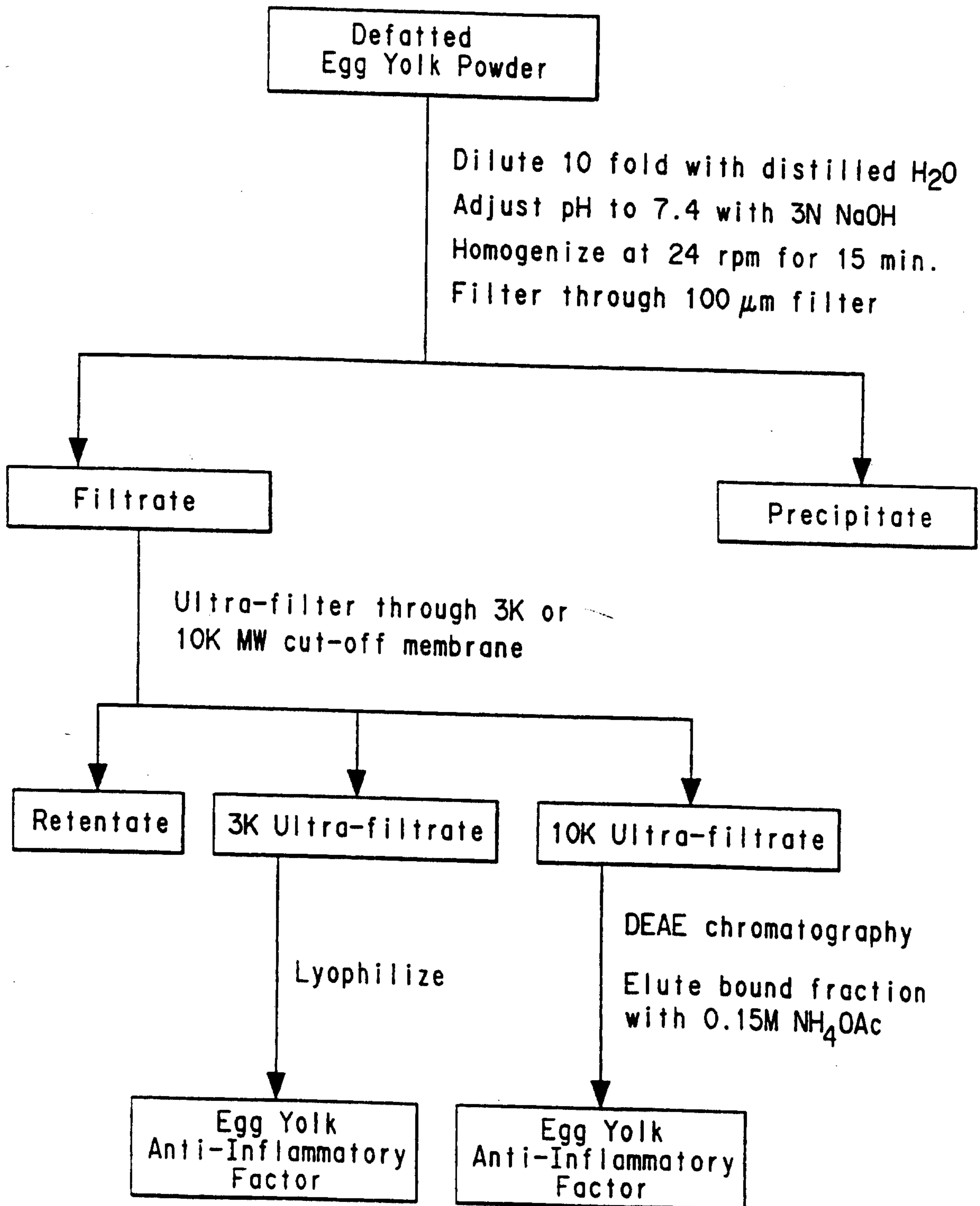


FIG. 2

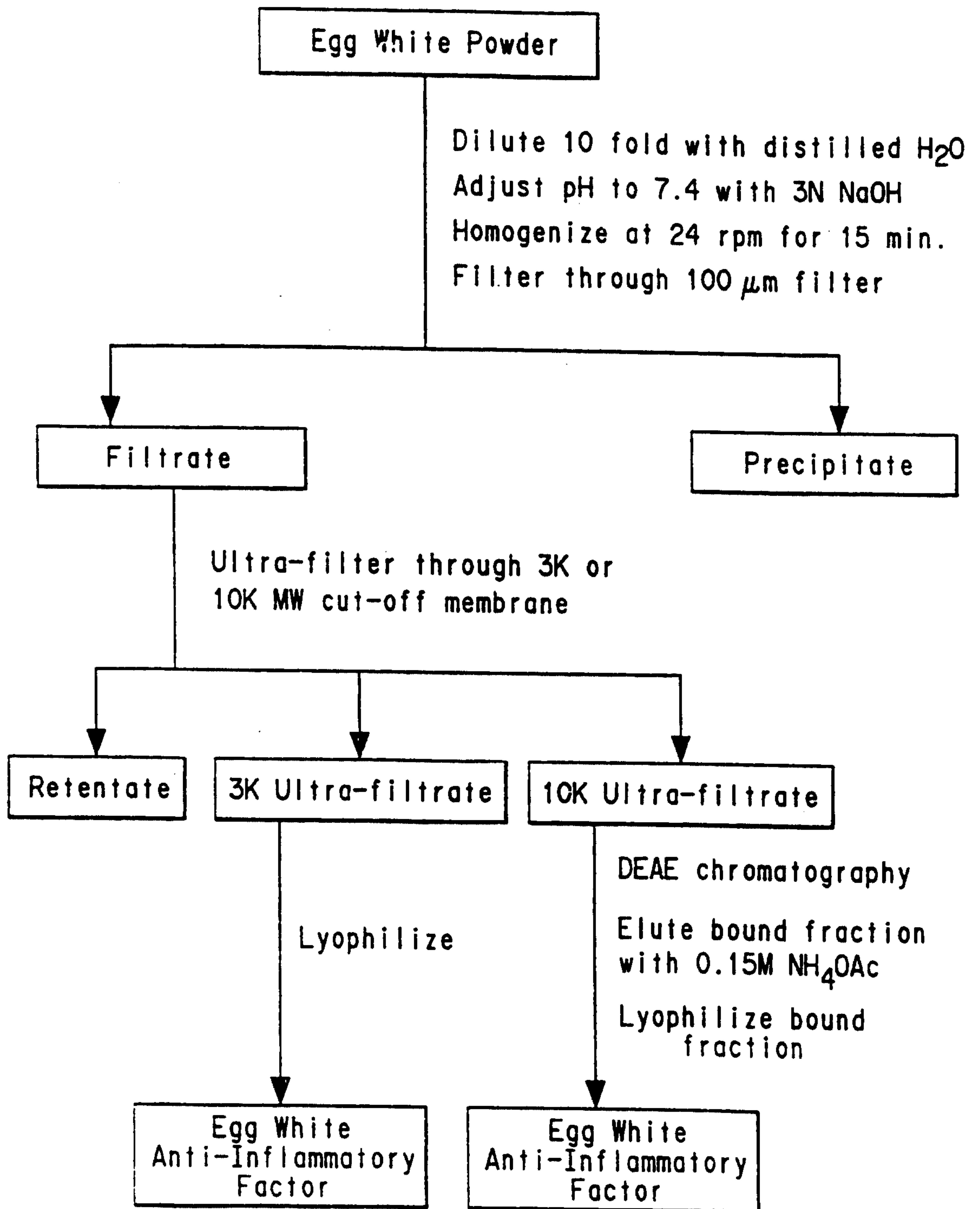
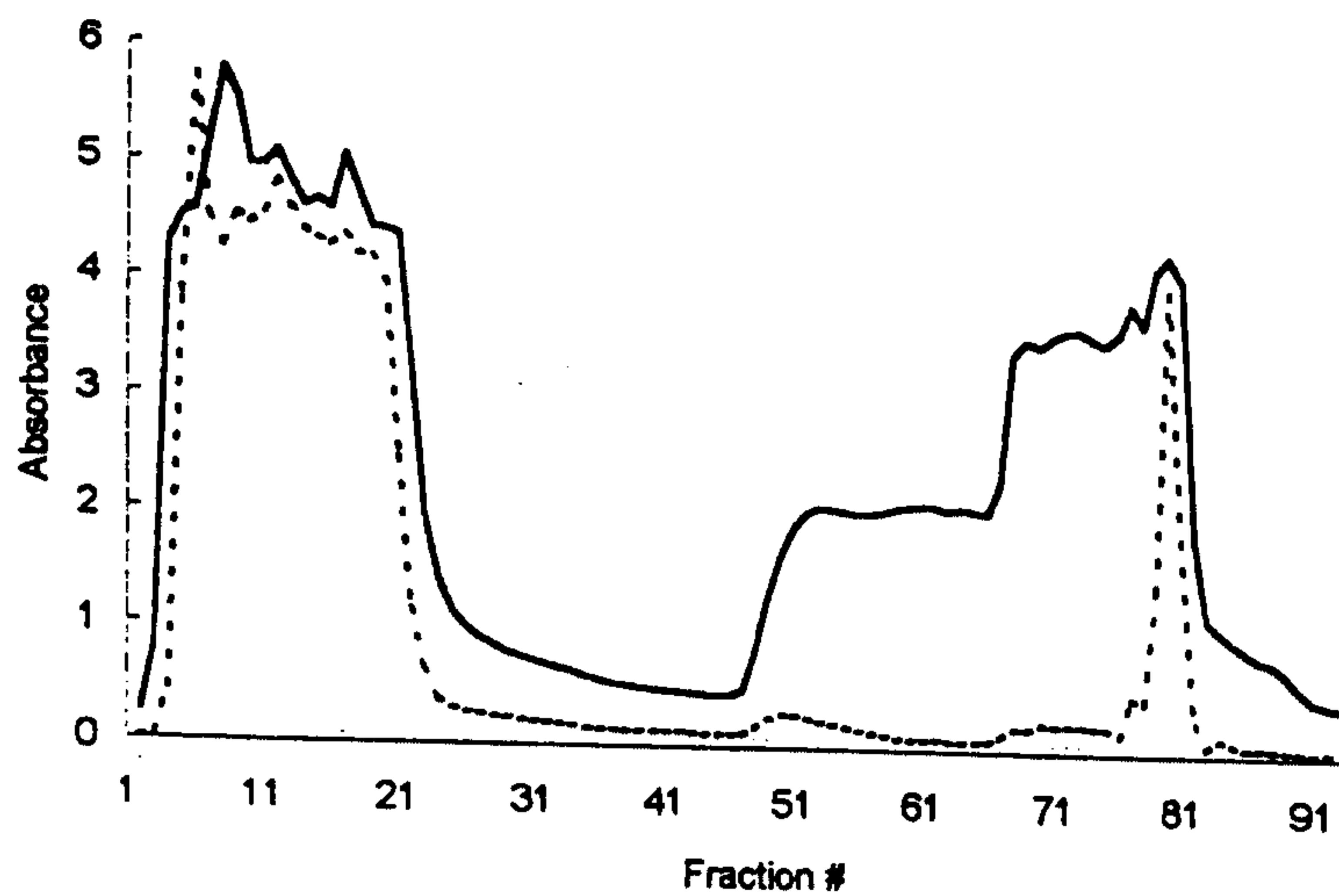
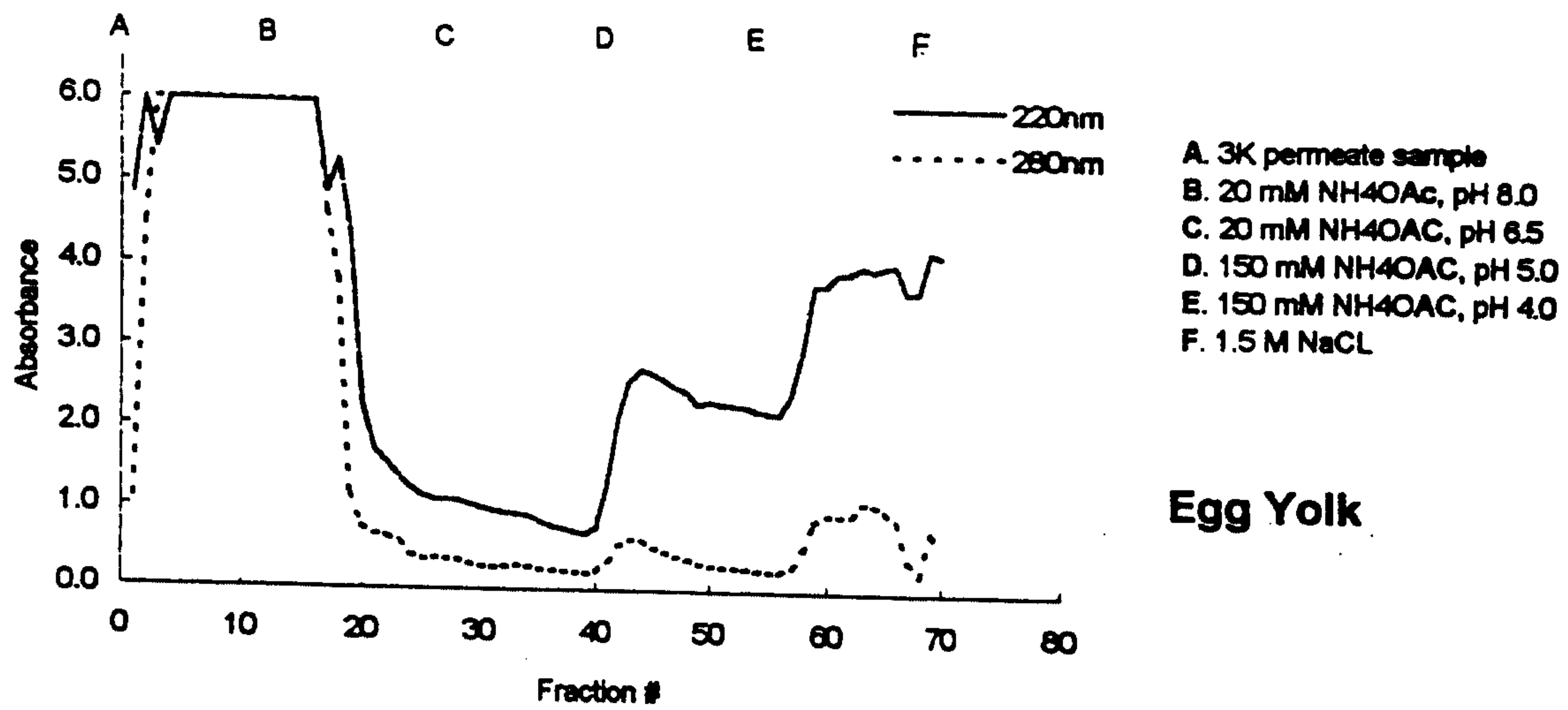


FIGURE 3



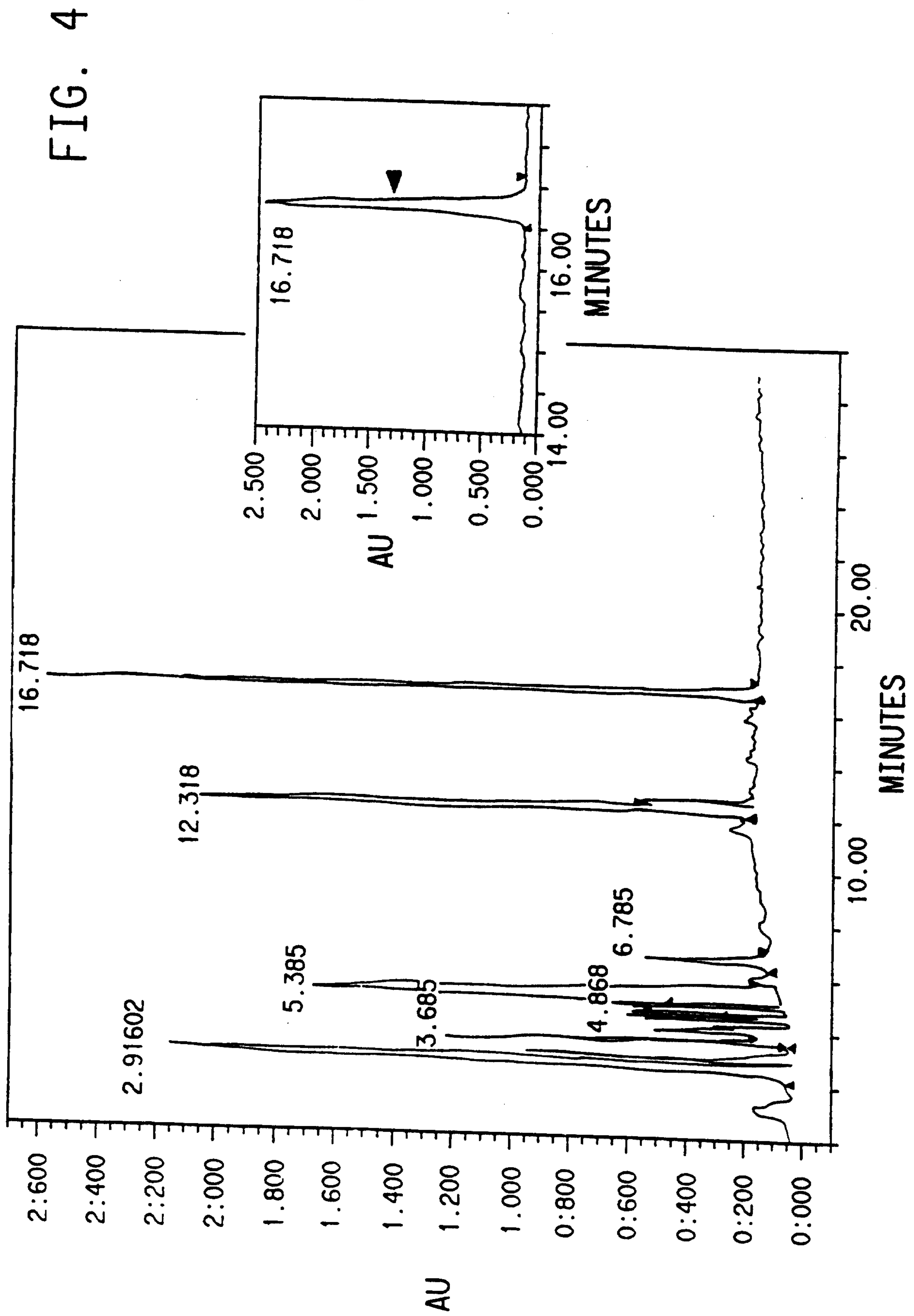


FIGURE 5

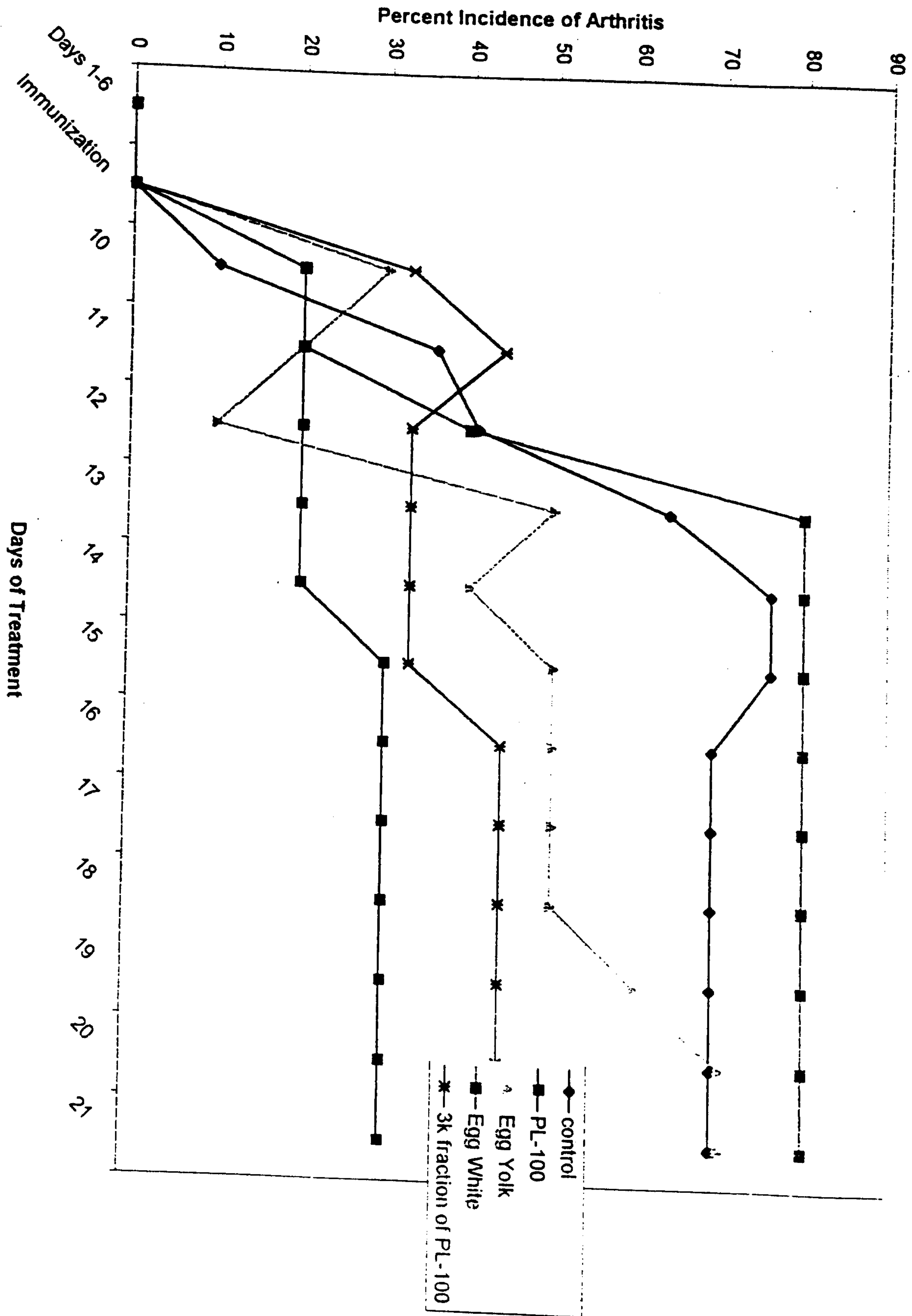


FIGURE 6

