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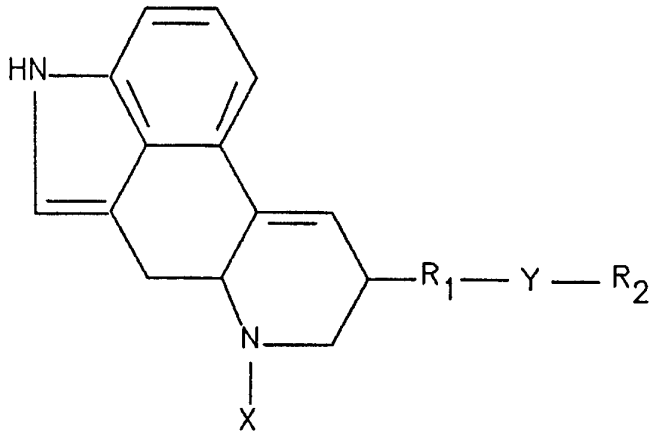
(54) Title: VACCINES AND METHODS FOR PREVENTING AND TREATING FESCUE TOXICOSIS IN HERBIVORES

(57) Abstract

The present invention provides an immunogenic compound comprising formula (I), wherein X is selected from the group consisting of methyl and hydrogen; wherein R_1 is a suitable functional group of the lysergic ring; wherein R_2 is an immunogenic protein; and wherein Y is a bridge to link R_1 to R_2. The present invention also provides purified monoclonal antibodies specifically reactive with the immunogenic compound and reactive with the lysergic ring of ergopeptine and clavine alkaloids. The present invention further provides an antibody which is an anti-idiotype of the monoclonal antibody. Also provided are methods of prevention and treatment of fescue toxicosis utilizing the immunogenic compounds and antibodies of the present invention.



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VACCINES AND METHODS FOR PREVENTING AND TREATING FESCUE TOXICOSIS IN HERBIVORES

FIELD OF THE INVENTION

This invention relates to vaccines useful to prevent and treat fescue toxicosis in herbivores. More particularly, this invention provides immunogenic compounds, a hybridoma cell line which produces monoclonal antibodies and an anti-idiotype vaccine to prevent and treat fescue toxicosis.

BACKGROUND ART

Fescue toxicosis is a condition of livestock associated with grazing tall fescue (*Festuca arundinacea* Schreb) infected with its fungal endophyte (*Acremonium coenophialum* Morgan-Jones and Gams). Inasmuch as tall fescue is widely adapted and utilized (*Burns* and *Chamblee*, 1979) and incidence of endophyte infection within pastures is high (*Hill* and *Stringer*, 1985; *Shelby* and *Dalrymple*, 1987), fescue toxicosis is a widespread problem and represents a major economic loss to the livestock industry in the United States.

Tall fescue plantings occupy over 35 million acres in the United States and Canada wherein over 90% of this acreage is infected with *Acremonium coenophialum* (*Bacon et al.*, 1988). Extreme expense and environmental concerns make it impractical, if not impossible to approach the problem of fescue toxicosis by replanting affected acreage with endophyte-free cultivars. Moreover, the endophyte and plant appear to exist in a symbiotic relationship, suggesting potential problems with the long term survival of endophyte-free cultivars.

Signs of fescue toxicosis include lethargy, reduced weight gain, increased respiration, gangrenous lesions on the extremities, and roughened hair coats. Physiologically, affected livestock have reduced serum cholesterol and alkaline phosphatase (*Bond et al.*, 1984; *Stuedemann et al.*, 1985; *Lipham et al.*,

1989), decreased visceral and peripheral blood flow (*Garner et al.,* 1978), increased D2 receptor affinity in the corpus striatum (*Mizinga*, 1991), and reduced prolactin secretion (*Lipham et al.,* 1989). Therefore the pathophysiology of fescue toxicosis includes effects on basal metabolism, cardiovascular, central nervous, and endocrine systems.

The toxic compounds of *Acremonium coenophialum* infected fescue have yet to be conclusively identified (*Putnam et al.*, 1991). Four classes of alkaloids produced by the endophyte/tall fescue association (loline, peramine, clavine, and ergopeptine) are potentially toxic to grazing livestock. The endophyte, a clavicepitaceous organism, produces ergopeptine alkaloids. Ergopeptine alkaloids are lysergic acid derivatives including agroclavine, elymoclavine, ergovaline, ergosine, and ergocornine (*Siegel et al.,* 1991). In addition, plant derived loline alkaloids are produced in response to the fungal endophyte which are presumed to have a regulatory role in limiting endophyte habitat within the plant. These loline alkaloids are also suspected of being involved with the toxicosis syndrome (*Bush et al.,* 1979). The loline alkaloids include the pyrrolizidine bases N-formyl loline, N-acetyl loline, and loline alkaloids (pyrrolizidine alkaloids).

Many attempts have been made to identify the alkaloid components of endophyte infected tall fescue which produce fescue toxicosis without a definitive answer. Although the unsaturated pyrrolizidine alkaloids have hepatotoxic activity (*Mattocks*, 1971), intraruminal infusion of saturated pyrrolizidine alkaloids found in tall fescue produced no symptoms of fescue toxicosis (*Yates,* 1973). However, in combination with acetylcholine, saturated pyrollizidine alkaloids are endowed with smooth muscle contracting capabilities which could result in gangrenous conditions (*Bruce et al.,* 1971).

University of Kentucky researchers reported pyrrolizidine alkaloids as the major toxin associated with fescue toxicosis and demonstrated that

thiamine treatment partially alleviated symptoms associated with the disease (*Doughtery et al.*, 1991) (See also U.S. Patent No. 4,755,519).

However, another group has previously shown that dorsal pedal veins contracted *in vitro* when exposed to various concentrations of purified lysergic acid derivatives but a mixture of pyrrolizidine alkaloids failed to produce contractile responses (*Solomons et al.*, 1989).

Circumstantial evidence exists that the ergopeptine alkaloids are responsible for fescue toxicosis (*Testereci et al.*, 1991). It has been shown that serum prolactin is frequently decreased in cattle grazing endophyte-infected tall fescue (*Thompson et al.*, 1987) and ergot alkaloids are potent inhibitors of prolactin secretion (*Goldstein et al.*, 1980). In addition, administration of metoclopramide, a type D2 receptor antagonist, increased serum prolactin and weight gains, and changed hair coats from classical fescue toxicosis appearances of long, rough and bronzed to black and shiny in Angus steers (*Lipham et al.*, 1989). Metoclopramide, however, has minor cross reactivity with other receptors which does not exclude effects other than type D2 activity. Likewise, *in vitro* research evaluating the vasoconstrictive effects of alkaloids assumes that ingestion of tall fescue results in their presence in serum (*Solomons et al.*, 1989).

Therefore, as can be appreciated by a review of the literature, the cause of fescue toxicosis remains unresolved. In addition, despite great economic losses, no economically feasible method to prevent or treat fescue toxicosis exists.

The present invention satisfies this long felt need to protect livestock from fescue toxicosis by providing antibodies and compounds for immunization against fescue toxicosis as well as a method of therapy for affected animals.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1a depicts percentage changes in serum prolactin from three basal values collected at 30 min intervals prior to time 0 following monoclonal antibody (treated) or bovine serum albumin (control) with bolus injection at time 0 followed by infusion of treatments into steers grazing endophyte-infected fescue.

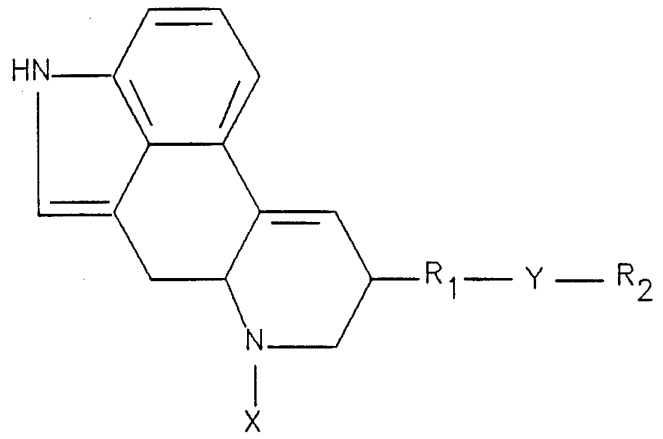
Fig. 1b depicts absolute serum prolactin changes from three basal values collected at 30 min intervals prior to time 0 following monoclonal antibody (treated) or bovine serum albumin (control) with bolus injection at time 0 followed by infusion of treatments into steers grazing endophyte-infected fescue.

Fig. 2 depicts an antibody response of Angus heifers immunized on three separate days with lysergic acid derivatives conjugated to human serum albumin.

Fig. 3 depicts antibody response over time in Angus steers immunized with varying amounts of lysergol-glutarate-human serum albumin conjugate.

SUMMARY OF THE INVENTION

The present invention provides an immunogenic compound comprising the formula:



wherein *X* is selected from the group consisting of methyl and hydrogen;
wherein R_1 is a suitable functional group of the lysergic ring;
wherein R_2 is an immunogenic protein; and
wherein *Y* is a bridge to link R_1 to R_2.

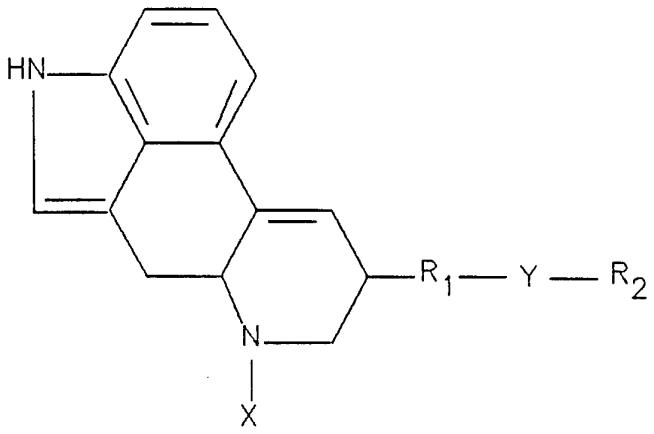
The present invention also provides purified monoclonal antibodies specifically reactive with the immunogenic compound and reactive with the lysergic ring of ergopeptine and clavine alkaloids. The present invention further provides an antibody which is an anti-idiotype of the monoclonal antibody. Also provided are methods of prevention and treatment of fescue toxicosis utilizing the immunogenic compounds and antibodies of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of specific embodiments and the Examples and Figures included therein.

As used in the claims, "a" means one or more.

The present invention provides an immunogenic compound comprising the formula:

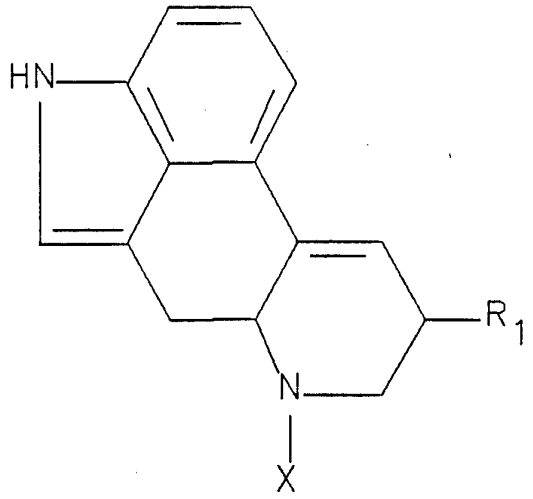


wherein *X* is selected from the group consisting of methyl and hydrogen;
wherein R_1 is a suitable functional group of the lysergic ring;
wherein R_2 is an immunogenic protein; and
wherein *Y* is a bridge to link R_1 to R_2.

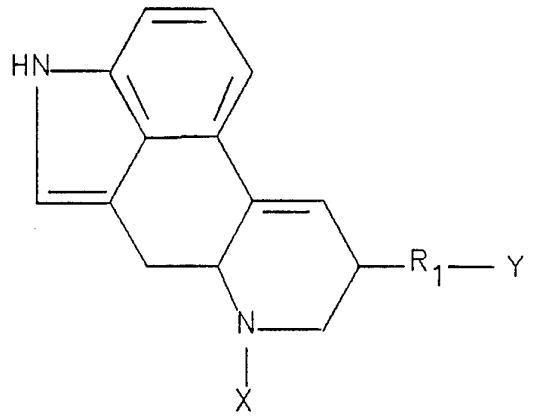
The immunogenic compound embodied by the present invention is synthesized by conjugating a hapten containing the lysergic ring to an immunogenic protein. As it exists in toxins produced by the endophyte *Acremonium coenophialum*, the lysergic ring structure in non-immunogenic. Therefore, it is necessary to conjugate the lysergic ring to an immunogenic protein to produce a compound capable of eliciting a immune response. It can be appreciated by one skilled in the art based on the teachings set forth in the

Examples that many variations of the R_1, R_2, X, and Y groups can be utilized as embodied by the present invention to produce a structure which still carries the core lysergic ring structure as the epitope to stimulate antibody production. The choice of Y as a bridge will ultimately depend upon the choices of R_1 and R_2.

The core lysergic ring structure is:



A number of suitable functional groups of the lysergic ring can be utilized at R_1 as a starting point for construction of a hapten:



By "suitable functional group of the lysergic ring" is meant to include all groups at the R_1 position of the lysergic ring which form natural or

synthetically produced derivatives of lysergic acid including, but not limited to alcohol, amine, amide, carboxyl, and hydroxyl. For example, a carboxyl group located at the R_1 position of the lysergic ring structure produces lysergic acid, whereas methanol substituted at the R_1 position results in lysergol. Lysergamine and lysergamide are functional compounds likewise produced by substitution of amine or amide groups at R_1.

In one embodiment of the present invention, the R_1 functional group is an alcohol forming lysergol. Compounds which are suitable to effectuate a bridge (*Y*) to the immunogenic protein R_2 include but are not limited to organic acids such as succinic acid, glutaric acid, and dichlorides such as sebacoyl dichloride or trans 1,4-cyclohexan-dicarboxydichloride.

The alcohol and acid can be joined by a mixed anhydride reaction as described in the examples. In another embodiment, if an amide group is positioned at R_1, then sulfonyl chlorides of organic acids such as succinic sulfonyl chloride can be utilized as bridges to the immunogenic protein. Likewise, acrylic acid can be used to effectuate the bridge when R_1 is an amine. Alternatively, acids such as 6-amino-N-hexanoic acid or other suitable amino acids can be utilized to effectuate the bridge when R_1 contains a carboxyl group.

Another embodiment of the present invention utilizes ergovaline conjugated to human serum albumin or another suitable immunogenic protein by a dicarbonyl bridge (*Y*) constructed from a suitable organic acid such as succinic or glutaric acid.

The immunogenic compound of this invention can be synthesized by bridging the lysergic ring to an immunogenic protein, R_2, wherein R_2 is selected from the group, including but not limited to human serum albumin, bovine serum albumin, chicken globulin, ovalbumin, keyhole limpet hemocyanin,

polyarginine, polyhistidine, polytyrosine, polyserine, polyaspartate, and polylysine or combinations of the polyamino acids.

Depending upon the choice of R_1 functional groups, one skilled in the art can link R_2 utilizing a suitable bridge (Y) by following methods described in Robbins, R.J., "The Measurement of Low-Molecular-Weight, Non-immunogenic Compounds by Immunoassay," *In* H.F. Linskens and J.F. Jackson (eds.), *Immunology in Plant Sciences*, p. 86−140 (1986).

The compounds, once synthesized can be screened to determine immunogenicity utilizing the methods set forth in the Examples or by other methods known in the art.

This invention also provides the immunogenic compound described above in a sustained release preparation. This preparation can be utilized as a prophylactic to protect animals from the toxic effects of ergopeptine and clavine alkaloids. Alternatively, the preparation can be used as a therapeutic. As can be appreciated by one skilled in the art, there are many suitable ways to incorporate the immunogenic compound into a sustained release preparation including but not limited to microcapsule and microsphere polymers, liposomes, and polylactic acid preparations. Alternate embodiments of the present invention utilize the immunogenic compounds described above in a biocompatible, biodegradable microsphere polymer or copolymer of polylactide or polyglycolide.

To produce an immunizing agent that will result in prolonged release of the antigen and therefore induce a long term immune response, the antigen can be incorporated, for example, into biodegradable microspheres. The most common agents to make vaccine containing microspheres are polyesters of polylactic acid and polyglycolic acid or co-polymers of both. The microspheres are produced using mild conditions that do not degrade or damage the antigens.

The antigens are trapped in the biodegradable matrix. The three basic methods used to produce these microspheres are as follows:

1. Phase separation - drug and polymer are dispersed or dissolved in a solvent. The microspheres are precipitated out by addition of silicon oil.

2. Solvent extraction - drug and polymer in solution are added to an aqueous solution of poly - (vinyl alcohol) to produce an oil - in - water emulsion. The solvent is then eliminated by adding water and the microspheres dried.

3. Spray drying - drug and polymer are dissolved in a solvent and then sprayed dried.

In all procedures after the spheres are formed, they are dried and then separated into various sizes by sieving.

Factors which affect antigen release are erosion and breakdown of the particles, diffusion of the drug out of the matrix, solubility of the antigen, antigen molecular weight, antigen loading of the spheres and polymer molecular weight. A given antigen release rate is related to particle size; small particles release the antigen sooner than large particles. For prolonged release and immunization a mixture of small and large particles appears to be desirable.

The immunogenic compounds, whether or not contained in a biodegradable microsphere, can be placed in a pharmaceutically acceptable carrier, including but not limited to sterile buffered saline or sterile distilled water. Likewise, the immunogenic compounds can be mixed with a suitable adjuvant, including but not limited to Freund's incomplete adjuvant, saponins or dextrans. Such carriers and adjuvants are well known in the art (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Laboratory, Cold Spring Harbor, New York (1988)).

The present invention also provides a method of preventing fescue toxicosis in a herbivore comprising administering a protective amount of the immunogenic compound described above to the herbivore. By "protective amount" is meant an amount sufficient to elicit an immune response to prevent fescue toxicosis in the herbivore. Likewise, a "therapeutic amount" means an amount sufficient to improve the condition of a herbivore having fescue toxicosis. Such an amount can be determined by a skilled artisan given the teachings set forth herein. The immunogenic compound can be used to prevent or treat fescue toxicosis in a herbivore.

The immunogenic compound is capable of stimulating an immune response in all herbivores including but not limited to cattle, horses, sheep, llamas, deer and goats. In one embodiment, the immunogenic compound is administered in an amount ranging from about 1mg up to about 40mg. This dose will vary based on the weight, type and condition of the animal. This dose can be administered by intramuscular or subcutaneous injection or can be administered as a sustained release implant. In a more preferred embodiment, the protective amount is about 20mg. It can be appreciated by one skilled in the art that the total protective amount can be divided into a loading dose followed by booster doses at suitable intervals, usually boosters are repeated at two-week intervals.

In a presently preferred embodiment, the invention provides a method of preventing fescue toxicosis in a herbivore (e.g., a cow) comprising administering a vaccine comprising an immunogenic amount of lysergol-glutarate-human serum albumin conjugate to the herbivore. Alternatively, the immunogen of the vaccine can comprise ergonovine maleate conjugated to human serum albumin utilizing the conjugation methods taught herein. The immunogenic amount of the immunogen in the vaccine can vary between about 1 mg and 40 mg but is preferably about 20 mg. Optimization of the dosage will depend upon the species of herbivore, and size, weight and condition of the animal but can be determined utilizing the methods taught, e.g., in Example 4.

This invention also provides purified monoclonal antibodies reactive with the lysergic ring of the immunogenic compound, e.g., reactive with the lysergic ring of ergopeptine and clavine alkaloids. These antibodies can be prepared, for example, according to the following examples.

In a presently preferred embodiment of the invention, monoclonal antibodies are produced from the hybridoma cell line designated 15F3.E5. This antibody is specifically reactive with the lysergic ring of the immunogenic compound. The 15F3.E5 antibody was produced from mouse B lymphocytes which formed antibody to a compound of lysergol conjugated to human serum albumin via a glutamic acid bridge. It should be appreciated by one skilled in the art that other monoclonal antibodies designed from the possible combinations for the immunogenic compound as described herein could be utilized to make a purified polyclonal or monoclonal antibody reactive with the lysergic ring. General methods of making various antibodies are described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Laboratory, Cold Spring Harbor, New York (1988).

The invention also provides a method of protecting a herbivore from fescue toxicosis either therapeutically or prophylactically comprising administering a protective or therapeutic amount of a monoclonal antibody reactive with the immunogenic compound described above to a herbivore. The antibody can be administered intravenously in amounts between about 0.1 mg/kg and 1.3 mg/kg of body weight. In a presently preferred embodiment, the antibody is administered at a dosage of about 0.5 mg/kg of body weight daily.

This invention also provides an antibody which is an anti-idiotype of the monoclonal antibodies of this invention. This invention also provides a method of preventing fescue toxicosis in a herbivore comprising administering a protection inducing amount of the anti-idiotype antibody to the herbivore. The anti-idiotype antibody can be utilized in a vaccine with a suitable adjuvant and/or pharmaceutical carrier as previously described. A preferred embodiment of the

invention comprises the anti-idiotype antibody in a sustained released preparation such as a microsphere polymer as discussed above. This preparation can be used as a vaccine to immunize any herbivore including but not limited to cattle, horses, sheep, llamas, deer and goats. As above, the vaccine can be administered, for example, intramuscularly or subcutaneously to the herbivore.

The following considerations are utilized in production of the anti-idiotype antibody. Idiotypic determinants are unique antigenic determinants associated with the antibody binding of antibodies to an antigen. Anti-idiotype antibodies are antibody molecules that react with the idiotypic determinant of a specific antibody (anti-idiotype -Ab_2 that reacts with the idiotype of Ab_1). Thus, anti-idiotypic antibodies mimic the three dimensional structure of the antigen used to induce Ab_1 . With these anti-idiotypic antibodies one can induce the production of antibodies (Ab_3) which will bind to the same antigen as Ab_1.

Anti-idiotype antibodies can be produced by immunizing mice with a monoclonal antibody (all binding sites on all antibody molecules are the same). The spleen cells from this mouse are used to make hybridomas and then the clones produced are selected for the production of anti-idiotype antibodies. The anti-idiotype antibodies are then screened to determine the antibodies which are the mirror image of the antigen, i.e., will block Ab_1 from binding to the antigen. These anti-idiotype antibodies can then be used to induce antibody responses to the original antigen (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Laboratory, Cold Spring Harbor, New York (1988)).

EXAMPLES

Example 1. Development of a monoclonal antibody to the lysergic ring common to the ergot and clavine alkaloids.

Generation of Lysergol and Ergonovine Haptens

Two haptens were generated for ergonovine and lysergol using the mixed anhydride method (*Anderson et. al.,* 1955). Briefly, glutaric and succinic anhydrides were used to make the haptens by placing them with the lysergic derivative and mixing in 1 mL pyridine in 2 mL screw cap vials. Vials were flushed with nitrogen, capped, and placed in the dark for 72 h. Quantities of reagents used to generate each hapten are presented in Table 1.

After the reaction, a stream of N_2 was used to evaporate the pyridine, the haptens were resuspended in 2 mL of 0.1 M HCl and extracted extensively with ethyl ether. The acidic solutions were neutralized with 0.1 M NaOH, frozen and freeze dried. Haptens were resuspended in 1 mL distilled water and loaded onto 20 mL disposable C18 Mega Bond Elut columns (Varian, Harbor City, CA; Cat. no. 1225-6023). The columns were washed with 5 mL water and the hapten eluted with 8 mL of 50% aqueous methanol. The hapten solutions were diluted with distilled water to 10% methanol, frozen at -70° C, and freeze dried.

Conjugation of Haptens to Human Serum Albumin

Haptens were conjugated to human serum albumin through the carboxylic residue using the carbodiimide reaction (*Robbins,* 1986). Thirty-five milligrams of each hapten was added to 40 mg human serum albumin (HSA) in separate 6 ml vials. The hapten and protein was dissolved in 3 mL of 0.03 M KH_2PO_4 buffer (pH=7.6). The solution was stirred continuously while 1 mL of water containing 20 mg 1-Ethyl-3(Dimethylaminopropyl) carbodiimide

hydrochloride (EDC) was added dropwise. Three milligrams of N-hydroxy sulfosuccinimide (NHS) was added and the pH adjusted to 7.6 with .1 N HCl. The vials were flushed with N, capped and sealed, and stirred continuously for 24 h in the dark at room temperature. Conjugates were dialyzed using 10,000 MW exclusionary tubing in the dark for 24 h at 4° C. The dialyzing solution was 3 L distilled water (pH=7.6) which was changed every 8h. The dialized protein conjugates were frozen at -70°C and freeze dried.

Hapten/protein ratios of the protein conjugates were determined by diluting the protein conjugate 1:1,000,000 in water and measuring fluorescence of the ergopeptine radical using a Shimadzu (Columbia, MD) HPLC. The HPLC configuration was a Model LC10AS pump equipped with a Model RF535 fluorescence detector. An Alltech (Deerefield, IL) Adsorbosphere HS C18 reverse phase column (cat no. 28930) was used with a mobile phase of 38% acetonitrile with a 0.001 M solution of ammonium carbonate per L H_2O. Fluorescence of non-conjugated protein was subtracted from that of the protein conjugate and the difference compared to standard dilutions of the respective haptens for quantitation. Conjugate:protein ratios are presented for each hapten in Table 1.

Conjugation of Lysergic Acid to Poly-L-Lysine

For use as an antigen to coat ELISA plates, D-lysergic acid was conjugated to poly-L-lysine hydrobromide (PLL) using the carbidiimide reaction sequence (*Robbins,* 1986). Twenty-five milligrams of poly-L-lysine hydrobromide and 5 mg lysergic acid were dissolved in 3 mL of 10% aqueous methanol in a 6 mL screwcap vial. The solution was stirred constantly while 1 mL water containing 20 mg EDC was added dropwise. Three milligrams of dry NHS was added and the pH adjusted to 7.6 with 1 N HCl. The vial was flushed with nitrogen, capped, and stirred in darkness for 24 h. The PLL conjugate solution was dialyzed exhaustively for 24 h using 10,000 MW exclusionary tubing, frozen

at -70° C, and freeze dried overnight. The PLL-lysergic acid conjugate was stored in a desiccator, in the dark, at room temperature.

Mouse Immunization

All mouse handling and cell suspensions, fusions, and cultures were conducted by personnel in the University of Georgia Monoclonal Antibody Facility (Miller Plant Sciences Building, Athens, GA 30602). One hundred fifty micrograms of each hapten/HSA conjugate was suspended in 100 μL phosphate buffer solution (PBS) (1.14 g Na_2HPO_4, 0.244 g NaH_2PO_4, 8.2 g NaCl, 1.0 L H_2O, pH 7.4) and Freund's complete adjuvant and injected into 3, 4-week old Balb/c mice (Dominion Laboratories, Dublin, VA) at the base of the tail. Mice were reimmunized 28 d later with a 50 ug hapten/HSA interperitoneal injection using Freund's incomplete adjuvant. Mouse blood was collected by tail bleeding, the serum diluted 50x with ELISA diluent (10 g bovine serum albumin, 1.17 g Na_2HPO_4, 0.244 g NaH_2PO_4, 8.2 g NaCl, 0.2 g NaN_3, 0.5 mL Tween-20, 1 L H_2O, pH 7.4) and tested for presence of antibody using ELISA techniques as described below. The mouse with greatest antibody reaction was injected with another 25 ug hapten/HSA suspended in Freund's incomplete adjuvant 14 d after the previous intraperitoneal injection.

Hybridization of B cells and Myelomas

Three days following final hapten/HSA injections, mice were sacrificed by cervical dislocation, their spleens removed, and a suspension of B cells made by macerating the spleen in 40 mL of sterile PBS. The suspension was centrifuged at 300 g for 5 m. Supernatant was poured off and the B cell pellet saved for hybridization with myeloma cells.

Mouse SP2/0 myeloma cells were centrifuged and 10^7 cells suspended in 10 ml IMDM solution (Sigma Chemical Co., St. Louis, MO; cat. no. I7633). Suspended myeloma cells were added to the B cell pellet and

centrifuged at 300 g for 5 m. The supernatant was poured off and 0.7 mL of 45% polyethylene glycol was added for 90 s. Fifteen milliliters of IMDM was added and the suspension centrifuged at 300 g for 5 m and supernatant poured off. The cells were resuspended in 90 mL IMDM containing 20% fetal calf serum with HT (FCS/HT) (Sigma Chemical Co., St. Louis, MO; cat. no. H0137), and 100 μL of the cell suspensions transferred to 96-well ELISA plates. Cell lines were nourished for 10 days by replenishing the IMDM/FCS/HT medium daily.

Screening Mice and Hybridomas for Antibodies

Immulon2 96-well ELISA plates (Dynatech Laboratories, Chantilly, VA) were treated with 50 μL of PBS buffer containing 10 ug PLL-lysergic acid conjugate for 2 h at 21°C. The plates were washed 3 x's using a squirt bottle containing ELISA wash (1.21g Tris, 0.5 mL Tween 20, 0.2 g NaN_3, 1.0 L H_2O, pH 8) and blocked with 100 μL bovine serum albumin (BSA) blocking solution (10 g BSA, 0.2 g NaN_3, 1.0 L PBS) for 30 m. The plates were washed 3 x's with ELISA wash and 50 μL of diluted mouse serum or hybridoma cell cultures added. After 2 h, the plates were washed 3 x's with ELISA wash and 50 μL of a 1:500 dilution of rabbit anti-mouse antibodies conjugated with alkaline phosphatase (Sigma Chem. Co., St. Louis, MO; cat. no. A1902):ELISA diluent added. After 2 h, 50 μL of alkaline phosphatase substrate (0.1 g $MgCl_2$, 96 mL diethanolamine, 1.2 g Disodium p-Nitrophenyl Phosphate, 1 L H_2O) was added. The yellow color reaction indicative of presence of mouse antibody binding to PLL-lysergic acid was measured spectrophotometrically at 405 nm using a Titertec Multiskan Model MCC/340 plate reader (Flow Laboratories Inc., McClean, VA).

One hybridoma cell line, identified as 15F3.E5 tested positive and was expanded to 1.0 L of hybridoma antibody in 12 well culture plates while feeding them with IMDM/HT medium containing 50 mL L^{-1} FCS. Hybridoma solutions from the 24-well culture plates were combined into a common antibody

solution to which 0.2 g NaN_3 were added to lyse the hybridomas and stabilize the antibody solution.

Determining Cross-reactivity of the Monoclonal Antibody

Mouse monoclonal antibody was diluted 1:50 in ELISA diluent. Working dilutions of various lysergic acid derivatives ranging between 1.78×10^{-6} to 3.93×10^{-15} M were made in 1:10 increments in PBS and 50 μL aliquots were pre-incubated for 1 h with 50 μL of diluted antibody at room temperature. To determine presence of unbound antibody, pre-incubated antibody/lysergic derivatives were transferred to ELISA plates pre-coated with PLL-lysergic acid for 2 h at room temperature. After washing, ELISA plates were treated with 1:500 dilution of rabbit derived antimouse antibody conjugated to alkaline phosphatase:ELISA diluent for 2 h at room temperature and washed. Fifty microliters of alkaline phosphate substrate was added to each well and the characteristic yellow color indicating binding of mouse antibody to the PLL-lysergic acid measured at 405 nm on a Titertek Multiskan Model MCC/340 plate reader.

Example 2. Passive Immunization of Monoclonal Antibody 15F3.E5 into Steers Suffering from Fescue Toxicosis.

Preparation of Monoclonal Antibody

Three liters of mouse monoclonal antibody were prepared in 12 well culture plates by feeding the hybridoma 15F3.E5 cell lines with IMDM/HT medium as previously described. The antibody solution was dialyzed against 150 L water over a 24 h period using 12,000 MW exclusionary dialysis tubing to remove excess salts and NaN_3. The antibody solution was lyophilized and reconstituted in 300 mL of 0.01M PBS and sterile filtered through a 0.45 micron filter. Protein concentration of the reconstituted antibody solution was 15 mg mL^{-1}.

Preparation of Animals Prior to Monoclonal Infusion

Eight yearling Angus steers were randomly assigned to each of two endophyte-infected paddocks 106 days prior to treatment with monoclonal antibody. Endophyte infection rates of the tall fescue plants within the two pastures were 65 and 77.5%. Ergovaline concentration of herbage samples taken immediately prior to monoclonal antibody infusion was 651 and 725 ug kg^{-1}, respectively. For a period of four days prior to implementation of the experiment, animals were gentled by tethering them in stalls in a pole barn for 3 to 4 hours where the experiment was to occur.

On July 22, 1992, steers were withheld from water for a 16 h period and weighed. A cannula was placed into each jugular vein for administration of antibody and collection of blood samples. The cannulas were protected by neck wrap and the steers returned to the assigned paddocks.

On July 23, steers were tethered in individual stalls within a pole barn to facilitate administration of the antibody and collection of blood samples.

The stalls were arranged parallel to one another with sufficient space for investigators to walk between animals for antibody treatment and sample collection. Steers remained in the stalls for approximately eight hours during the experiment. Bermudagrass hay and water was provided during animal confinement.

Infusion of Monoclonal Antibody

The four steers within each tall fescue paddock were ranked by the 16 h off water weights. Steers were assigned to groups of two based upon rank, and two treatments (control and antibody) were randomly assigned within the groups of two. The antibody treatment group averaged 297 kg (S.D.=19.7) and the control treatment group averaged 304 kg (S.D.=11.6). The control treatment group received a bovine serum albumin placebo at the same rate as the group which received the monoclonal antibody 15F3.E5. Prior to administration of treatments, three sham blood samples were collected at 30 min intervals to accustom the animals to blood collection. Three more samples were taken from which basal circulating prolactin could be determined. Immediately after the final basal blood sample was collected, the steers were given IV bolus injections of 0.62 mg kg^{-1} of reconstituted monoclonal antibody or bovine serum albumin in sterile phosphate buffer solution (SPBS) (8.5 g NaCl, 2.18 g $Na_2(HPO_4)\cdot 7H_2O$, 0.32 g $Na(H_2PO_4)\cdot H_2O$, pH=7.1). The antibody and BSA treatments were diluted in SPBS to 3 mg protein mL^{-1} and infused IV at a rate of 30 mg $steer^{-1}$ h^{-1} for a 5 h period. Infusions were administered using Ismatec-SA Model 7613-30 programmable pumps (Basel, Switzerland). Antibody dosage was based upon a report of circulating ergovaline at a concentration of 40 ng L^{-1} (*Savary et al.,* 1990). Antibody dosage was derived as being approximately 1000 x's the concentration of circulating ergovaline.

Blood was collected at 30 min intervals after initial administration of the treatments. At the end of the infusion period, all steers were given 33 ug kg^{-1} thyrotropin releasing hormone (TRH) IV as a test challenge for prolactin

secretion. Blood was collected at 10 and 20 min intervals after TRH treatment. All blood samples were permitted to clot at ambient temperature, stored on ice in the field, and then at 4°C overnight. Serum was harvested following centrifugation and stored at -20°C for prolactin determination.

Measurement of Prolactin and Prolactin Response

The concentration of prolactin in the serum was determined by radioimmune assay procedures adapted from *Wallner et al.*, (1983) and used by *Thompson et al.*, (1992). Reagents used were USDA-b-Prl-B-1-s bovine prolactin as a standard, iodinated USDA-b-Prl-I-s bovine prolactin (I^{125}) and DJB-7-0330 rabbit anti-bovine prolactin antisera for binding. The prolactin-bound rabbit anti-sera was precipitated using 6% polyethylene glycol with guinea pig anti-rabbit antisera. All prolactin measurements were made in a single assay. Two control serum pools (high and low) were included for quality control (n=6). Means and coefficients of variation for each were 14.17 ng mL^{-1} and 6.5%, and 7.74 ng mL^{-1} and 10.4%, respectively, which were considered acceptable. Data were analyzed by analysis of variance using a split-plot model with antibody treatment as the main effect and time after initial administration as the split. A significant time x treatment interaction occurred. Therefore, linear and quadratic coefficients were determined using regression analysis to relate percent and absolute changes from basal values over time.

Example 3. Active immunization of Angus heifers against fescue toxicosis.

Nine yearling Angus heifers were maintained on cereal rye (*Secale cereale* L.) pastures from mid-December, 1991 through May, 1992. The heifers received 2.7 kg head^{-1} day^{-1} of a concentrate diet at the onset of grazing through March, 1992. Heifers were randomly assigned to three treatment groups which were immunized with either the ergonovine-glutaric, lysergol-glutaric, or lysergol-succinic haptens conjugated to HSA (Table 1).

TABLE 1. Quantities of reagents used to generate haptens for conjugation to immunizing proteins and their molar ratios of the protein conjugates.

Hapten/Protein Alkaloid Derivative Ratio	mg	Anhydride	mg	Molar
Ergonovine	55	Glutaric	67	8.52
Ergonovine	20	Succinic	13	3.15
Lysergol	40	Glutaric	49	12.20
Lysergol	40	Succinic	13	8.50

The first injection was given on January 16, 1992 using 1.0 mg of immunogen dissolved in 1.0 mL SPBS plus 1.0 mL of Freund's complete adjuvant IM in the neck region. Subsequent injections using 0.5 mg of immunogen were given on January 30 and February 14. Animals were maintained in a single group after each injection. Serum titer for antibody was conducted by the ELISA technique outlined in the methods for the monoclonal antibody. Blood was collected prior to initial immunization and at 2 week intervals through March 26, 1992 and again on May 18, 1992.

RESULTS

Example 1. Development of a monoclonal antibody to the lysergic ring common to the ergopeptine and clavine alkaloids.

A mouse immunized with lysergol linked to HSA via glutaric anhydride gave the greatest immune response. Only 29 hybridoma lines were viable, one of which had affinity to PLL-LYS as indicated by ELISA. The cell line, 15F3.E5, expressed cross reaction to lysergol, lysergic acid, ergovaline, ergonovine, and ergotamine tartrate, suggesting that the antibody recognized the lysergic ring structure common to the ergopeptine and clavine alkaloids (Table 2).

TABLE 2. Molar concentration of lysergic acid derivatives needed to give 50% maximum absorbance when analyzed in a competitive ELISA assay.

Lysergic Acid Derivative	Molar Concentration at 50% Maximum Absorbance
Lysergol	3.93×10^{-15}
Lysergic Acid	3.73×10^{-11}
Ergovaline	1.00×10^{-11}
Ergonovine	1.69×10^{-9}
Ergotamine tartrate	7.69×10^{-9}
2-bromo-a-ergocryptine	$> 1.33 \times 10^{-6}$
dihydro-ergocornine	$> 1.68 \times 10^{-6}$
dihydro-ergocristine	$> 1.78 \times 10^{-6}$

Hydrogenation or bromation of the ring structure resulted in no cross reaction with those compounds at the concentration at which they were tested. It is likely that hydrogenation or bromation resulted in conformational

changes of the lysergic ring structure. Therefore, the monoclonal antibody 15F3.E5 was highly specific to the intact lysergic ring.

Example 2. Passive immunization of monoclonal antibody 15F3.E5 into steers suffering from fescue toxicosis.

Mean serum PRL prior to bolus treatment was 23.8 and 18.8 ng mL^{-1} in control and treated steers respectively. Because circulating PRL was variable among steers, the data was analyzed as percentage and absolute change from the basal measurements for each animal. The regression equation describing increased percent serum PRL in response to monoclonal antibody infusion had an intercept not different from 0.0 ($p=0.70$), and positive linear and negative quadratic coefficients ($p=0.02$). This suggests that the rate of percent increase in serum prolactin decreased as time progressed (Fig. 1a). Conversely, control animals had a negative linear coefficient and positive quadratic coefficient ($p=0.02$). The regression equation describing absolute increased serum PRL in animals receiving antibody was linear with a positive coefficient ($p=0.01$) and an intercept not different from 0.0 ($p=0.25$) (Fig. 1b). Control animals had negative linear and positive quadratic coefficients. All animals responded to TRH (97.1−100% increase in control and treated steers, respectively).

It is important to note that an increase in serum PRL was immediate among the steers receiving the monoclonal antibody. While absolute serum PRL increased at a linear rate, the rate of percent change in PRL decreased in steers receiving antibody as time progressed. Serum PRL increased in control steers receiving BSA, but after 150 min only. We attribute the increase serum PRL in the control steers to anxiety among animals from their confinement.

These results demonstrate that the monoclonal antibody, specific for the lysergic ring of the ergopeptine and clavine alkaloids, reversed a sign of

fescue toxicosis. Since the antibody binds ergopeptine alkaloids, specifically ergovaline, and the antibody neutralized circulating ergopeptine alkaloids, this resulted in increased PRL. This is the first definitive evidence that the ergopeptine alkaloids are directly involved in animal responses to endophyte-infected tall fescue.

Example 3. Active immunization of Angus heifers against fescue toxicosis.

Antibodies to the lysergic ring were stimulated in all animals regardless of the immunogen used (Fig. 2). Titer increased linearly with subsequent injections and continued until 2 weeks after final immunization. Titers demonstrate that animal responses to the immunogens were not different from one another.

It should be noted that the ELISA procedure used to determine antibody production uses lysergic acid as the hapten. Therefore, antibodies recognizing the lysergic acid hapten are likely to have broad spectrum activity on other compounds with the lysergic ring. Therefore, antibodies generated by active immunization have activity similar to that of the mouse monoclonal antibody and provide protection to the clavine and ergopeptine alkaloids.

Example 4. Immune Response of Cattle Dosed With Lysergol-Glutarate-Human Serum Albumin Conjugate

The purpose of this study was to optimize the dosage of a vaccine needed to generate anti-lysergic antibodies in livestock.

Twenty black angus steers were divided into five treatment groups. Treatment groups were assigned to vaccinations of either 1, 10, 20, or 40 mg/steer of lysergol-glutarate-human serum albumin conjugate; or 20 mg of human serum albumin (control group). After 3 weeks a secondary administration of vaccine was performed with one-half the dose of the primary

administration for each treatment group. All treatments were administered with a saponin:aluminum hydroxide adjuvant. Animals were bled prior to primary vaccination, at the time of secondary vaccination, and every 7 days following for 42 days. Serum was harvested from the blood samples and analyzed for antibodies to lysergic acid using an ELISA test. Maximum and most prolonged antibody response was found when the primary vaccination was a 20 mg administration of the immunogen (See Fig. 3). Ten milligrams of immunogen was slightly lower in antibody production over time, but 1 and 40 mg immunogen treatments clearly elicited inferior antibody responses by the cattle. Human serum albumin elicited no anti-lysergic response. Therefore, a dose of between about 10 and 20 mg of a vaccine comprising a lysergol-glutarate human serum albumin appears optimum for immunization of herbivores against fescue toxicosis.

Fifty picograms of tritiated LSD were administered to a 50 μL aliquot of 1:20 dilutions of serum collected either 14 or 28 days following secondary injection. After incubation for 1 hour, the antibody was precipitated and sorbed to activated charcoal to separate bound and unbound tritiated LSD. Radioactive counts of the bound fraction were then determined to quantify antibody binding of the tritiated compound. Treatment responses to binding of tritiated LSD were similar to the antibody titers (see Table 3).

Assuming blood volume to be approximately 8.5% of body weight and serum to be 60% of the volume of blood, quantity of bound tritiated LSD was calculated per 100 kg of animal live weight. Intake and metabolism of ergopeptine alkaloids was calculated based upon daily dry matter intake of 2.5% of animal body weight with a ergopeptine alkaloid concentration of 1 ppm. Based upon previous research, 1 ppm ergopeptine alkaloid concentration would be the maximum concentration in field grown herbage to which an animal would be exposed. Potential daily intake of ergopeptine alkaloids is 1.25 μg per 100 kg body weight of animal (see Table 4). The binding potential of LSD compared to the daily intake of ergopeptine alkaloids is varied between 9.1 and 27.1 times

as much at day 14 and from 4.2 to 30.9 times as much on day 28. The immunogen treatment in which 20 mg of immunogen was administered during primary vaccination gave the greatest ratio of binding potential:alkaloid intake.

TABLE 3. Percent binding by serum antibodies of 50 pg administration of tritiated LSD to 50 μL of a 1:20 dilution of blood serum from animals treated with different amounts of immunogen.

Day/Post Injection	Immunogen Treatment	% binding LSD
14	1 mg	32.35
	10 mg	28.97
	20 mg	33.60
	40 mg	11.82
28	1 mg	19.75
	10 mg	31.82
	20 mg	37.85
	40 mg	5.18

TABLE 4. Antibody binding of tritiated LSD per 100 kg of animal live weight, quantity of antibody absorbed per 100 kg of animal live weight, and the ratio of antibody binding potential:absorbed ergopeptine alkaloids.

Day post injection	Immunogen treatment	Antibody binding potential	Alkaloid absorbed	Binding potential:absorbed ratio
		- μg per 100 kg live weight -		
14	1 mg	33.0	1.25	26.4
	10 mg	29.5	1.25	23.6
	20 mg	34.3	1.25	27.4
	40 mg	11.4	1.25	9.1
28	1 mg	20.1	1.25	16.1
	10 mg	32.5	1.25	26.0
	20 mg	38.6	1.25	30.9
	40 mg	5.3	1.25	4.2

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

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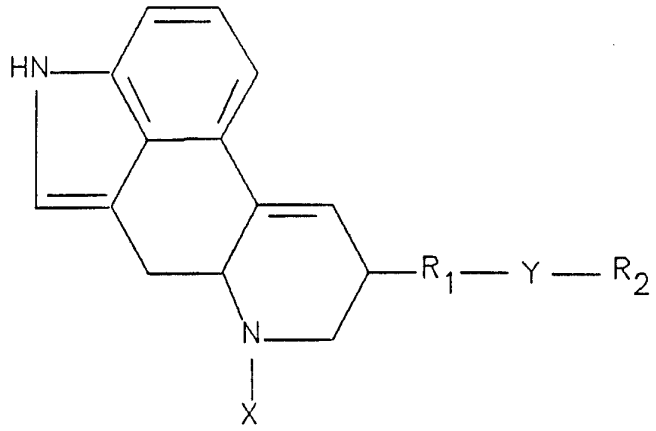
What is claimed is:

1. An immunogenic compound having the formula:



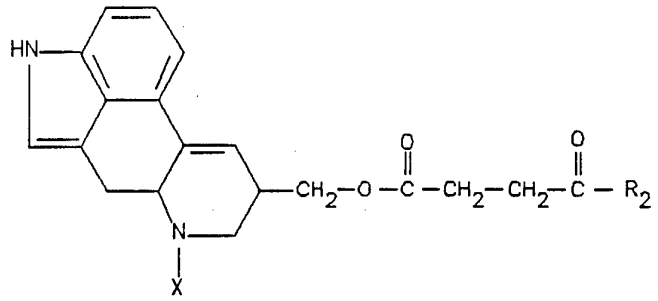
wherein *X* is selected from the group consisting of methyl and hydrogen;
wherein R_1 is a suitable functional group of the lysergic ring;
wherein R_2 is an immunogenic protein; and
wherein *Y* is a bridge to link R_1 to R_2.

2. The compound of Claim 1, wherein R_1 is selected from the group consisting of alcohol, amine, amide, carboxyl, and hydroxyl.

3. The compound of Claim 1, wherein R_2 is selected from the group consisting of human serum albumin, bovine serum albumin, chicken globulin ovalbumin, keyhole limpet hemocyanin, polyarginine, polyhistidine, polytyrosine, polyserine, polyaspartate, and polylysine.

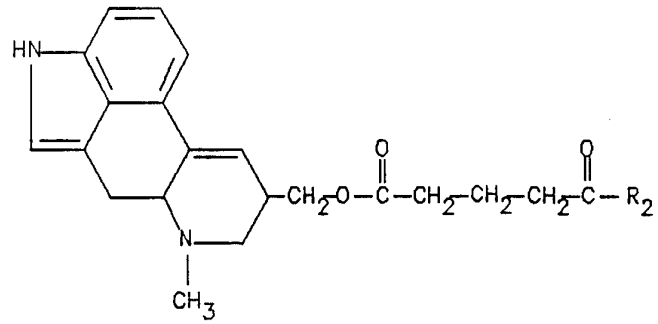
4. The compound of Claim 1, wherein R_1 is selected from the group consisting of alcohol and hydroxyl; and
wherein *Y* is selected from the group consisting of dicarbonyls of succinic acid, glutamic acid, fumaric acid, adipic acid and maleic acid and other organic acids.

5. An immunogenic compound having the formula:



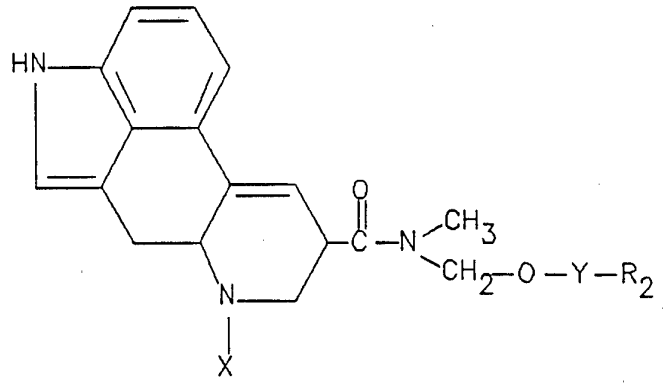
wherein X is selected from the group consisting of methyl and hydrogen; and wherein R_2 is selected from the group consisting of human serum albumin, bovine serum albumin, chicken globulin, ovalbumin, keyhole limpet hemocyanin, polyarginine, polyhistidine, polytyrosine, polyserine, polyaspartate, and polylysine.

6. An immunogenic compound having the formula:



wherein R_2 is selected from the group consisting of human serum albumin, bovine serum albumin, chicken globulin, ovalbumin, keyhole limpet hemocyanin, polyarginine, polyhistidine, polytyrosine, polyserine, polyaspartate, and polylysine.

7. An immunogenic compound having the formula:



wherein *X* is selected from the group consisting of methyl and hydrogen; wherein *Y* is selected from the group consisting of dicarbonyls of succinic acid, glutamic acid, fumaric acid, adipic acid, maleic acid and other organic acids; and wherein R_2 is selected from the group consisting of human serum albumin, bovine serum albumin, chicken globulin, ovalbumin, keyhole limpet hemocyanin, polyarginine, polyhistidine, polytyrosine, polyserine, polyaspartate, and polylysine.

8. The compound of Claim 1, wherein the compound is in a sustained release preparation.

9. The compound of Claim 1, wherein the sustained release preparation is selected from the group consisting of microsphere polymers, liposomes, polylactic acid and microcapsules.

10. The compound of Claim 1, in a pharmaceutically acceptable carrier.

11. A composition comprising the compound of Claim 1 and an adjuvant.

12. A method of preventing fescue toxicosis in a herbivore comprising administering a protective amount of the compound of Claim 1 to the herbivore.

13. The method of Claim 12, wherein the protective amount is between about 1 mg and 40 mg.

14. The method of Claim 13, wherein the protective amount is about 20 mg.

15. The method of Claim 12, wherein the herbivore is a cow.

16. A method of treating fescue toxicosis in a herbivore comprising administering a therapeutic amount of the compound of Claim 1 to the herbivore.

17. The method of Claim 16, wherein the therapeutic amount is between about 1 mg and 40 mg.

18. The method of Claim 17, wherein the therapeutic amount is about 20 mg.

19. The method of Claim 16, wherein the herbivore is a cow.

20. A method of preventing fescue toxicosis in a herbivore comprising administering an immunogenic amount of a vaccine comprising lysergol-glutarate-human serum albumin conjugate to the herbivore.

21. The method of Claim 20, wherein the immunogenic amount is between about 1 mg and 40 mg.

22. The method of Claim 21, wherein the immunogenic amount is about 20 mg.

23. The method of Claim 20, wherein the herbivore is a cow.

24. A purified antibody reactive with the lysergic ring of the immunogenic compound of Claim 1.

25. The antibody of Claim 24, wherein the antibody is monoclonal.

26. The antibody of Claim 25, wherein the monoclonal antibody is produced by the hybridoma cell line designated 15F3.E5.

27. A method of preventing fescue toxicosis in herbivores comprising administering a protective amount of the antibody of Claim 25 to the herbivore.

28. The method of Claim 27, wherein a protective amount is between about 0.1 mg/kg and 1.3 mg/kg of body weight daily.

29. The method of Claim 27, wherein the protective amount is about 0.5 mg/kg of body weight daily.

30. The method of Claim 27, wherein the herbivore is a cow.

31. A method of treating fescue toxicosis in a herbivore comprising administering a therapeutic amount of the antibody of Claim 28 to the animal.

32. The method of Claim 31, wherein the therapeutic amount is between about 0.1 mg/kg and 1.3 mg/kg of body weight daily.

33. The method of Claim 31, wherein the therapeutic amount is about 0.5 mg/kg of body weight daily.

34. The method of Claim 31, wherein the herbivore is a cow.

35. A hybridoma cell line producing the monoclonal antibodies of Claim 25.

36. The hybridoma cell line of Claim 35, wherein the hybridoma cell line is designated 15F3.E5.

37. An antibody which is an anti-idiotype of the monoclonal antibody of Claim 25.

38. A method preventing fescue Toxicosis in a herbivore comprising administering a protection inducing amount of the antibody of Claim 37 to the herbivore.

39. The method of Claim 38, wherein the herbivore is a cow.

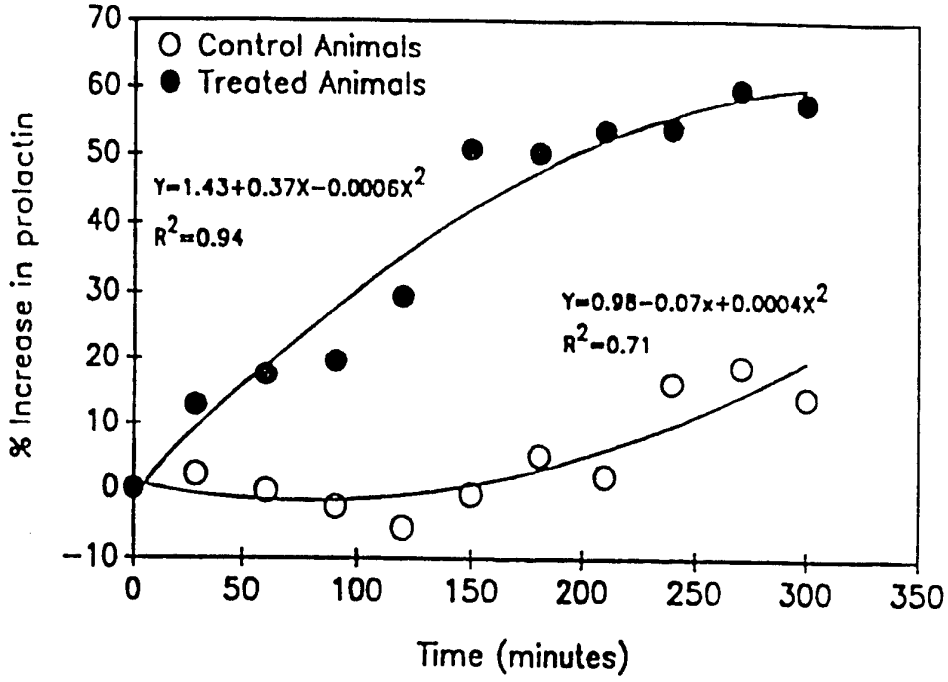

O Control Animals
● Treated Animals
Y=1.43+0.37X−0.0006X²
R²=0.94
Y=0.98−0.07x+0.0004X²
R²=0.71
% Increase in prolactin
Time (minutes)
70
60
50
40
30
20
10
0
−10
0
50
100
150
200
250
300
350

Fig. 1a

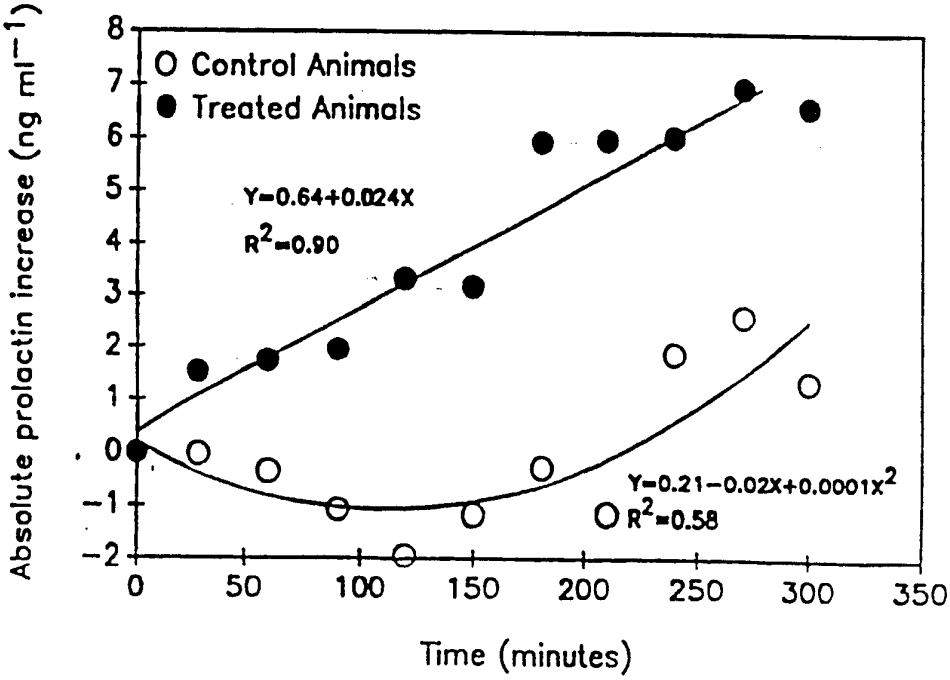

O Control Animals
● Treated Animals
Y=0.64+0.024X
R²=0.90
Y=0.21−0.02X+0.0001x²
O R²=0.58
Absolute prolactin increase (ng ml⁻¹)
Time (minutes)
8
7
6
5
4
3
2
1
0
−1
−2
0
50
100
150
200
250
300
350

Fig. 1b

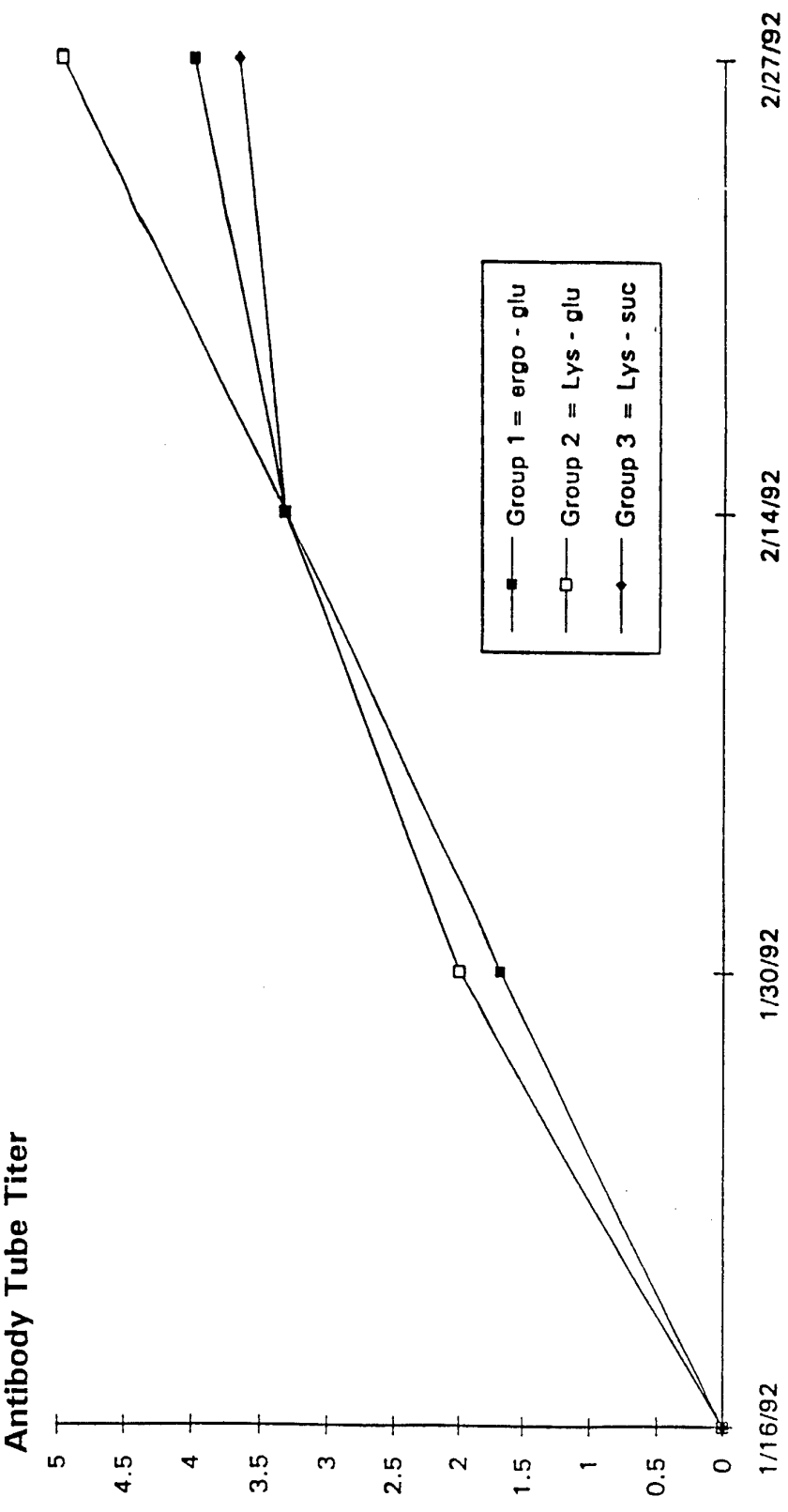
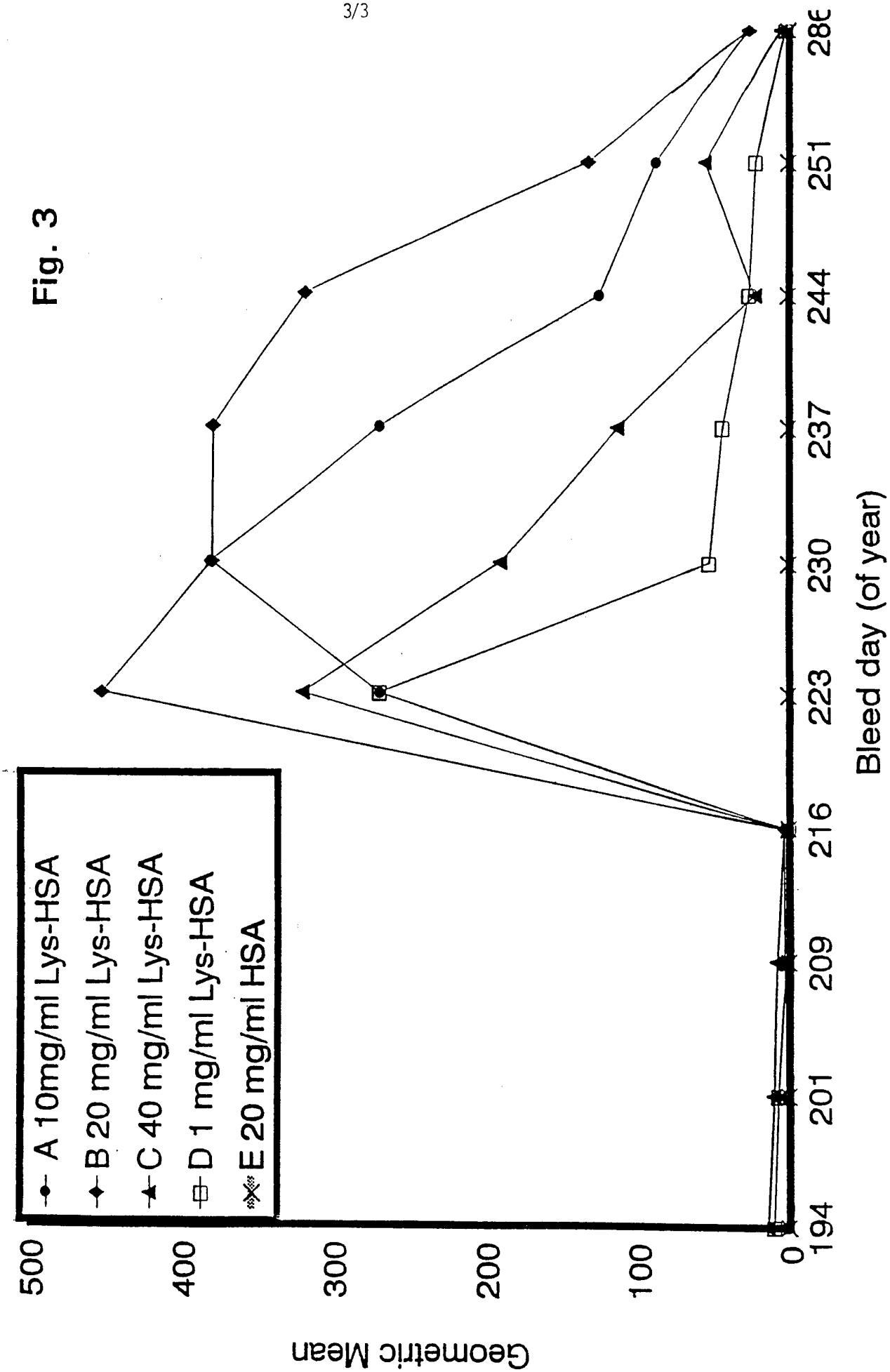

Antibody Tube Titer
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1/16/92
1/30/92
2/14/92
2/27/92
Group 1 = ergo - glu
Group 2 = Lys - glu
Group 3 = Lys - suc

Fig. 2

Fig. 3


Geometric Mean Titers values + 1
A 10mg/ml Lys-HSA
B 20 mg/ml Lys-HSA
C 40 mg/ml Lys-HSA
D 1 mg/ml Lys-HSA
E 20 mg/ml HSA
500
400
300
200
100
0
Geometric Mean
194
201
209
216
223
230
237
244
251
286
Bleed day (of year)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/00812

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 15/00; A61K 31/48, 37/02, 39/385
US CL : 530/350, 807; 514/12; 424/88
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 807; 514/12; 424/88

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 3,224,945 (TYLER) 21 December 1965, col. 1, lines 9-10.	1-23
Y	IN PROC. AMER. Foliage and Grassland Conference, issued 01-04 April 1991, H. Testereci et al, "Fescoe Toxicosis: Cattle Response to IV Infusion of an Ergovaline Enriched Fraction in a Climate Controlled Chamber", pages 244-247, especially page 244.	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
04 April 1994

Date of mailing of the international search report
APR 15 1994

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/00812

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Int. Symposium on Acremonium/Grass Interactions, Louisiana State University, New Orleans, Louisiana, issued 1990, B. J. Savary et al, "Analysis of Ergopeptine Alkaloids in Bovine Serum", pages 263-266, especially page 263.	1-23
Y	Fudenberg et al EDS., "Basic and Clinical Immunology", published 1976 by LANGE Medical Publications, (Los Altos, California), pages 32-40 and 596-605, especially pages 33 and 597.	1-23
Y	Food and Agricultural Immunology, Volume 3, issued 1991, R. A. Shelby et al, "Detection of Ergot Alkaloids in Tall Fescue by Competitive Immunoassay with a Monoclonal Antibody", pages 169-177, especially page 169.	1-23
Y	J. Agric. Food Chem., Volume 38, No. 4, issued 1990, R. A. Shelby et al, " An Immunoassay for Ergotamine and Related Alkaloids", pages 1130-1134, especially page 1130.	1-23
Y	Linskens et al EDS., "Immunology in Plant Sciences", pubished 1986 by Springer-Verlag, (Berlin), pages 86-141, especially pages 92-94 and 97.	1-23
Y	Morris et al EDS., " Immunoassays in Food Analysis", published 1985 by ELSEVIER APPLIED SCIENCE PUBLISHERS LTD, (Essex, England), pages 197-211, especially pages 197-198 and 206.	1-23
Y	Immunobiol., Volume 184, issued 1992, M. T. Aguado et al, "Controlled-Release Vaccines-Biodegradable Polylactide/Polyglycolide (PL/PG) Microspheres as Antigen Vehicles", pages 113-125, especially page 114.	1-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/00812

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

I. Claims 1-23, drawn to an immunogenic compound and a method of preventing or treating fescue toxicosis, classified in classes 530 and 424, subclasses 350 and 88, respectively.

II. Claims 24-36, drawn to an antibody and a method of preventing or treating fescue toxicosis, classified in classes 530 and 424, subclasses 388.5 and 85.8, respectively.

III. Claims 37-39, drawn to an anti-idiotype antibody and a method of preventing fescue toxicosis, classified in classes 530 and 424, subclasses 387.2 and 85.8, respectively.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-23

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.