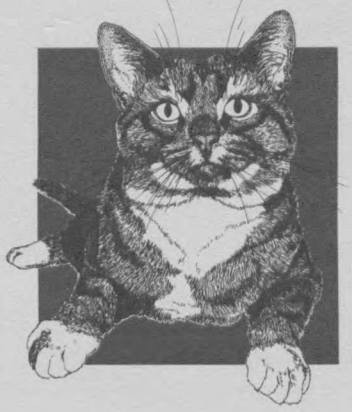

Feline Health Topics

for veterinarians

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Vaccines and Adjuvants

Jeffrey E. Barlough, D.V.M., Ph.D.

In ancient times certain sage physicians claimed that they could protect healthy people against illness by treating them with tissues or fluids obtained from diseased individuals. This seemingly wild notion was based on the observation that patients recovered from a disease often were resistant when subsequently re-exposed. Many successes, but also many failures, were counted among the results of these earliest of medical experiments in immunization. In relatively recent times, it is sad to relate, public outrage against the ludicrous and dangerous practice of "inoculation" even threatened abandonment of some lines of medical inquiry.[1] From our presumably enlightened perspective at the latter end of the twentieth century, we can only marvel at the insight (and often the courage) of some of these pioneers in medical and veterinary research - - the early Chinese and Turks, Edward Jenner, Louis Pasteur, even Cotton Mather, and others - - for it is on the shoulders of their accomplishments that our society's unprecedented high level of health and relative freedom from infectious disease rest.

In veterinary medicine, as in human medicine, inoculation represents the single most important preventive health measure available today. The excellent vaccines against rabies, canine distemper, canine parvovirus, and feline panleukopenia are only a few of the spectacular successes achieved in this area over the past several decades. And the future holds the promise of even greater successes to come.

Types of Vaccines

Two major types of vaccines are currently available to practitioners for use in their feline patients: **modified-live** vaccines and **inactivated** ("killed") vaccines. Modified-live vaccines contain an attenuated (weakened) strain of the particular disease agent of concern. Attenuated strains have

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an advantage in that they replicate within the host (thus increasing antigenic mass) without producing serious clinical illness, yet still are able to stimulate protective host defenses. Modified-live vaccines often require only a single dose to provide an immunity that is both solid and enduring. The immunity develops relatively swiftly, and both humoral and cellular immune responses are stimulated. Modified-live vaccines closely mimic infection with the virulent disease agent, and thus provide the best immune stimulation.

Inactivated vaccines have advantages too. They are much more stable than are modified-live vaccines (i.e., they have a longer shelf-life); they never spread from the vaccinated host to other animals; they cannot revert to a virulent state; they are safe for use in pregnant animals. Although more than a single dose of vaccine is required and the duration of immunity is shorter, inactivated vaccines and their experimental first cousins, **subunit vaccines** - - vaccines containing discrete immunogenic polypeptides, rather than entire disease agents - - are regaining importance in this age of retrovirus and herpesvirus infections and concern about the safety of genetically customized microbes.

Designing a successful inactivated vaccine requires that the agent in the vaccine is inert and thus will not replicate in the host. Over the years it has become evident that artificial means must be employed to heighten the response of the host to the injected material. Earlier in this century the basic mechanics for eliciting this improved immune response were devised, using certain specialized mixtures to which the vaccine material was then added. These mixtures are known as adjuvants (Latin *adjuvans*, "aiding").

Types of Adjuvants

The secret of success of many a commercial vaccine lies in the composition of its adjuvant. To be technically accurate, an adjuvant is any substance that nonspecifically enhances the immune response to a given antigen. A good adjuvant should be safe and should produce an earlier, better, and more enduring immune response than the inactivated vaccine material alone. Unfortunately, the adjuvant component of inactivated vaccines often is responsible for some post-vaccination side-effects (fever, malaise, vomiting, muscle soreness). A really good adjuvant thus must minimize side-effects while maximizing its immune boost. This boost can be achieved in a number of ways. Adjuvants can enhance the immune response by sequestering antigen at the site of inoculation; by optimizing presentation of antigen to im-

Feline Health Topics

A publication for veterinary professionals

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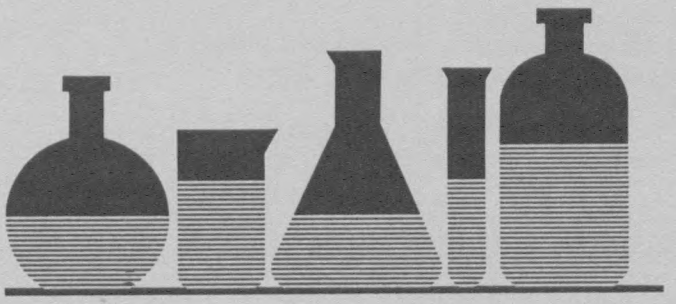
munocompetent cells; and by encouraging production of important cytokines - - molecules that signal immune cells and help direct their activities.

Many different substances can serve as vaccine adjuvants. Those such as aluminum salts (aluminum hydroxide, aluminum phosphate) and water-in-oil emulsions act apparently by means of antigen sequestration. By slowing release of the antigen into the systemic circulation (and hence into the lymphatics as well), they provide a lengthened lifespan for the antigen in the body and hence greater exposure to immunocompetent cells - - the so-called depot effect. Sequestration of antigen at the inoculation site also promotes an influx of immunocompetent cells secondary to inflammation. A problem with aluminum salt-based adjuvants is that, although they are considered safe, they can produce local reactions in a small percentage of cases. They tend also to promote IgE responses, and are better at stimulating humoral rather than cell-mediated immune responses. Despite this, aluminum salt-based adjuvants remain among the most popular adjuvants employed in veterinary vaccines. Mineral oil adjuvants are disadvantageous, however, in that they have a shorter shelf-life, can induce severe granulomatous reactions, and will track through muscle and fascial planes.

Some adjuvants act by stimulating macrophages to produce interleukin-1. Interleukin-1 is a cytokine that promotes hepatic synthesis of the acute-phase proteins of inflammation -- C-reactive protein, serum amyloid A, fibrinogen, transferrin, haptoglobin, and others. Interleukin-1 also is responsible for producing a febrile response, which assists in the functioning of the alternate pathway of complement and in antibody synthesis and T-cell proliferation. Interleukin-1 also induces T cells to make interleukin-2 and other cytokines necessary for the development of proper T-cell and B-cell responses. Such reponses as these are responsible for the most important quality of a good adjuvant - - its ability to boost both humoral and cell-mediated immune responses.



One of the most famous (but least clinically useful) adjuvants is **Freund's complete adjuvant**. It consists of a preparation of killed mycobacteria in mineral oil. Aqueous vaccine antigen is emulsified with the adjuvant and then inoculated as a water-in-oil emulsion. Freund's complete adjuvant is a potent stimulator of immune responses and is considered a classical adjuvant. Unfortunately some of its relatively unpleasant side-effects (severe local irritation and massive granuloma formation) preclude its widespread use in domestic animals or human beings. Another disadvantage is the presence of the killed mycobacteria, which can convert the vaccinated host to tuberculin-positive status. A modification of Freund's complete adjuvant -- consisting of a similar preparation without the mycobacteria, and called, predictably, **Freund's incomplete adjuvant** - - is available but is much less potent than the complete adjuvant. Without the mycobacterial component, the majority of the adjuvant effect is lost.

Proponents of Freund's complete adjuvant can still take heart, however. The adjuvant-active ingredient in the mycobacterial fraction has been isolated and identified as N-acetylmuramyl-L-alanyl-D-isoglutamine, otherwise known as muramyl dipeptide. Early clinical trials have been promising. A great advantage of using the isolated peptide (or, preferably, its synthetic derivatives) alone is that a complete adjuvant reponse can be elicited without most of the side-effects and without sensitizing the vaccinated individual to tuberculin. *(continued on page 7)*

Part 1: Lead P

Leonard

Editor's Note: Part 1 will discuss clinical, signs, physiology and diagnosis of lead poisoning. Part 2 will provide information on treating lead poisoning in cats.

Lead toxicity is one of the most common toxicities associated with clinical signs in domestic animals. Since small animals have a high potential risk for ingesting lead, this toxicity may have a greater role than previously thought in patients with non-specific neurologic and gastrointestinal symptoms, and in animals with subclinical elevation of blood lead. The onset of clinical signs is often insidious and diagnosis may be difficult.[6]

Epidemiologic data from Australia suggests that lead poisoning accounts for 22 percent of accidental posionings in cats.[7] Limited studies indicate that there are no obvious geographic trends in the United States, but there is a seasonal correlation -- with lead poisoning occurring more frequently in the summer and autumn. This may be due to increased exposure to outdoor sources of lead or an increase absorption of lead from the gastrointestinal tract due to higher endogenous vitamin D levels.

Clinical Signs

Clients that present cats with nonspecific gastrointestinal and/or neurologic signs should be questioned thoroughly about the cat's possible exposure to toxins (table 1). However, often there is no history of lead ingestion, even in confirmed cases. Chronic exposure to lead, and even acute ingestion, may result in a gradual onset of clinical signs. Vomiting, diarrhea, anorexia, constipation, and tenderness on abdominal palpation are usually the first signs of lead toxicity. Subsequent neurologic abnormalities such as hysteria, blindness, convulsions, ataxia, mydriasis, aggression and head pressing may occur, progressing to tonic-clonic seizures and death.

Table 1. Sources of Lead

Lead-based Paint	**Rug Padding**
Solder	**Linoleum**
Putty	**Lead Foil**
Batteries	**Pesticides**
Gasoline	**Roofing**
Lead Acetate Lotions	**Newspaper**
Contaminated Soil & Water	
Hard water from lead pipes	

Physiology

In adult cats approximately 10 percent of ingested lead is absorbed, while kittens may absorb as much as 90 percent.[4] The soluble diphosphate form enters erythrocytes and is transported to soft tissues. It then equilibrates with bone where it is stored as insoluble lead triphosphate. After equilibration the relative distribution is 60 percent in bone, 25 percent kidney, 3 percent intestinal wall, 3 percent reticuloendothelial system, and 4 percent other tissues including erythrocytes. Almost all circulating lead is in erythrocytes. Excretion slowly occurs through biliary (99 percent) and urinary (0.24 percent) routes, although elevated

oisoning in Cats

Rice, D.V.M.

levels may still be present years after initial exposure. Lead can also cross the placenta and may also be found in queen's milk.[1]

Kittens are more susceptible to lead poisoning due to the immaturity of the blood-brain barrier; less efficient detoxification, metabolic and excretory pathways; increased vulnerability of growing tissues; altered GI absorption; and a higher calcium flux rate in growing bones. Poor condition, iron and zinc deficiency, hypoproteinemia, excess vitamin D and coexistant systemic disease may contribute to general severity of the toxicity. Diseases which can cause acidosis, such as distemper and calcium deficiency, may increase lead mobilization from bone. Similarly, treatment with acidifiers such as ammonium chloride or potassium iodide can cause acute lead toxicity in animals with high lead levels in bone tissue.[2]

Lead acts by inhibiting enzymes with free sulfhydryl groups. One of these enzymes is aminolevulinic acid (ALA) dehydrase which converts delta-ALA into porphobilinogen. Lead also inhibits the synthesis of heme from protoporphyrin in erythrocytes. Inadequate heme synthesis causes decreased hemoglobin and anemia. The decreased ALA dehydrase activity causes ALA to be excreted in higher quantities in the urine. There is a greater correlation between clinical signs of lead toxicity and urine ALA levels than with blood lead levels.[8] However, urinary ALA may also be elevated in certain porphyrias.

Diagnosis

Routine tests may rule out several of the differential diagnoses (table 2) and may provide specific evidence of lead poisoning. Characteristic hematologic changes include anemia, nucleated red blood cells (nRBCs), and basophilic stippling of erythrocytes along with anisocytosis, poikilocytosis, polychromasia, and target cells. Nucleated RBCs and basophilic stippling are usually much more marked in lead poisoning than in endotoxemia, neoplasia, myelofibrosis, marrow hemorrhage, extramedullary hematopoeisis and some anemias.[3] Nucleated RBCs may range from 5 to 40/100 WBC in lead poisoning and are seen regardless of the stain used. Their presence reflects damage to the barrier that normally prevents their release from the bone marrow.

Special stains, such as the Romanowski stain, are needed to see the fine to coarse basophilic granulation or stippling that occurs with ineffective erythropoeisis. The stippling is due to the aggregation of ribosomes in young erythrocytes

Table 2. Differential Diagnosis

Distemper
Gastrointestinal Parasitism
Encephalitis
Epilepsy
Acute Pancreatitis
Rabies
Other Poisonings
Nonspecific GI & Neurologic Diseases

during drying. Stippling decreases if sodium EDTA and potassium oxalate are used to preserve blood samples. Lead poisoning is presumed if there are greater than 40 stippled RBCs per 10,000 RBCs and the patient presents with typical clinical signs of lead poisoning.[5] White blood cell counts are artificially increased if the nRBCs are not accounted for in automated leukograms.

Bone marrow erythroid hyperplasia might be seen in response to mild anemia. Serum chemistry is usually normal.

Since ingested lead is often in the form of lead dust that has been ingested while grooming, GI lead particles are not very radiodense. Occassionally, increased metaphyseal density (lead lines) in the radius, ulna and fibula may be seen. These are not actually due to lead, but reflect increased mineralization of cartilage at the sites of endochondral ossification (eg. phosphorus and vitamin D intoxication.) These changes are difficult to see and thus are not very useful in diagnosis. Electroencephalography may reveal nonspecific irregular high-amplitude, slow-wave activity in cases with significant neurologic abnormalities, but it is most useful prognostically in assessing permanent sequelae.[3] Cerebrospinal fluid analysis may be useful in distinguishing the encephalopathy of lead poisoning from encephalitis.

Definitive diagnosis is obtained by measuring blood lead levels in the patient, or liver lead levels in the postmortem animal. In general, any value greater than 60 µg/dl, or any value greater than 40 µg/dl with associated clinical symptoms, is diagnostic of lead poisoning in small animals. Blood lead values less than 40 µg/dl must be interpreted in light of clinical and laboratory findings. (Toxicologists at the Cornell College of Veterinary Medicine regard any lead value greater than 10 µg/dl as suggestive of lead toxicity.) Whole blood for testing should be submitted heparinized (do not preserve with EDTA or potassium oxalate). Liver lead greater than 10 µg/dl is also diagnostic.

Blood lead is an excellent indicator of lead absorption, however delta-ALA correlates better with clinical signs. Therefore, urinary ALA can also be used to diagnose and monitor lead poisoning. A wide range of normal values are reported for the cat and dog, but in general any value greater than 600 µg/dl is strongly suggestive of lead poisoning. When testing, measure 24-hour ALA excretion, single sample ALA and urine specific gravity, or ALA:creatinine ratio to compensate for urine concentration. ALA measurements can be done using an in-house testing kit (Davis Urinary ALA Test, BioRad Laboratories, Richmond, California). ■

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Vaccines and Adjuvants

(continued from page 3)

Liposomes - - evanescent microscopic spheres made of phospholipid bilayers separated by aqueous compartments - - represent a promising new approach to vaccine antigen delivery. In this system the antigen is trapped within the liposomes, which are then injected into the host. The liposomal delivery system acts not only as a depot for antigen delivery but also enhances the activation of macrophages - - an important event in the control of many intracellular disease agents. Liposomes may be given by the conventional intramuscular route or by intravenous injection.

A new and related area of adjuvant research involves the so-called **immune-stimulating complexes**, or **ISCOM**. The ISCOM are analogous to liposomes, consisting primarily of a glycoside, Quil A, within which the antigenic material is incorporated. An experimental ISCOM-based feline leukemia virus vaccine has been developed by European researchers.[2] This new vaccine has shown great promise thus far in early vaccine trials; the results of further ISCOM research - - and other adjuvant research as well - - are awaited with anticipation. ■

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In the News ...

Lyme Disease Testing for Cats

The Diagnostic Laboratory at Cornell College of Veterinary Medicine can now test cats for Lyme disease using indirect fluorescent antibody (IFA) test. The cost is $12. Approximately 0.5 ml of serum is required to run the test. Additional information on submitting samples for testing can be obtained by contacting the Diagnostic Laboratory (607) 253-3900.

Robert H. Winn Foundation Funds Nutrition Study at Cornell

Funds are being provided to complete a study that focuses on the mechanism of vitamin E utilization during taurine deficiency and fat absorption and metabolism in deficient cats. Investigators on this project are Dr. Harold Hintz, Dr. Herbert Schryver, and Joyce Carnevale. The first part of the project was funded by the Birmingham Feline Fanciers, Inc.

Feline Specialist Seminar

The Cornell Feline Health Center, the Office of Continuing of Education of Cornell's College of Veterinary Medicine and the American Association of Feline Practitioners are sponsoring a comprehensive seminar in feline medicine August 7 to 11 in Ithaca, NY. Topics scheduled for the five-day seminar include:

- *Abdominal Ultrasound*
- *Adrenal Disorders*
- *Bronchial Disease*
- *Cardiomyopathy and New Cardiotherapeutic Drugs*
- *Contrast Cystography*
- *Dental and Oral Diseases and Treatment*
- *Dermatology*
- *Diabetes*
- *Feline Practice Management*
- *Hyper'ıyroidism*
- *Infectious Disease Update*
- *Liver Disorders and Bile Salts*
- *Pharmacology*
- *Reproductive Endocrinology and Disorders*
- *Salmonellosis*
- *Toxicology*

Cost for the seminar is only $250 and includes admission to all sessions, a wine/cheese social and a catered dinner at a state park. Additional details are available by contacting **Linda Ritzler, Office of Continuing Education, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853.** Space is limited, so you are urged to register early for this comprehensive seminar.

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