Veterinary Medical Specialization

Bridging Science and Medicine

Edited by

W. Jean Dodds

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and Comparative Medicine

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Veterinary Medical Specialization: Bridging Science and Medicine

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Dr. W. Jean Dodds
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PREFACE

This volume is the first of the series for which I am privileged to serve in the capacity of Series Editor. The subject, veterinary medical specialization, is the bridge between practicing clinical veterinarians and academic scientists that generates new knowledge to further the art of veterinary medicine. Of course, much of the scientific discovery that benefits animal medicine is derived from the basic and applied sciences with the original purpose of benefitting human health. This often includes biomedical research on animals along with *in vitro* alternatives to animal testing. Much of the information gathered from the biomedical research effort can be applied equally to human and veterinary medicine.

It is not surprising that the veterinary profession has evolved a series of subspecialties over the past two decades that parallels specialization in human medicine. This follows the explosion of knowledge in basic science and medicine from the 1960s to the era of molecular biology and gene therapy we have entered today. My own career, which spans 30 years, attests to this change. As a biomedical scientist who developed an interest and expertise in comparative hemostasis, I have seen the field develop from a clinical specialty with rather unsophisticated techniques for manually monitoring whole blood coagulation activity in glass and silicone-coated test tubes to the most advanced applications of biochemical and molecular techniques. Today, scientists working in academia and private industry are cloning the genes that produce individual coagulation factors and sequencing the gene products. They can even manipulate experimental animals through gene therapy to correct inherited bleeding disorders. Coagulation factor concentrates are routinely produced by recombinant technology for treatment of diseases such as hemophilia to avoid the serious risk of transfusion-transmitted disease associated with the use of blood plasma concentrates.

To be able to see a particular medical specialty evolve during my career has been a stimulating and challenging experience. During this time, scientific advances in hemostasis research have been translated

into clinical benefits such that the diagnosis, management, and treatment of bleeding diseases in both human and veterinary medicine have advanced considerably. In veterinary medicine today, blood components available for treating animals with bleeding disorders include packed red blood cells, fresh-frozen plasma, platelet-rich plasma, and cryoprecipitate. Perhaps the most gratifying experience for me personally has been a growing awareness of the value of all sentient life, which evolved from an appreciation of the fact that one can pursue a fruitful biomedical research career without undertaking invasive experimentation on animals. These studies focused on animals born with naturally occurring genetic defects to learn more about the biochemistry and pathophysiology of their disorders, develop new diagnostic tests for clinical diagnosis and research investigations, and perfect better treatment methods to prevent and control the disorders. The current interest in identifying and screening for genetic diseases in veterinary medicine is exemplified by this research effort. We have entered a time of great promise in applying molecular techniques and genetic engineering to correcting many animal and human diseases.

The present volume reviews the historical, current, and future needs for specialization in the veterinary profession, discusses the emerging importance of appropriate informed consent for all clinical and experimental trials, and deals with veterinary medical ethics as applied to specialization in clinical medicine. I thank authors Clinton Lothrop, Dawn Boothe and Margaret Slater, and Jerrold Tannenbaum for their insightful contributions to these subjects. My own chapter reviews current information from health surveys and genetic screening of selected dog breeds for inherited and other diseases, and George Happ presents a timely review of autoimmune thyroiditis as a model canine autoimmune disease. Thyroid disease is considered by veterinarians and purebred dog fanciers to be a major problem of increasing prevalence, as well as an area of my own special interest. It is hoped that basic research into the mechanisms of thyroid disease and dysfunction in the dog will provide more insight into the equivalently common hyperthyroid disorder of the cat.

W. JEAN DODDS

Overview: Bridging Basic Science and Clinical Medicine

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I. Background

A. Basic and Applied Animal Research

During the past century, advances in medical knowledge have contributed not only to basic science but also to clinical medicine. With respect to veterinary medicine, biomedical research on experimental animal subjects along with basic science using nonanimal methods have enhanced our understanding of the physiology and pathophysiology of animal health and disease. Because a vast data base has been generated from animal-based experiments designed primarily to benefit human health and well-being, parallel benefits have been accorded to animals (Dodds, 1988; Patterson *et al.*, 1988; Wagner, 1992; Lawrence, 1994). The research field of comparative medicine evolved from

this perspective and was based on the study of naturally occurring or induced animal models of human disease (Dodds, 1988; Patterson *et al.*, 1988). As alluded to in the Preface and discussed in the reviews by Jolly *et al.* (1981), Dodds (1988), Patterson *et al.* (1988), and Smith (1994), investigations of animal models have provided important basic information about the mechanism of specific disease states, allowed for development and improvement in diagnostic tests for these conditions, and have led to advances in management and treatment methods. For the past three or more decades, studies of animal disease models have contributed significantly to the understanding of analogous human diseases. Examples include the inherited bleeding disorders studied by this author and others (Jolly *et al.*, 1981; Dodds, 1988, 1989), congenital cardiac disease and inborn errors of metabolism (Patterson *et al.*, 1988), neuromuscular and copper storage disorders (Kramer *et al.*, 1981; Brewer *et al.*, 1992), and the inherited eye diseases (Smith, 1994).

The net effect of these basic and comparative medical advances has been to translate the findings to improve diagnostic and treatment modalities in clinical veterinary medicine. This has fostered the development of veterinary specialization, which brings existing knowledge from the basic sciences and clinical human medicine to clinical veterinary medicine, and investigates new basic and applied research initiatives. As might be expected, the evolution of this new area has sparked not only scientific and medical benefits but also controversy, as the specialties have become officially recognized and a certification process has been created to establish guidelines for the entry of new members (Stromberg and Schneider, 1994). A more detailed look at veterinary medical specialization can be found in the chapter by Lothrop in this volume.

B. Early Practices

Over the years, individuals with specific interests have developed expertise in defined fields of veterinary medicine. These pioneers, through teaching seminars at regional and national meetings, writing scientific medical articles and textbooks, and training interns, residents, and other graduates, served as mentors for the formal definition of veterinary medical specialties. The founders of this movement included colleagues such as Drs. Stephen J. Ettinger, William F. Jackson, William J. Kay, and Robert W. Kirk. This group of esteemed colleagues served as a nucleus for ongoing support of the development of specialization in veterinary medicine, and has encouraged the more

widespread introduction of specialists into clinical veterinary practice (Stromberg and Schneider, 1994). Some of the first specialties to evolve and be recognized by the American Veterinary Medical Association were the American College of Veterinary Pathologists and American Board of Veterinary Public Health, both in 1951 (the latter group was renamed the American College of Veterinary Preventive Health in 1978); American College of Laboratory Animal Medicine in 1957; American College of Veterinary Radiology in 1962; American College of Veterinary Microbiology in 1966; and the American College of Veterinary Surgeons and American Board of Veterinary Toxicology, both in 1967 (AVMA, 1995). Since then, other specialties developed, including those for theriogenology, ophthalmology, and veterinary internal medicine with its subspecialties of cardiology, internal medicine, neurology, veterinary medical oncology, and anesthesiology. New specialties continue to be added and these are approved and governed by the American Veterinary Medical Association through the American Board of Veterinary Specialties. (For more details on these specialties, refer to the chapter by Lothrop in this volume.) The first board devoted to general veterinary practice specialties was formed in 1978 (AVMA, 1995). This is called the American Board of Veterinary Practitioners and includes the specialties of avian, canine and feline, dairy, equine, food animal, and swine health management practices.

In 1982, a new organization called the National Academies of Practice was established in Washington, DC. Patterned after the National Academy of Sciences, the purpose of this organization is to recognize various medical clinical specialties, and membership is based upon election by one's peers as a Distinguished Practitioner in a specific medical specialty. The National Academies of Practice specialties include Dentistry, Medicine, Nursing, Optometry, Osteopathic Medicine, Podiatric Medicine, Psychology, Social Work, and Veterinary Medicine. Veterinary Medicine became one of the nine Academies of Practice in 1984. The current Executive Director is a veterinarian, Dr. John B. McCarthy, and there are presently 105 active and emeritus Distinguished Practitioners of the National Academy of Practice in Veterinary Medicine (McCarthy, 1995). For the past two years, a special symposium on Veterinary Medicine and Human Health has been sponsored by the Academy and held in conjunction with the annual meeting of the American Veterinary Medical Association. A second program was sponsored by the Academy in 1995 in conjunction with the silver anniversary symposium of the Student Chapters of the American Veterinary Medical Association (McCarthy, 1995).

II. Emergence of Veterinary Medical Specialization

A. Introduction

Since the early days of veterinary medical specialization, 19 recognized colleges and specialty boards of the American Veterinary Medical Association have evolved with more than 4,400 certified diplomats (AVMA, 1995). Over the years, veterinary specialists were primarily employed by academia, industry, government agencies, and large veterinary specialty practices or institutions. The present increasing trend for the development of clinical specialty practices in the private sector should be encouraged, as general practitioners benefit from working closely with specialist colleagues in the community. As might be expected, however, this emphasis on specialization has resulted in "growing pains." The first of these arose from the need of specialists and generalists to follow appropriate guidelines for their roles in the practice of veterinary medicine, in order to minimize overlap and the perceived or actual encroachment on their respective turfs. A second, more difficult challenge related to the training and standards required for entry into a specialty with the goal of subsequent board certification in that specialty. Because most of the training programs are offered by veterinary medical teaching institutions, one could argue that these standards may not necessarily reflect the needs in specialty clinical practice. Thus, there has been a need to diversify training programs, specify the board certification process and professional certifying examinations that reflect the state of the art in each specialty, and ensure fair and legally defensible standards (Stromberg and Schneider, 1994).

As pointed out in a recent review by Stromberg and Schneider (1994), the law of due process requires that any standards upon which an individual's economic opportunities may be affected must be "rationally related" to the stated purpose of the process of certification. This means that the requirements for candidates to become certified must accurately measure their competence in the specialty to which they request certification. While it is clear that the appropriate written and oral examinations may test a candidate's skill in the field and that certain educational requirements are necessary to satisfy eligibility, several of the specialty organizations also require that the candidate prepare case reports, publish a minimum number of articles as first author, or spend some time away from clinical practice performing research. As stated by Stromberg and Schneider (1994), "These requirements may not be supportable under the law, because they do not

necessarily measure or ensure practitioner clinical competence." The objective of requiring case reports may be to demonstrate that candidates have managed a variety of appropriate cases during their residency or other training, whether the cases have been managed properly, and whether the candidate can write an appropriate description of the clinical laboratory and treatment records for the case. However, the question remains about how many case reports a candidate would be expected to prepare to be truly reflective of the variety of cases more commonly seen in specialty practice. If the selected cases represent rarely encountered clinical disorders, one could argue that this does not reflect the ability of the candidate to deal with the more common cases seen in a typical specialty practice. With respect to publishing case reports, writing skills may be less important than oral communication in the practice of a clinical specialty. With respect to the certifying examination, an argument can be made that merely being accepted and successfully completing a clinical residency program should lead the way to certification, for only about 10% of all licensed veterinarians pursue specialty training and not all of these complete a formal residency program or the specialty examination process (Stromberg and Schneider, 1994). Finally, these investigators outline a series of due process requirements that ensure procedural fairness (Stromberg and Schneider, 1994):

- Are certification requirements clearly set out and conveyed to potential candidates?
- Are rules and requirements for certification followed equally in all cases?
- Is the grading system unbiased?
- Is there a clearly stated, meaningful appeal process that is strictly adhered to?
- Do rules governing retaking a portion or all of the examination result in equal treatment of candidates?

Answers to these questions have been offered by the authors who indicate that they should "provide guidelines for modifying existing certification programs to make them more useful to the profession and the public" (Stromberg and Schneider, 1994).

B. Scientific Advances

1. Basic and Clinical Immunology

During my 30-year career in biomedical research, the scientific advances made in the field of hematology and immunology have been

remarkable (Dodds, 1988, 1992b). Interest in basic immunology has increased over this period and has been further sparked by the discovery of a group of retroviral agents affecting various mammalian species and inducing profound immunological dysfunction and suppression as well as hematopoietic and other cancers. The discovery in the 1980s of human lenteviruses that produce adult T-cell leukemia and acquired immune deficiency syndrome, with its devastating effects throughout the world, has increased research efforts and funding for this area of science and medicine (Marx, 1990). Early studies of the immune system were focused on the phenomenon of the body's ability to generate specific protective immunity following exposure to infectious or toxic agents. This basic knowledge has progressed to an understanding of the cellular molecular components involved in the immune system, definition of the B- and T-cell systems, and the role of genetic determinants mediated through the major histocompatibility complex (Marx, 1990). Today, the molecular basis of antigenic recognition by T-cells and their pathways of activation, inactivation, and exhaustion have been defined (Lanzavecchia, 1993). The importance of T-lymphocytes in immune functions is underscored by their central role in the immune response. In this capacity, they kill infected cells, control inflammatory responses, and help B-lymphocytes to make antibodies. The T-cell receptor on the cell surface recognizes antigens presented to it as a complex of a short peptide bound to a molecule of the major histocompatibility complex present on the surface of another cell. This latter cell is called an antigen presenting cell. The major histocompatibility complex is made up of two molecules: class I determinants which are expressed on all cells, and class II determinants which are expressed on macrophages, dendritic cells, B-cells, and occasionally on other cells. The major histocompatibility complex is highly polymorphic, and different allelic forms of the molecules have different specific peptide binding characteristics (Lanzavecchia, 1993; Shoenfeld, 1994).

The fact that antigenic peptides derived from intact proteins bind directly to major histocompatibility class I or class II molecules present on cell surfaces offers potential targets for immune intervention, because it allows selected antigenic peptides to be added to T-cells exogenously (Lanzavecchia, 1993). Knowledge of these basic immune mechanisms has made it possible to identify strategies for immune intervention in order to design protective vaccines; for example, to induce effective responses to tumor antigens and even to control graft rejection and autoimmune diseases (Lanzavecchia, 1993). These situations provide exciting possibilities for future research. I have a specific

interest in vaccine immunology not only because of the need to develop new approaches to protecting the host from immunological and infectious challenge (Shoenfeld and Cohen, 1987; Tomer and Davies, 1993), but also to better understand the earlier and current increases in adverse reactions to vaccines in both human and animal populations (Tizard, 1990; Dodds, 1995b). While the goal of vaccination was originally to protect against infectious diseases, this approach has now been broadened to include treatment of tumors, allergies, and even for treatment of autoimmune diseases. However, it is quite clear that in some cases vaccination may result in exacerbation of disease (Tizard, 1990; Oehen *et al.*, 1991; Dodds, 1995a,b).

2. *Immunological Effects of Vaccines*

Combining viral antigens, especially those of modified-live virus (MLV) type which multiply in the host, elicits a stronger antigenic challenge to the animal (Tizard, 1990). This is often viewed as desirable because a more potent immunogen presumably mounts a more effective and sustained immune response. However, it can also overwhelm the immunocompromised or even a healthy host that is continually bombarded with other environmental stimuli and has a genetic predisposition that promotes adverse response to viral challenge (Phillips and Schultz, 1992; Dodds, 1995a,b). This scenario may have a significant effect on the recently weaned young animal that is placed in a new environment. Furthermore, while the frequency of vaccinations is usually spaced 2–3 weeks apart, some veterinarians have advocated vaccination once a week in stressful situations. While young animals or even children exposed frequently to vaccine antigens at the dosages given to adults may not demonstrate overt adverse effects, their relatively immature immune systems can be temporarily or more permanently harmed by such antigenic challenges (Moyes and Milne, 1988; Garenne *et al.*, 1991; Phillips and Schultz, 1992; Stratten, 1993; Dodds, 1995b). Consequences in later life may be the increased susceptibility to chronic debilitating diseases. Some veterinarians trace the increasing current problems with allergic and immunological diseases to the introduction of MLV vaccines some 20 years ago (Tizard, 1990; Dodds, 1995a). While other environmental factors no doubt have a contributing role, the introduction of these vaccine antigens and their environmental shedding may provide the final insult that exceeds the immunological tolerance threshold of some individuals (Dodds, 1995b).

Recent studies with MLV herpes virus vaccines in cattle have shown them to induce necrotic changes in the ovaries of heifers that were vaccinated during estrus (Smith *et al.*, 1990). The vaccine strain of this

virus was also isolated from control heifers that apparently became infected by sharing the same pasture with the vaccinates. Furthermore, vaccine strains of these viral agents are known to be causes of abortion and infertility following herd vaccination programs. Another example of the dangers inherent to vaccinating animals during periods of sex hormonal change was the abortion and death seen following vaccination of pregnant dogs with a commercial canine parvovirus vaccine that was contaminated with blue tongue virus (Wilbur *et al.*, 1994).

The future will evolve new approaches to vaccination including subunit vaccines, recombinant vaccines using DNA technology, and killed products with new adjuvants to boost and prolong protection (Lanzavecchia, 1993; Stratten, 1993; Shoenfeld, 1994). These are not simple solutions to the problem, however, because early data from recombinant vaccines against some human and mouse viruses have shown potentially dangerous side effects by damaging T-lymphocytes. Contributing factors were shown to be the genetic background of the host, the time or dose of infection, and the makeup of the vaccine (Oehen *et al.*, 1991). We are obviously still a long way from producing a new generation of improved and safe vaccines (Cohen, 1994a). In the meantime, we should use inactivated vaccines whenever they are available and should consider giving them more often (twice yearly rather than annually) for high-risk exposure situations (Dodds, 1995b). Vaccines, while necessary and generally safe and efficacious, can be harmful or ineffective in selected situations (Tizard, 1990; Phillips and Schultz, 1992). The most recent alarming adverse vaccine reactions have been the tragic mortalities following use of high-titered measles vaccines in infants (Garenne *et al.*, 1991), refractory injection-site fibrosarcomas in cats (Kass *et al.*, 1993), and the abortions and deaths of pregnant dogs vaccinated with a blue tongue virus-contaminated commercial vaccine (Wilbur *et al.*, 1994).

C. Health Surveys and Genetic Screening

Epidemiologic and demographic studies of human populations have yielded important information about worldwide trends in human health and disease, and have contributed to the long-standing debate about the relative influences of environment and genetics on such factors as intelligence, behavior, physical characteristics, and longevity (Gibbons, 1995). During the same period, epidemiological studies of animal populations were directed primarily at issues related to public health and control of infectious diseases. More recently, comparative

epidemiologists and geneticists have turned their attention to studying populations of related animals to identify biochemical markers to be used as screening tests for genetic diseases, and to performing population health surveys to more accurately describe the health problems affecting the group as a whole. Over the past two to three decades, these approaches have been applied to the study of companion animal populations with the goals of learning more about the diseases themselves and also reducing or eliminating the number of affected and carrier individuals (Jolly *et al.*, 1981; Dodds, 1988; Patterson *et al.*, 1988; Smith, 1994). Established national screening programs for hip dysplasia; inherited blood, cardiac, and eye diseases; and screening for congenital deafness are examples of the more widely appreciated screening programs. (Specific details of these and other population screening programs are discussed in Chapter 2 of this volume.)

D. Nutrition and the Immune System

Wholesome nutrition is a key component to maintaining a healthy immune system and resistance to disease (Sheffy and Schultz, 1979; Corwin and Gordon, 1982; Tengerdy, 1989; Alexander and Peck, 1990; Burkholder and Swecker, Jr., 1990; Turner and Finch, 1991; Berdanier, 1994a,b; Dodds and Donoghue, 1994). Many environmental factors trigger immune dysfunction leading either to immune deficiency states or immune stimulation (reactive states or autoimmunity) (Shoenfeld and Cohen, 1987; Dodds, 1992b). Autoimmunity literally means immunity against self and is caused by an immune-mediated reaction to self-antigens (i.e., failure of self-tolerance)(Sinha *et al.*, 1990). Susceptibility to autoimmune disease has a genetic basis in humans and animals (Marx, 1990; Carson, 1992; Shoenfeld, 1994). Numerous viruses, bacteria, chemicals, toxins, and drugs have been implicated as the triggering environmental agents in susceptible individuals (Marx, 1990). This mechanism operates by a process of molecular mimicry and/or nonspecific inflammation (Sinha *et al.*, 1990). The resultant autoimmune diseases reflect the sum of the genetic and environmental factors involved. Autoimmunity is most often mediated by T-cells or their dysfunction. As stated in a recent review, "perhaps the biggest challenge in the future will be the search for the environmental events that trigger self-reactivity" (Sinha *et al.*, 1990).

Affected individuals have generalized metabolic imbalance and often have associated immunological dysfunction. An important facet of managing these cases is minimizing exposure to unnecessary drugs, toxins, and chemicals and optimizing nutritional status with healthy

balanced diets (Marx, 1990; Alexander and Peck, 1990; Sinha *et al.*, 1990). Because of the genetic predisposition to autoimmune disorders the same recommendations apply to family members (Trence *et al.*, 1984). Individuals susceptible to these disorders are at increased risk for adverse effects from immunological challenges of many kinds including polyvalent modified-live or inactivated vaccines and other chemicals, drugs, and toxins. Related to these events is the susceptibility to and development of cancer, which reflects a disruption of cell growth control (Dodds, 1995a,b).

1. Immune-Suppressant Viruses

Immune-suppressant viruses of the retrovirus, parvovirus, and other classes have recently been implicated as causes of bone marrow failure, immune-mediated blood diseases, hematologic malignancies (lymphoma and leukemia), dysregulation of humoral and cell-mediated immunity, organ failure (liver, kidney), and autoimmune endocrine disorders especially of the thyroid gland (thyroiditis), adrenal gland (Addison's disease), and pancreas (diabetes) (Young and Mortimer, 1984; Trence *et al.*, 1984; Shoenfeld and Cohen, 1987; Dodds, 1988; Krieg *et al.*, 1992; Tomer and Davies, 1993). Viral diseases and recent vaccination with monovalent or polyvalent vaccines are increasingly recognized contributors to immune-mediated hematologic and other autoimmune diseases, bone marrow failure, chronic degenerative disorders, and organ dysfunction (Shoenfeld and Cohen, 1987; Tizard, 1990; Oehen *et al.*, 1991; Tomer and Davies, 1993; Dodds, 1995a,b). Genetic predisposition to these disorders in humans has been linked to the leucocyte antigen D-related gene locus of the major histocompatibility complex, and is likely to have parallel associations in domestic animals (Marx, 1990; Carson, 1992; Dodds, 1992b).

2. Nutritional Factors Influencing Immunity

Nutritional influences are important in managing a variety of inherited and other metabolic diseases as well as for a healthy immune system. Examples where nutrition plays a significant role in disease include: adding ingredients to the diet to make it more alkaline for miniature schnauzers with calcium oxalate bladder or kidney stones; use of the vitamin A derivative etretinate in cocker spaniels and other breeds with idiopathic seborrhea; management with drugs and/or diet of diseases such as diabetes mellitus and the copper-storage disease prevalent in breeds like the Bedlington terrier, West Highland white terrier, and Doberman pinscher; wheat-sensitive enteropathy in Irish setters; and treatment of vitamin B-12 deficiency in giant schnauzers

(Dodds and Donoghue, 1994). Other nutritional influences include the vitamin K-dependent coagulation defect elicited in Devon rex cats following vaccination; hip dysplasia in puppies fed excessive calories; osteochondritis dissecans in dogs fed high levels of calcium; and hypercholesterolemia in inbred sled dogs fed high-fat diets (Dodds and Donoghue, 1994).

Nutritional factors that play an important role in immune function include zinc, selenium and vitamin E, vitamin B-6 (pyridoxine), and linoleic acid (Hayes *et al.*, 1970; Sheffy and Schultz, 1979; Corwin and Gordon, 1982; Tengerdy, 1989; Burkholder and Swecker, Jr., 1990; Turner and Finch, 1991). Deficiencies of these compounds impair both circulating (humoral) as well as cell-mediated immunity. The requirement for essential nutrients increases during periods of rapid growth or reproduction and also may increase in geriatric individuals, because immune function and the bioavailability of these nutrients generally wanes with aging. As with any nutrient, however, excessive supplementation can lead to significant clinical problems, many of which are similar to the respective deficiency states of these ingredients (Diplock, 1976; Burk, 1983; Tengerdy, 1989; Turner and Finch, 1991).

3. Nutrition and Thyroid Metabolism

Nutritional factors can have a significant effect on thyroid metabolism (Berry and Larsen, 1992; Ackerman, 1993). The classical example is the iodine deficiency that occurs in individuals eating cereal grain crops grown on iodine-deficient soil. This impairs thyroid metabolism because iodine is essential for formation of thyroid hormones. Another important link has recently been shown between selenium deficiency and hypothyroidism (Berry and Larsen, 1992). Cereal grain crops grown on selenium-deficient soil contain relatively low levels of selenium. While commercial pet food manufacturers compensate for variations in basal ingredients by adding vitamin and mineral supplements, it is difficult to optimize levels for so many different breeds of animals having varying genetic backgrounds and metabolic needs (Cargill, 1993; Cargill and Thorpe-Vargas, 1993, 1994; Berdanier, 1994a,b; Dodds and Donoghue, 1994).

The selenium-thyroid connection has clinical relevance, because blood levels of total and free thyroxine (T4) rise in selenium deficiency (Berry and Larsen, 1992). This effect does not get transmitted to the tissues, however, as evidenced by the fact that blood levels of the regulatory thyroid stimulating hormone (TSH) are also elevated or unchanged. Thus, selenium-deficient individuals showing clinical signs of hypothyroidism could be overlooked on the basis that blood levels of

the T4 hormones appeared normal (Ackerman, 1993). The selenium issue is further complicated because synthetic antioxidants used to preserve pet foods have the potential to change the bioavailability of vitamin A, vitamin E, and selenium and alter cellular metabolism by inducing or lowering cytochrome P450, glutathione peroxidase (a selenium-dependent enzyme), and prostaglandin levels (Parke *et al.*, 1972; Combs, Jr., 1978a,b; Langweiler *et al.*, 1983; Rossing *et al.*, 1985; Kagan *et al.*, 1986; Kim, 1991; Meydani *et al.*, 1991). As manufacturers of many premium pet foods began adding the synthetic antioxidant ethoxyquin in the late 1980s, its effects along with those of the other synthetic preservatives, discussed in Section *4* following, may well be detrimental over the long term (Cargill, 1993; Cargill and Thorpe-Vargas, 1993, 1994). The way to avoid this potential risk is to use foods preserved with natural antioxidants such as vitamin E and vitamin C or feed only home-cooked fresh, natural ingredients (Cargill and Thorpe-Vargas, 1994; Dodds and Donoghue, 1994).

4. Effects of Synthetic Antioxidants

Synthetic antioxidants like butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) have been used as preservatives in human and animal foods for more than 30 years. A more potent chemical antioxidant 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin) has also been used during this period but only recently has become the preferred antioxidant for preserving the premium commercial dog and cat foods (Cargill, 1993; Cargill and Thorpe-Vargas, 1993). Many pet food manufacturers choose ethoxyquin because of its excellent antioxidant qualities, high stability, and reputed safety. However, ongoing controversy surrounds issues about its safety when regularly fed at permitted amounts in dog and cat foods. The only chronic feeding trials in dogs were completed 30 years ago and were medically and scientifically flawed by today's standards, and no feeding trials to address the safety of this preservative have been conducted in cats (Cargill and Thorpe-Vargas, 1993). Most of the safety questions pertain to genetically susceptible breeds of inbred or closely linebred dogs. Toy breeds may be particularly at risk because they eat proportionately more food and preservative for their size in order to sustain their metabolic needs (Cargill, 1993; Cargill and Thorpe-Vargas, 1993; Dodds and Donoghue, 1994).

Ethoxyquin is absorbed into the body via the gastrointestinal tract and then exerts its antioxidant effect (Skaare and Nafstad, 1979). This changes the overall balance of oxidation/reduction in the body so that functions dependent upon oxidation, especially those involving per-

oxides, are reduced (Parke *et al.*, 1972; Kahl, 1984; Rossing *et al.*, 1985; Kagan *et al.*, 1986; Kim, 1991). This in turn decreases prostaglandins and other eicosanoids (thromboxanes in platelets and leukotrienes in leukocytes) (Meydani *et al.*, 1991). Thus, synthesis of hormones like progesterone, estrogen, and testosterone can be impaired and thereby could alter reproductive performance in males and females (Dunkley *et al.*, 1968; Steele *et al.*, 1974). Ethoxyquin has also been shown to cross the placenta, thereby exposing the developing fetuses which would be continuously reexposed in their closed amniotic environment until birth. Effects of ethoxyquin on other steroid hormones such as the glucocorticoids and aldosterone could alter responses to stress and kidney function. Alteration of cytochrome P450 affects hydroxylation of foreign substances and drugs (Rossing *et al.*, 1985). Diminished ability to hyroxylate would impair the body's capacity to detoxify and excrete toxic or pharmacological compounds (Kahl, 1984).

Theoretically, imbalances of essential vitamins and minerals could occur when the body's natural antioxidant system is disrupted by the presence of synthetic antioxidants (March *et al.*, 1968; Hayes *et al.*, 1970; Mathias and Hogue, 1971; Combs, Jr., 1978a,b; Langweiler *et al.*, 1983). Ethoxyquin simulates vitamin E *in vivo* and apparently can raise hepatic levels of vitamin A severalfold while lowering bioavailability and tissue requirements for vitamin E and selenium (Skaare *et al.*, 1977; Nafstad and Skaare, 1978; Combs, Jr., 1978b; Kim, 1991; Cargill and Thorpe-Vargas, 1993, 1994). These biological effects are troublesome as vitamin A is essential for many biochemical pathways including thyroid metabolism, and vitamin E and selenium are critical to maintain integrity of the immune system. As the clinical signs of toxicity and deficiency of these important nutrients are similar, any observed clinical effects could be related to either an excess and/or a deficiency state (Diplock, 1976; Sheffy and Schultz, 1979; Burk, 1983; Tengerdy, 1989). Some pet food manufacturers have addressed these concerns by lowering the levels of ethoxyquin added to the finished products from 120–150 ppm (the legal limit) to as low as 30–40 ppm. The cumulative antioxidant load needs to be considered, however, because use of BHA or BHT to preserve animal fat sources is additive to the ethoxyquin incorporated into the finished product.

Antioxidants also can induce both toxic and protective effects on biomembranes (Parke *et al.*, 1972; Kagan *et al.*, 1986). Natural antioxidants (tocopherals or vitamin E, and ubiquinols) contain hydrocarbon tails and so do not disturb the membrane lipid bilayer, whereas synthetic antioxidants which are devoid of hydrocarbon tails can exert toxic and destructive effects on biomembranes (Rossing *et al.*, 1985;

Kagan *et al.,* 1986). Selected examples include effects on erythrocyte membranes which induce red cell hemolysis, on sarcoplasmic reticular membranes which inhibit calcium transport, and on platelet membranes where they inhibit calcium ion-dependent platelet aggregation (March *et al.,* 1969; Diplock, 1976). As these antioxidants are the substrates for cytochrome P450, oxidative hydroxylation occurs which produces a relatively short half-time in biomembranes and the body (Rossing *et al.,* 1985; Kagan *et al.,* 1986). This makes synthetic antioxidants ten- to twentyfold more potent as inhibitors of lipid peroxidation. However, the side effects from changes in membrane function can have important biological consequences (Kagan *et al.,* 1986).

Naturally occurring antioxidants (such as tocopherol and ascorbic acid) are also used in pet foods, and have become more popular in response to consumer and professional queries about the effects of chronically feeding chemical antioxidants to pets (Cargill and Thorpe-Vargas, 1993, 1994; Dodds and Donoghue, 1994). While naturally occurring antioxidants are somewhat less effective and more expensive than the synthetic antioxidants, proponents believe their safety outweighs these drawbacks. It should be appreciated, however, that pet foods devoid of chemical antioxidants added at the time of processing often contain ingredients (such as animal tallow or other fats and oils) that are preserved with antioxidants. Thus, claims made about the use of "all natural" antioxidant preservatives should also apply to preservatives used in the raw materials (Dodds and Donoghue, 1994).

The synthetic antioxidants (BHA, BHT, propyl gallate, and ethoxyquin) have been linked to inducing, promoting, and protecting against a variety of cancers, although the literature is both disturbing and contradictory in this regard (Skaare *et al.,* 1977; Pearson *et al.,* 1983; Kahl, 1984; Ito *et al.,* 1986; Manson *et al.,* 1987; Cargill and Thorpe-Vargas, 1994). Synthetic antioxidants induce cytochrome P450 and glutathione peroxidases which results in increased levels of the reactive hydrogen peroxides and oxygen radicals that affect cellular metabolism (Burk, 1983; Pearson *et al.,* 1983; Rossing *et al.,* 1985). Increases in these potentially harmful activated oxygen molecules are counterbalanced during normal cellular metabolism by a complex natural antioxidant defense system including the glutathione peroxidase enzymes, catalase, superoxide dismutase, and vitamins C and E (Prestera *et al.,* 1993; Rose and Bode, 1993). Oxidative stress occurs in the body when the balance between free radical fluxes and the antioxidant defense system is impaired. But oxidative stress plays an important role in the initiation and promotion of oncogenesis and may contribute to genetic instability and an increase in mutations (Prestera *et al.,*

1993; Rose and Bode, 1993). The genetic consequences of exposure to increased oxidative stress include a rising number of chromosomal aberrations (DNA breakage) and genetic mutations. In a recent study of induced hyperoxia, ascorbic acid and ethoxyquin potentiated the clastogenic effect (breaking of DNA/RNA) and increased chromosomal aberrations in ovarian cells. However, in another study, simultaneous administration of a mutagen and ethoxyquin reduced the clastogenic effects of the mutagen (Renner, 1984).

The most commonly used synthetic antioxidants mentioned above have been shown to increase not only the toxicity of other chemicals, but also mutagenicity, sensitivity to exposure to radioactivity, and tumor yield from chemical carcinogens (Ito *et al.*, 1986; Manson *et al.*, 1987). Production of reactive oxygen species, particularly those of hydroxy-radicals, appears to be a critical determinant. It is tempting to speculate that the rising incidence of leukemias, lymphomas, hemangiosarcomas, and chronic immunosuppressive disorders among companion animals is due at least partially to the widespread use of chemical antioxidants and other additives in commercial pet foods. In genetically predisposed individuals, these environmental chemicals that promote immune suppression or dysregulation and oncogenesis can contribute to the failure of immune surveillance mechanisms which protect the body against the vast array of infectious and other agents that induce immunologic or neoplastic change (Dodds, 1995a). Clearly, additional longterm controlled feeding trials are needed that incorporate modern toxicological, medical, and epidemiological assessments of these chemicals, to evaluate their interactions with the other genetic and environmental factors that affect the health and performance of inbred and closely linebred companion animals (Dodds and Donoghue, 1994).

E. Medical and Legal Aspects of Clinical Trials

The need to pay more careful attention to the design and regular monitoring of large-scale clinical trials in human medicine has been underscored recently by the misconduct surrounding the multicenter breast cancer trial in North America (Cohen, 1994b). Large-scale clinical trials of this type are considered to be the only means of gathering information from thousands of patients at various clinical centers into large enough groups to test specific types of therapy, devices, vaccines, drugs, and other health questions. The very magnitude of these trials, however, leaves them wide open to negligence and even deliberate misconduct in their execution and ultimate evaluation. While some of

these instances of mishandling or misconduct may not actually affect the final outcome of a particular trial, the negative publicity generated within both the medical and scientific communities and the public often prejudices acceptance of their outcomes (Cohen, 1994b). With respect to the misconduct associated with the breast cancer trials sponsored by the National Cancer Institute, the major conclusion that lumpectomy is as effective as mastectomy has now been called into question. To what extent this type of misconduct will continue to plague clinical trials is unclear, because there is no overall system in place for monitoring these trials in North America. Unfortunately, if such a system were in place it would significantly increase the already burdensome costs of large-scale clinical trials. This could be a major drawback, and is one of the reasons why previous trials are unlikely to be repeated.

Unlike the National Institutes of Health where requirements for more extensive on-site monitoring have not been implemented because of lack of funds to do so, more frequent and thorough monitoring has taken place in the pharmaceutical industry, primarily to ensure that data generated would satisfy requirements of the federal Food and Drug Administration for approval of new drugs or devices. Despite these concerns, clinical trials do undergo on-site monitoring to review patient records and case report forms; the collected data are sent to a coordinating center; Institutional Review Boards made up of researchers, ethicists, and lay people review trial protocols and informed consent forms to protect the interests of participants; and Data Safety Monitoring Boards made up of researchers, preferably not involved in specific trials, periodically review the data obtained. These Boards are responsible for evaluating incoming data in blinded, placebo-controlled trials so that they can halt a trial at any time if toxicities are found or a particular treatment is proven to be beneficial (Cohen, 1994b). Hopefully, this renewed emphasis on the need for oversight of clinical trials will translate into the implementation of a new national policy to renew medical and public trust in the information generated.

Closer monitoring of clinical trials is not the only need facing medicine today, as other concerns have focused on the adequacy of research methodology (Nowak, 1994). Most of the problems related to research methodology have occurred because of ignorance about the proper system to employ or the reasons why particular approaches may invalidate the conclusions. The complexity of this situation is illustrated by the recent experience of investigators examining data from a large trial of drugs used to treat patients infected with human immune deficiency virus. The overall conclusion of the trial was that the com-

bined use of two drugs offered no greater benefits to patients than giving just one. When the researchers compared data from many different patient subsets, however, they found an apparent benefit of the combination approach in a small number of them (Nowak, 1994). A huge outcry accompanied this report at a scientific meeting, because clinicians, scientists, and statisticians were upset by the apparent statistical manipulations of the data during an attempt to arrive at a positive conclusion. Unfortunately, this statistical error in analyzing results of randomized controlled clinical trials is not that uncommon, largely because few researchers are trained in the basic understanding of their proper design and execution (Nowak, 1994; Waltner-Toews, 1989).

The key problems that occur all too frequently and lead to substandard performance in clinical trials include: failure to guarantee randomization; inappropriate method to ensure that patients are assigned to a particular treatment by chance alone; enrolling too few participants to detect differences in a particular treatment; inappropriate analysis of subgroups; post hoc removal of data from final analysis without appropriate reasons; and misleading substitution of "surrogate" biological markers for the clinical end-points originally designated (Nowak, 1994). Obviously, increased emphasis on this subject needs to be introduced in medical and specialty training in order to reduce the likelihood of these improper practices.

The extent to which flaws attend the conduct of randomized, controlled clinical trials was disturbing, because about one-third of these trials were published in prestigious medical journals and failed to establish criteria that assured patients were assigned to the different treatments by chance (Nowak, 1994). Without true randomization, a physician might inadvertently place healthier patients on the experimental treatment protocol due to a subconscious desire to see the treatment validated. Many commonly employed methods used to attempt randomization can be easily bypassed or abused. Thus, there are many ways in which bias can enter the conduct and interpretation of clinical trials and thereby undermine the validity of results. Furthermore, one of the most hotly disputed areas of clinical trials involves the intention-to-treat approach to which the majority of biostatisticians adhere. With this protocol, the data from every patient assigned to a particular treatment must be included whether or not the patient complies with the designated treatment. Experience with some completed clinical trials has led to different conclusions when the data were analyzed by two different methods, one in which patients who did not always take the treatment were excluded from the analysis, and the other in which all patients were analyzed (Nowak, 1994). Despite the

many flaws that could apply to the conduct of clinical trials, the current emphasis on using statistical metaanalysis to pool data from many trials in order to seek answers or conducting massive megatrials where 10,000 or more patients are enrolled will undoubtedly have a beneficial impact. At this time there is no better alternative to large clinical trials for evaluating relatively small differences between new drugs or therapy (Nowak, 1994).

While these medical and statistical concerns about misconduct and methodological flaws have received increasing attention, another hotly disputed area of clinical trials is the ethical concern in using placebo controls (Taubes, 1995). The major debate is whether it is "ethical to compare a potential new disease treatment with inactive placebo controls if an accepted treatment for the disease already exists." There is little agreement on the answer (Taubes, 1995). Many scientists say that it is unethical to use placebo controls in these situations and blame the government for requiring them, whereas federal officials defend the present system because they believe the suggested alternatives to be even less acceptable. The issue surfaced again in 1994 when a position paper stated that clinical trials commonly violate the 1964 Declaration of Helsinki, World Medical Association Proclamation on Biomedical Research Ethics. This proclamation states that it is unethical to use a placebo control if a proven therapy already exists, because patients may suffer unnecessarily and may even risk death (Taubes, 1995). Many Institutional Review Boards struggle with this issue on a continuing basis, and members have stated their belief that use of placebo control groups is neither scientifically necessary nor ethically sound. Nevertheless, other bioethicists and the federal government have taken the opposing view that the Declaration of Helsinki is the wrong standard for assessing the ethics of clinical trials because the Helsinki Declaration was intended to guide physicians in treating patients and not to perform controlled trials. Regardless of the differing positions on this issue and the current heated debate, it is clear that strong justification is needed for including placebo controls as well as more flexibility in interpreting broad-based ethical standards.

The background summarized above has looked at many important issues in the conduct of human clinical trials. But how does this apply to veterinary medicine where one might presume that the conduct of clinical trials would not be subject to the same degree of constraints and ethical considerations? (A detailed look at the current situation in veterinary medicine can be found in Chapters 5 and 6 in this volume by Drs. Boothe, Slater, and Tannenbaum.) Over the past 10 or more years, more emphasis has been placed on concerns for the welfare of animals

used in biomedical research, teaching, and testing. Also, society has demanded improved veterinary patient care, and more attention has been paid to ethical and legal considerations in veterinary and comparative medicine (Boothe *et al.*, 1992; Lund *et al.*, 1994). While major changes have been promulgated during this period to benefit animals used in nonclinical research and implement specific guidelines for research investigators in these settings, there has been less emphasis on the conduct of clinical trials from methodological, statistical, ethical, and legal perspectives (Dohoo and Thomas, 1989; Waltner-Toews, 1989).

The advent of veterinary medical specialization has focused our attention on this important topic; namely, to assure that client-owned animals are ethically treated in the conduct of veterinary clinical trials. There is no reason to presume that clinical trials in veterinary medicine should proceed under guidelines that differ significantly from those that are or should be implemented for humans. The basic principles to be adhered to are outlined in the review by Boothe *et al.* (1992) and in Chapter 5 of this volume. These include development of a Hospital Review Committee whose goals are to address for animals the ethical concerns being considered for humans by Institutional Review Boards, the need for peer and departmental review of clinical research proposals before they are submitted or considered for funding, and the necessity for informed consent from the animal's owner. In designing clinical trials to assess new drugs, forms of therapy, or veterinary biologics, Ribble (1989) summarized the three key steps as: choosing an outcome measure, preventing bias, and establishing the role of chance.

Controlling or eliminating chance as a factor in the analysis of results of clinical trials is one of the most important concerns. Both type I errors in which an investigator incorrectly concludes that a particular modality is effective and type II errors in which the same investigator concludes that the modality was ineffective can occur. The probability of committing a type I error is usually set at the 5% level of significance, whereas the probability of committing a type II error depends on the size of the trial. The larger the trial group the less likely that an incorrect conclusion will arise, although type II errors are often ignored (Ribble, 1989). The third important consideration affecting chance events is the need for determining a power calculation before entering a trial. This can be done by either presetting the power of the trial you wish to achieve and calculating the number of animals needed to achieve it, or by starting with the number of animals you have available for the trial and determining the power of this number in enabling you to reach a statistically valid conclusion. The

importance of this situation is exemplified by the chronic feeding trials with the synthetic antioxidant ethoxyquin discussed in Section D. Two trials of 1 and 5 years in length were completed in dogs in the early 1960s. The number of animals entered into the treatment and control groups was not large enough to give sufficient statistical power to any conclusions drawn about the efficacy or safety of this chemical preservative. Nevertheless, results of the studies have been interpreted since then to indicate that no significant adverse affects could be attributed to ethoxyquin in comparison to the control groups. Design flaws in the undertaking and evaluation of these results have contributed to the lingering controversy about the safety of chemical antioxidant preservatives used in pet foods. Until such time that a more appropriately designed chronic feeding trial is executed—which is unlikely given the cost involved and the fact that foods can be preserved adequately with natural antioxidants instead—results of the early trials will continue to be challenged (Cargill and Thorpe-Vargas, 1993, 1994).

Recently, Lund *et al.* (1994) reviewed the potential and importance of implementing randomized clinical trials in veterinary clinical research. They discussed the differences between observational study to investigate causative agents and risk factors, and experimental design which involves manipulating the clinical state of patients in some manner to more easily establish causality. Experimental interventions are less likely to be affected by confounding extraneous variables than are observational retrospective or prospective studies. (Various study designs are also used in evaluating treatments and these are outlined in Chapter 5 of this volume.) Uncontrolled and nonrandomized controlled studies are often used, and the latter may have concurrent controls or use historical data to define similar groups of patients to serve as a control group. These two methods of experimental study have serious design flaws, however, and may not be much better than observational studies in their ability to eliminate confounding variables. As discussed by Lund *et al.* (1994) and Boothe and Slater in Chapter 5 of this volume, properly designed and executed randomized clinical trials constitute the preferred approach for veterinary clinical studies. As veterinary medical specialization increases, not only in the academic sector but also in private clinical practice, owners of animal patients entered into trials are going to demand more accountability for their conduct and the outcome of their pet's health. The extent to which the investigator and institution or practice where clinical trials are conducted will be held liable, should problems arise that harm the patient, is difficult to predict. Animals are still considered a form of property under the law in most states, and clients would be awarded

only the replacement value of the animal rather than any consideration for the emotional pain and distress they and the animals suffered. Nevertheless, as medical professionals we have an ongoing commitment to offer the best care and advice possible regardless of any legal consequences. The frequency of situations in which owners take legal recourse for actual or perceived misconduct is likely to increase, as our profession becomes more specialized and undertakes to enter animals into observational and experimental clinical trials using treatments that may be approved for humans but have not yet been established to benefit animals. The legal and ethical challenges involved in this growth are discussed in detail in Chapter 6 of this volume.

III. Recommendations for the Future

A. Integrating Basic and Clinical Research

Other chapters in this volume outline how basic research investigations have and can lead to the design and implementation of clinical trials to test the validity of research findings in patients. Perhaps the most important aspect of this transition from the bench to the patient is the execution of appropriately designed and randomly controlled clinical trials, especially when they evaluate the potential benefits of therapeutic modalities already in use in human clinical medicine. As more veterinarians become trained in specialties in the private sector, this should promote collaboration with academic institutions to accomplish metaanalysis of a trial size sufficient to achieve the statistical power to be able to detect differences of a significant magnitude between groups (Ribble, 1989; Wagner, 1992; Lund *et al.*, 1994).

A second area of need in planning the symbiotic relationship between basic science and clinical medicine has been recognized for many years. Basic scientists tend to be highly specialized research investigators trained in a particular postdoctoral specialty area and, in turn, train graduate students in the same discipline, whereas relatively few clinicians have independent scientific specialty training. Despite the obvious fact that these two groups need to work closely together to achieve the clinical advances made possible by new scientific findings, this marriage of disciplines has always been more difficult to achieve in practice than in theory. In academic settings, there has to be a conscious effort made by senior administrative officials to foster interaction between the basic and clinical sciences and to identi-

fy individuals that can be leaders in this regard (Wagner, 1992). There is also the tendency of some medical and veterinary clinicians to fail to appreciate that equal weight should be accorded to the basic science discoveries within a collaborative effort. Additionally, where human medical and veterinary clinical scientists are collaborating on projects, the contribution of the veterinary component may be undervalued, especially if the human clinical component of a trial reaches an interim conclusion that obviates the need for further study when the veterinary component of the trial is still under way. In these situations, it is conceivable that different outcomes would pertain when the same modality is tested in a particular animal species in comparison to humans. The above statements reflect my personal point of view based on 30 years of experience in basic and clinical comparative hemostasis research. These comments are not intended to dissuade veterinary clinical scientists from collaborating actively in research with other health professionals.

B. Molecular Approaches and Gene Therapy

As alluded to in the Preface and Chapter 2 of this volume, we are entering an exciting area of molecular approaches in veterinary medical research with the goal of implementing gene therapy. In this area of study, animal experimentation will, by necessity, provide the essential early data for using gene therapeutic approaches for advancing human health and well-being (Ostrander *et al.*, 1993). Two examples of diseases that commonly affect humans and companion animals and lend themselves to molecular and gene therapy approaches are autoimmune thyroid disease (McGregor, 1992) and the inherited bleeding disorders such as hemophilia and von Willebrand's disease (Dodds, 1988; Kay *et al.*, 1993). With respect to autoimmune thyroiditis (as discussed in Chapter 3 of this volume), most of the factors that contribute to the process that recognizes thyroid autoantigens have been identified, and so a number of strategies are feasible for their blockade or modification. One could even envision that vaccination against organ-specific autoimmune disease could be considered a mode of prevention for individuals that are known to be genetically susceptible to these disorders (McGregor, 1992).

A concerted effort is currently under way to evaluate gene therapy for hemophilia B, because the factor IX molecule is smaller than the factor VIII and von Willebrand factor molecules and is less complex in its biological expression (Dodds, 1988, 1989; Kay *et al.*, 1993). Several collaborative gene therapy studies between academic institutions and

industry are being conducted with factor IX using dogs with hemophilia B. The initial efforts reported by Kay *et al.* (1993), although remarkable, achieved only minimal increments in factor IX activity after incorporating the normal canine factor IX gene into the livers of partially hepatectomized hemophilic puppies. Even though this incremental increase was not sufficient to be clinically protective and the nature of the invasive experimental protocol precludes its use in human infants, the importance of this work lies in the proof that such approaches are feasible. Other less invasive strategies are now being explored for this type of gene therapy. As pointed out by Mulligan (1993),

> despite substantial progress, a number of key technical issues need to be resolved before gene therapy can be safely and effectively applied in the clinic. Future technological developments, particularly in the areas of gene delivery and cell transplantation, will be critical for the successful practice of gene therapy.

C. Strategies for Research Funding

As the pool of government funds available to support basic and clinical research for the medical sciences continues to dwindle, there will be more competition for these research dollars from agencies such as the National Institutes of Health, National Science Foundation, U.S. Department of Defense, and U.S. Department of Agriculture. Research targeted to improve animal health will usually take second priority to studies where the primary goal is directed toward human health. Other sources for funding veterinary medical research generate relatively small amounts of money in comparison to the larger governmental funding agencies. These include the Morris Animal Foundation, American Animal Hospital Association, American Veterinary Medical Association Research Foundation, American Kennel Club, Winn Foundation, and others (Wagner, 1992). In the last decade, there has been a much greater emphasis on soliciting funds from industry to support the medical sciences, especially now that definition and resolution of concerns about conflict of interest have largely been resolved. The increased focus on proper design and validation of clinical trials, while essential, will inevitably increase their cost. A multidisciplinary approach is needed where basic scientists and clinical specialists from various medical disciplines work together, particularly when the veterinary pharmaceutical and biologics industry needs to obtain clinical trial data prior to licensing new products. These financial constraints on current research also put pressure on investigators to select more applied research topics rather than addressing basic

science questions. While this approach is likely to generate a higher priority for funding from both the government and private sectors wishing to target research to the more common problems needing resolution, it has one negative aspect—it tends to stifle basic inquiry into the fundamental principles underlying scientific phenomena. These basic findings form the backbone of knowledge that can eventually translate into clinical benefits (Dodds, 1988; Patterson *et al.,* 1988; Ostrander *et al.,* 1993).

Finally, in order to compete more successfully for current research dollars, academic veterinary institutions need to capitalize on the expertise of their existing faculty and new recruits, rather than trying to cover all disciplines less effectively. This will involve the setting of priorities to concentrate research efforts not only to focus on certain species for study, but also to set the emphasis for the topics to investigate. Often the proximity to a medical school or other basic science departments will assist in focusing an effective collaborative program (Wagner, 1992). One of the most successful long-standing examples of this approach has been the medical genetics research program conducted by Dr. Donald Patterson and colleagues at the University of Pennsylvania (Patterson *et al.,* 1988; Smith, 1994). Their program has served as a model for more than three decades to structure the comparative medical research of experts in other veterinary teaching institutions, as well as for our own research in comparative hemostasis which continues today under the direction of my colleagues, Drs. James Catalfamo and Marjorie Brooks at Cornell University, College of Veterinary Medicine in Ithaca, New York.

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Estimating Disease Prevalence with Health Surveys and Genetic Screening

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I. Background

A. Historical Perspective

Most purebred dogs raised today evolved from a relatively small gene pool that established each of the original breeds (Jolly *et al.*, 1981; Patterson *et al.*, 1988; Bell, 1993; Clark and Stainer, 1994; Smith, 1994). Over the years, the common practices of line-breeding

and inbreeding descendants of this foundation stock promoted genetic mutations, which led to the increased transmission and recognition of genetic diseases (Dodds *et al.*, 1981; Jolly *et al.*, 1981; Patterson *et al.*, 1988; Bell, 1993; Association of Veterinarians for Animal Rights (AVAR), 1994). Even though a particular genetic defect may initially have been recognized in a specific line or family within a breed, all important breeding stock of the breed should be screened for the defect because of their similar genetic background (Dodds *et al.*, 1981; Dodds, 1982; Cargill, 1991). If this approach is not followed, the frequency of genetic defects will inevitably increase and have a negative impact on the overall health and longevity of many dog breeds (Corley and Hogan, 1985; Bell, 1993; Smith, 1994).

This prediction has already come true for many inherited disorders including inherited eye diseases (Dodds *et al.*, 1981; Patterson *et al.*, 1988; Rubin, 1989); copper toxicosis in Bedlington and West Highland white terriers (Brewer and Yuzbasiyan-Gurkan, 1989); sebaceous adenitis in standard poodles (Laratta, 1991, 1992); pyruvate kinase deficiency (Searcy *et al.*, 1979; Giger and Noble, 1991); and Fanconi syndrome in basenjis (Brown, 1989; Noonan and Kay, 1990; Gonto, 1993); phosphofructokinase deficiency in English springer spaniels (Giger and Harvey, 1987); GM_1 gangliosidosis (storage disease) of Portuguese water dogs (Greenfield, 1990; Bell, 1993); congenital cardiac diseases (Patterson *et al.*, 1988); hip dysplasia (Corley and Hogan, 1985; Keller *et al.*, 1991; Corley, 1992, 1994); deafness (Holliday *et al.*, 1992, Strain *et al.*, 1992; Greibrokk, 1994), abnormal purine metabolism, and urate bladder stones in dalmatians (Schaible, 1986; Sorenson and Ling, 1993a,b, Bartges *et al.*, 1994); cystine and urate uroliths in bulldogs (Bartges *et al.*, 1994); calcium oxalate uroliths in miniature schnauzers (Lulich *et al.*, 1991); neuromuscular disease in Labrador retrievers (Kramer *et al.*, 1981; Patterson *et al.*, 1988); hereditary kidney diseases (Picut and Lewis, 1989); gastric dilatation-volvulus in Great Danes, weimaraners, Saint Bernards, Gordon setters, and Irish setters (Glickman *et al.*, 1991); and the bleeding disorders, especially von Willebrand's disease (Dodds *et al.*, 1981; Dodds, 1982, 1984, 1988, 1989, 1992c,f; Raymond *et al.*, 1990; Brooks *et al.*, 1992). An example of the latter case is von Willebrand's disease in Doberman pinschers and Shetland sheepdogs, in which an alarming increase in the incidence of severely bleeding dogs arose because animals carrying this genetic defect were bred together despite the warning to avoid such matings (Dodds, 1982, 1984; Raymond *et al.*, 1990; Brooks *et al.*, 1992).

Large-scale screening programs for the identification of affected and

carrier animals are an effective mechanism to discover and eventually control the frequency of specific genetic defects within the population at large (Dodds *et al.*, 1981; Jolly *et al.*, 1981; Dodds, 1982; Corley and Hogan, 1985; Rubin, 1989; Keller *et al.*, 1991; Corley, 1992). Screening programs of this type have been used successfully in humans for many years (e.g., Tay-Sachs disease, phenylketonuria) and more recently have been applied to animals (e.g., mannosidosis in cattle; hip dysplasia, ear, eye, blood, and heart diseases in dogs). Dr. Donald Patterson, a recognized authority on comparative medical genetics, recently stated "We are at the beginning of a revolution in veterinary medical genetics, where we will have a better understanding of how to diagnose genetic disease, detect carriers of recessive genes, and thus reduce the frequency of genetic disorders" (Smith, 1994).

B. Genetic Screening

In the mid-1960s veterinarians began screening dog populations for inherited eye diseases and hip dysplasia (Jolly *et al.*, 1981; Corley and Hogan, 1985). These programs have been an effective means of genetic control because companion animals live in daily contact with their owners, and thus illnesses are more likely to be noted and treated. Genetic screening may be essential to the survival of breeds in which mild or moderately severe defects have been propagated unknowingly for many generations (Bell, 1993; Smith, 1994). The responsibility necessarily falls on the shoulders of established breeders because they set the example by requiring the health screenings that impact the rest of the breed.

It is important that the top-producing sires and foundation bitches of a breed be screened for conditions prevalent in that breed or in dogs generally, because they represent the major nucleus of genetic material for the current and future decades (Jolly *et al.*, 1981; Dodds, 1982; Padgett, 1992; Bell, 1993; Smith, 1994). Despite the proven benefits of this approach and intensive educational efforts, some breeders choose not to participate. Veterinarians and scientists who provide testing and genetic counseling services should be understanding in dealing with the concerns and sensitivities of these individuals. Attempts to pressure people into participation can be counterproductive to the overall goals of the program. Success depends upon an honest, voluntary commitment to the principles and ethics of breeding better companion animals (Dodds, 1982). One can even question the appropriateness of propagating purebreds if a significant proportion of a particular breed

suffers from one or more clinically expressed genetic traits, especially when several million unwanted or stray animals are killed annually in pounds or shelters.

Depending on the mode of inheritance, different approaches may need to be applied for the detection and control of genetic diseases (Patterson *et al.,* 1988; Keller *et al.,* 1991; Corley, 1992; Padgett, 1992; Bell, 1993; Ostrander *et al.,* 1993). It is advantageous to be able to select against heterozygotes (carriers) rather than have to eliminate affected individuals from a breeding program once the condition has already appeared. Control and elimination of the disease by testing are feasible and reliable in cases where a biochemical marker of the trait is expressed in genetic carrier animals (e.g., as measured in a blood, urine, or saliva test, electrocardiogram, skin biopsy, eye examination, or hair analysis) (Jolly *et al.,* 1981; Dodds, 1988; Patterson *et al.,* 1988). Some current examples include testing for the bleeding diseases such as von Willebrand's disease and hemophilia (Dodds, 1982; Dodds, 1988; Brooks *et al.,* 1992; Dodds, 1992e); autoimmune thyroid disease (Dodds, 1992a,b,e; Nachreiner and Refsal, 1992b); hip dysplasia (Corley and Hogan, 1985; Keller et al., 1991); red blood cell enzyme defects like phosphofructokinase and pyruvate kinase deficiencies (Bell, 1993; Smith, 1994); the various eye, heart, metabolic enzyme, and storage disorders (Jolly *et al.,* 1981; Patterson *et al.,* 1988; Noonan and Kay, 1990); and neuromuscular diseases (Ostrander *et al.,* 1993). Coupled with this approach to eliminating undesirable traits is the necessity to evaluate breeding stock regularly for overall soundness, reproductive health, and performance.

II. Health Surveys

A. Introduction

Every purebred dog fancier desires to breed and/or exhibit healthy, vigorous, long-lived, and attractive animals that conform closely to the breed's ideal. Despite the best intentions, however, breeders continually face the reality of health problems that impact the breed in general or are limited to specific families or members of the breed (Jolly *et al.,* 1981; Dodds, 1982; Noonan and Kay, 1990; Dodds *et al.,* 1991, 1992). The most significant problems arise when a disease develops gradually or subclinically for several generations until it has become widespread before being generally recognized (Dodds *et al.,* 1981; Dodds,

1982; Raymond *et al.*, 1990; Brooks *et al.*, 1992; Bell, 1993). Effective, practical solutions to this growing spectrum of problems will only be realized if veterinarians, geneticists, and other health professionals work closely together with breeders. An organized joint effort is needed to encourage cooperation, with rigorous attention being paid to the development of reliable, validated screening tests and appropriate medical and genetic counseling.

The increased recognition over the last decade of inherited and autoimmune diseases in both humans and animals is a convincing example of the present trend, as we become more adept at diagnosis, prevention, and treatment of nongenetic disorders but continue to alter and pollute our surroundings, thereby sustaining the environmental factors that influence or "trigger" disease (Dodds, 1983; Trence *et al.*, 1984; Fisher *et al.*, 1987; Patterson *et al.*, 1988; Sinha *et al.*, 1990; Utiger, 1991; Berry and Larsen, 1992; Dodds, 1992a,b; Bell, 1993; Steinman, 1993; Tomer and Davies, 1993; Dodds, 1994a,b; Glickman *et al.*, 1994).

Given this rather gloomy scenario, what approaches can be taken to assess the situation and develop a set of goals and priorities for the immediate future and longterm? Conducting formal health surveys or screenings of animal populations (e.g., individual dogs breeds) is one approach that has generated useful data to guide future efforts in addressing the identified major health concerns of these breeds (Boyer *et al.*, 1980; Dodds *et al.*, 1981; Corley and Hogan, 1985; Dodds, 1990a,b, 1992f; Noonan and Kay, 1990; Dodds *et al.*, 1991, 1992; Keller *et al.*, 1991; Brooks *et al.*, 1992; ISCA, 1992; Nachreiner and Refsal, 1992b; Bell, 1993).

The success of this approach is attested to by reduction in the prevalence of conditions such as the inherited eye (Rubin, 1989) and blood diseases (factor X deficiency and von Willebrand's disease) (Dodds, 1982; Brooks *et al.*, 1992), deafness in dalmatians (Holliday *et al.*, 1992); copper toxicosis in Bedlington and West Highland white terriers (Brewer and Yuzbasiyan-Gurkan, 1989); storage disease in Portuguese water dogs (Bell, 1993); and hip dysplasia in many breeds (Keller *et al.*, 1991; Corley, 1992). With respect to hip dysplasia, a specific example is the reduction in its prevalence in Old English sheepdogs between 1966–1973 (29.9%) and 1974–1989 (22.5%), a pattern shared by many other breeds as a consequence of breeder diligence in addressing this problem (Corley and Hogan, 1985; Corley, 1992).

By contrast, the frequency of autoimmune disorders such as autoimmune thyroiditis has been increasing dramatically to the extent

that some 50 breeds of dogs are commonly affected with thyroid dysfunction today (Haines *et al.,* 1984; Conaway *et al.,* 1985; Dodds, 1987, 1992a,b,c,e,f, 1994). Furthermore, hypothyroidism was considered the foremost health problem of the 90 parent breed clubs responding to a recent questionnaire from the American Kennel Club (AKC) Delegate's Health Research and Health Education (HRHE) Committee (AKC Delegate's HRHE, 1994). A similar increase in familial autoimmune hemolytic anemia has been reported in breeds such as the vizsla, Scottish terrier, American cocker spaniel, miniature schnauzer, and Old English sheepdog (Boyer *et al.,* 1980; Dodds, 1983, 1987, 1990a,b; Mills *et al.,* 1985; Dodds *et al.,* 1991, 1992; Day and Penhale, 1992; Barker *et al.,* 1993).

The results of the AKC Delegate's HRHE Committee questionnaire confirmed clinical observations gathered during the last decade or more by private veterinary practitioners and their academic colleagues. The diseases perceived by the respondents to be the most important overall were ranked as follows: hypothyroidism, arthritis, seizures/epilepsy, allergies, cataracts, and hip dysplasia (AKC Delegate's HRHE, 1994). These findings are summarized below.

The Delegate's HRHE Committee mailed a questionnaire to the 137 Parent Clubs of the American Kennel Club in April 1994. The purpose of this questionnaire was "to determine which health problems were of greatest concern to purebred dogs." By the end of July 1994, 114 responses were received from 89 different Parent Clubs (65% response rate), and 20 clubs sent in 2 to 4 responses. The responses from 21 Delegates, 18 Club Secretaries, and 15 other persons representing their respective clubs were entered into a computer-coded database and analyzed. As no documentation was requested to substantiate the importance of the various diseases listed, the answers reflect the perceptions of the respondents who may or may not be acting on behalf of the club as a whole or as individuals deemed knowledgeable and elected to answer for the club. Nevertheless, there is no reason to doubt the validity of the survey findings for two reasons. First, the diseases listed as most important to the breeders are the same ones that the veterinary profession has been diagnosing and treating most often in the same breeds. Second, this author contributed to the response for the Vizsla Club of America and can attest to the relevance of the survey.

The 10 most important diseases listed in rank order for the 90 Parent Clubs responding were as follows: (the number in parenthesis after the disease represents the number of breed clubs indicating that the problem was fairly important or very important).

1. hypothyroidism (73)
2. arthritis (63)
3. allergies (62)
4. seizures/epilepsy (60)
5. cataracts (59)
6. hip dysplasia (58)
7. breast cancer (55)
8. pyometra (52)
9. failure to conceive (50)
10. requirement for cesarean section (50)

In addition to the overall ranking of health concerns, the most common diseases were further broken down by the 7 groupings according to breed type. For example, results of the Terrier Group reflected reporting from 18 different terrier breed clubs. The Sporting and Working Groups each reported on 14 different breeds, the Hound Group reported on 11 groups, the Toy and Herding Groups reported on 10 breeds each, and the Nonsporting Group on nine breeds. Hypothyroidism was the first disease in overall ranking, and in all 14 breeds in the Sporting Group and 10 breeds in the Herding Group (100% responses); 93% of breeds in the Working Group; 82% of breeds in the Hound Group; 67% of breeds in the Nonsporting Group; and 61% of breeds in the Terrier Group. Only in the Toy Group was hypothyroidism not listed among the most important diseases. This probably does not reflect a truly lower incidence of hypothyroidism among the 10 Toy Group breeds responding, however, because in seven of them the clubs reported hypothyroidism to be fairly important (toy poodle, pomeranian, Yorkshire terrier, and Brussels griffon), and three declared it very important (Italian greyhound, pug, and shih tzu). As far as the total breed response is concerned, 27 breed clubs listed hypothyroidism as a fairly important problem, and a remarkable 42 breed clubs listed hypothyroidism as a very important problem. This number compares closely to the 50 or more breeds listed by this author as being subject to hypothyroidism of familial nature (Dodds, 1992a,d).

Bone and joint diseases constituted the second (arthritis) and sixth (hip dysplasia) diseases in importance. These were of most concern for the Sporting and Working Groups where hip dysplasia was considered important for 93% of the breeds in both groups and arthritis for 86% of breeds in the Working Group. In the Herding Group, arthritis and hip dysplasia were each considered important by 80% of breed clubs responding. Curiously, arthritis was also considered to be of significant importance to 90% of breeds in the Toy Group, but was not listed as

being important in the Terrier, Hound, and Nonsporting Groups. Fifty breeds considered arthritis to be a fairly important problem, whereas 12 breeds considered it to be very important. With respect to hip dysplasia, the numbers were about equal with 28 considering it fairly important and 29 very important. Seizures or epilepsy were the third most important diseases overall and were listed as a problem in 93% of Sporting Group breeds and 82% of Hound Group breeds. Seizures were not listed among the top diseases for the other groups. The fourth most common concern was allergies. Allergies were listed as the most important problem for 78% of the Terrier Group and 67% of the Nonsporting Group breeds, but were not listed as being important concerns for the other groups. Among the breed clubs listing allergies, 34 listed it as a fairly important problem and 27 listed it as very important.

The fifth most common disease was cataracts, and these were listed as the most important disease problems of 78% of breeds in the Nonsporting Group, and 80% of the breeds in the Herding Group. They were not listed as top conditions in the other groups. Finally, other diseases listed among the top diseases by breed group included: heart murmurs and seborrhea in the Terrier Group; breast cancer in the Sporting Group; bloat, which was ranked first in 93% of breeds in the Working Group; and panosteitis, which was also of concern for this group; caesarean section, heart failure and patellar luxation in the Toy Group; bloat, bone cancer, cystitis, and spinal problems in the Hound Group; entropion, failure to conceive, patellar luxation, and pyometra in the Nonsporting Group; and failure to conceive, irregular heat cycles, and progressive retinal atrophy in the Herding Group.

Other demographic information obtained from the questionnaire included lifespan and litter size expectancies. The average litter size of these breeds ranging all the way from toy to giant breeds was 6 puppies (range 2 to 10). The average number of puppies dying in the first 4 weeks of life per litter was 0.72 with a range of 0 to 3 pups. The average life expectancy was 11.8 years with a range of 6 to 15 years. The most frequent causes of death reported in the survey were cancer, heart failure, and kidney failure. The breed clubs responding to the survey indicated that most of them (72%) had a Health Committee, and 45% reported having a specific health clause in their Code of Ethics. However, only 5% of the clubs reported having any type of recognition program or policy for breeders who test and screen their breeding stock or progeny for various genetic defects, such as hip and elbow dysplasia, blood diseases, eye and hearing disorders. More than 50% of the responding clubs reported that they participate in health

education programs, and 44% of them make monetary donations to health-related organizations. Eighteen clubs reported having established tax-exempt foundations for purposes of supporting health research and health education. It is obvious from the results of this preliminary report that the Parent Clubs of the American Kennel Club are making a concerted effort to do something to prioritize the health concerns of their breeds. Thus, despite recent media attention to the contrary, the current movement to become more aware and responsive to the health concerns of purebred dogs should be recognized and encouraged.

Several national dog breed clubs have previously undertaken health surveys sponsored by their respective organizations. As examples, data collected from the Old English Sheepdog Club of America (OESCA), Irish Wolfhound Club of America (IWCA), Basenji Club of America (BCA), Newfoundland Club of America (NCA), and Dalmatian Club of America (DCA) are reviewed below. Studies for the first two clubs (OESCA and IWCA) were undertaken by the author between 1990 and 1992, and results are summarized here and elsewhere (Dodds, 1990b, 1992f; Dodds *et al.*, 1991, 1992).

B. Old English Sheepdogs

The purpose of the health survey conducted in 1990 and completed in 1991 (Dodds, 1990b; Dodds *et al.*, 1991, 1992) was to determine the type and prevalence of health problems affecting the Old English sheepdog breed. A comprehensive questionnaire was designed (Dodds, 1990b), and all completed surveys were submitted to this author and kept strictly confidential. Participants were also given the option to provide registered names, "call"-names, pedigree or family history information, and any other information they deemed pertinent. Alternatively, the completed survey forms could be returned without any name identifiers for the respondent or the animals. Study results were compiled and summarized to provide an overview of health problems currently affecting the breed for use by the OESCA, individual breeders, and potential buyers of Old English sheepdogs, as well as the veterinary profession. The findings have and should continue to increase awareness of breed-related health matters among Old English sheepdog fanciers, provide a baseline for future emphasis and genetic counseling, and offer educational material for current and future owners, breeders, and care providers.

The general outline for this survey was similar to one conducted in

1989 by the Health and Longevity Committee of the NCA. Their response rate was about 46% from a total of 1,512 questionnaires mailed to members. Results of the NCA study are summarized in Section II, G.

For survey purposes, any condition affecting or altering the animal's normal state of health was to be included, even if the condition had been diagnosed and was under control with treatment. For example, information about dogs being medicated for hypothyroidism and restored to good health was requested in order to estimate the prevalence of this relatively common canine disease. Similarly, over- and undershot jaws or other structural faults were to be listed as they provide insight into the extent and severity of these problems amongst breeding stock.

1. Study Design

The study was designed to determine the prevalence of health problems in the Old English sheepdog breed. Prevalence is defined as the number of dogs with a particular health problem, abnormality, structural fault, or disease present at any given point in time, divided by the estimated population at risk. Prevalence is usually expressed per 100 individuals and is actually a point prevalence rate (Alderson, 1976).

A cross-sectional study design was selected because data were to be collected during one time period from respondents by questioning, examining, or investigation (Alderson, 1976). A cross-sectional study typically surveys a random sample of the population. This provided background descriptive data to clarify the actual status of the breed's health and to guide planning of further more specific health studies.

Three issues arose when designing the study instrument: how suitable was the population for study?; how feasible was it to sample the population?; and how available was the population? In this case, we wished to survey the Old English sheepdog breed throughout North America. However, it was impossible to randomly survey the entire population because we could not identify nor locate everyone who owned this breed. Logically, identifiable populations could most easily be reached through the national breed club and its newsletter and potentially through veterinarians who examined and treated members of the breed.

The majority of OESCA members presumably have owned one or more Old English sheepdogs. Membership in this breed club obviously did not include all those owning Old English sheepdogs nor did it necessarily reflect the general population of owners. The extent whereby such differences in the owners or their dogs applied to the breed at

large was unknown. Therefore, the results of this survey could not be generalized to all Old English sheepdogs. Nevertheless, the data obtained were useful to both members and nonmembers.

With respect to identifying Old English sheepdogs seen by veterinarians, it would be unrealistic to expect a useful response rate from practitioners, not to mention the cost of mailing questionnaires to a randomly selected group of them throughout the country. Unless their records were computerized, it would have been very difficult for veterinarians to retrieve specific breed information. One way to access this data base was for survey respondents to include copies of the dog's veterinary clinical records along with the completed survey forms, and some respondents did so.

2. Response Rate

The response rate is always an important factor in any survey (Erdos, 1970; Alderson, 1976). The respondents must be representative of the universe as a whole or the validity of the research is threatened. If a high response rate is not obtained, verification that the respondents are representative is necessary. Generally, a mail survey cannot be considered reliable unless it has a response rate of at least 50%. The findings from a survey with a lower response can still be useful, however, especially if no other information is available and the replies are interpreted with this in mind.

3. Results

a. Response Rate. Questionnaires were mailed as an insert to the *Old English Times* newsletter beginning with the April 1990 issue and continuing bimonthly through the December 1990 issue. The circulation list for the newsletter was about 950 copies. Responses were received for the 1-year period, April 1990 through April 1991, from 217 individuals who reported on the health status of 740 dogs. There were 127 (59%) OESCA member respondents and 90 (41%) were nonmembers. The overall response rate (number of respondents versus number of questionnaires provided per newsletter mailing) was 217/950 or 23%. This response rate was considerably below the rate of 50% deemed necessary to validate the survey population as representing the larger universe of all Old English sheepdog owners (Erdos, 1970). Nevertheless, the data provided an accurate description of the health status of the 740 dogs reported by those people who responded. Finally, while assuring confidentiality of reporting may be perceived to be important, only three respondents chose to submit anonymous survey forms and two of these had return addresses on the envelope.

b. Demographic Variables. i. General description. There were 553 dogs (75%) owned by OESCA members, and the remaining 187 of the 740 total were nonmember owned. With respect to geography, the location of 10 dogs (1.4%) was not given. For those located in the United States (671 or 92% of 730), their zip codes of origin were: 00000–09999(109 or 16% of 671), 10000–19999 (98 or 15%), 20000–29999 (88 or 13%), 30000–39999 (53 or 8%), 40000–49999 (77 or 11%), 50000–59999 (55 or 8%), 60000–69999 (34 or 5%), 70000–79999 (42 or 6%), 80000–89999 (38 or 6%), 90000–99999 (77 or 11%). Another 55 dogs (8%) were located in Canada, and 4 (0.5%) were from Australia. These data indicated that respondents and their dogs represented all parts of the United States as well as Canada.

For the 740 dogs profiled, 210 (28%) had always been healthy and another 311 (42%) had had only one identified health problem to date. The remainder (219 dogs or 30%) had experienced two or more significant health problems, a finding that matches the number of healthy dogs as about 30% were allocated to each group. The group with two or more diseases included 17 dogs where the owners failed to indicate whether or not they were healthy but provided answers showing several health problems.

ii. Number of dogs per owner. Of the 217 respondents, 14 failed to report the number of dogs currently owned. Of the remaining 203, 90 (44%) owned only 1 Old English sheepdog, 85 (42%) owned 2 to 5 dogs, and 28 (14%) had more than 5 dogs at the time of reporting. As would be expected, most respondents owned fewer than 6 Old English sheepdogs with an approximately equal number having just one or several dogs. If the 90 owners known to have only 1 dog were subtracted from the total of 740 dogs, the remaining 127 owners collectively reported on 650 dogs.

iii. Sex. Of the 740 dogs reported, the sex of 3 dogs was not provided. Of the remaining 737, 453 (61%) were females and 284 (39%) were males. A total of 275 dogs (37%) were reported as neutered; 200 females (44%) were spayed and 75 males (26%) were castrated. This preponderance of females may reflect the tendency of breeders to keep more females as breeding stock, that pet owners prefer females to males, other unidentified reasons, or a population sampling bias.

As expected, more females than males had been neutered. The sex ratios of the healthy group of dogs (29% of the total), those with only one health problem at the time of reporting (43%), and those with two or more diseases (30%) were similar to those of the group as a whole (i.e., 3:2 in favor of females).

iv. Age. Ages were given for 710 of the 740 dogs reported in the

survey; these ranged from 10 weeks to 17 years. The frequency breakdown by age was 42 (6%) less than 1 year of age, 57 (8%) between 1 and 2 years old, 166 (23%) between 2 and 5 years, 221 (31%) between 5 and 9 years old, and 224 (32%) geriatrics above 9 years of age. This age breakdown showed that 445 or 63% of the animals surveyed were 5 years of age or older.

v. Birth and death statistics. Of the 740 dogs reported in the survey, 470 (64%) were alive at the time and 270 (36%) had died. The dates of birth by decade were as follows: 1950s, 2; 1960s, 27; 1970s, 216; 1980s, 463; and 1990–1991, 16. The birth date was not stated for 16 dogs. Of the 724 dogs with birth dates provided, 619 or 85% were born between 1975 and 1991. The earliest reported birth was 1956. For those dogs that had already died at the time of reporting, data were: 1960s, 3; 1970s, 28; 1980s, 207; 1990–1991, 32. Of the 270 deceased dogs, 239 or 89% had died between 1980 and 1991. The earliest reported death was 1967. Thus, the 15-year period between 1975 and 1991 included the majority of dogs surveyed (85% of the dogs still alive and 89% of those that had died). This information is further analyzed in Table I.

vi. Lifespan and causes of death. Table II describes the lifespan of dogs reported in the survey by sex and age at death. Table IB lists the causes of death by various categories; namely, natural causes, acciden-

TABLE I

Date	Number of births	Number of deaths
A. Birth and death statistics for OES[a] reported in this study		
Before 1970	29	3
1970–1974	74	8
1975–1979	144	20
1980–1984	190	72
1985–1989	271	135
1990–1991	16	32
Unknown	16	0
Totals	740	270

B. Causes of reported deaths in OES

	Natural	Accidental	Euthanasia	Specific disease	Unknown
Number	56	32	127	159	55

[a] Old English sheepdogs.

TABLE II

LIFESPAN OF OES REPORTED[a]

Sex (years)	Number and age at death (years)					Mean lifespan
	<1	1–2	2–5	5–9	>9	
Males, intact	1	7	9	30	37	8.1
Males, neutered	0	1	6	7	14	8.2
Females, intact	2	11	9	29	27	7.6
Females, spayed	0	0	7	14	56	10.6
Totals	3	19	31	80	134	8.7

[a] 267 dogs; the age of 3 others was not given.

tal death (injury, fire, storm, hit-by-car), euthanasia, and specific disease where this was provided. Fifty-five animals died of unspecified or unknown causes (20% of the 270 deaths). The mean lifespan of the total group was 8.7 years with a range of less than 1 year to 17 years. This included 156 females and 110 males. The data also show that the lifespan of intact and neutered males was essentially the same, and was only 6 months less than that of the total group mean. The data for females, however, was in striking contrast. Spayed bitches lived 3 years longer on average than did their intact counterparts. Furthermore, the mean lifespan of intact bitches was a year less than that of the total group mean. This underscores the well-recognized beneficial effects of ovariohysterectomy on reduction of prevalence of mammary cancer and other reproductive disorders in female dogs (Feldman and Nelson, 1987). These survey data provide significant justification for all Old English sheepdog breeders and owners to spay their bitches early in life, if they are not intending to use them for breeding purposes, or otherwise as soon as their career as a brood bitch has been completed.

Of the 270 dogs that were no longer alive at the time of reporting, 159 (59%) died from a specified type of disease. While some of these animals died naturally as a consequence of their disease state, most diseased animals were put to sleep at the request of the owners presumably after consultation with their veterinarian.

vii. Congenital malformations. Congenital malformations were reported with relatively low frequency except for umbilical hernia and bite problems. These included: deafness in 15 animals (7 males, 8 fe-

males); missing teeth in 25 (12 males, 13 females); retained deciduous or "milk" teeth in 4 (2 of each sex); monorchidism in 6 males and cryptorchidism in 5 males; undershot jaw in 36 (18 of each sex); overshot jaw in 8 (4 of each sex); and umbilical hernia in 63 (17 males, 46 females). Additional data for the jaw malformations are given in Table VII.

viii. Preventive health (vaccination history). Most owners with only one dog relied upon veterinarians to administer puppy and booster vaccinations, whereas those with more than five dogs usually gave their own vaccinations (except for rabies vaccine). Most dogs received modified-live combination vaccines, although some received only killed parvovirus vaccines given separately or simultaneously with the modified-live distemper-hepatitis-parainfluenza combination booster. Six dogs were reported to have experienced significant vaccine reactions (two to rabies vaccine, one to distemper, two to leptospirosis bacterin, and one to a polyvalent vaccine product). Seven dogs were reported to have had vaccine failures (five to parvovirus vaccine, one to leptospirosis bacterin, and one to a polyvalent product).

c. Disease in the Old English Sheepdog. i. Rank order and frequency. About 72% (530 of the 740 dogs profiled) reported one or more significant health problems (Table III). The rank order and frequency of the top 23 conditions (including several tie rankings) is compared to the total group of 740 dogs in Table III.

ii. Sex. The sex of dogs experiencing the top 17 health problems is shown in Table IV. Only three conditions had a sex ratio that differed significantly from the population as a whole (i.e., 3:2 in favor of females): umbilical hernia, mammary cancer, and cystitis, all of which were more common in the females. The other 17 of the top ranked disorders showed a sex distribution equivalent to that for the population as a whole. Cystitis affected females 87% of the time (Table IV). This finding probably reflected the fact that females more often had urogenital infections (6.8%) than males (<1%). The reason why umbilical hernias affected more females than males is unclear. Perhaps more strain occurs on the umbilical ring when the dam pulls at and severs the umbilical cord of a female pup than a male, where the nearby penile sheath may add support.

iii. Neutered animals. Table V compares the top 17 health problems of neutered dogs. The data revealed some interesting facts. For example, while more than half of the affected females were spayed in 11 of the 20 most common conditions, relatively few of the females were spayed in 6 of the other problems. Those disorders with about $\frac{1}{3}$ of the animals spayed were hypothyroidism (ranked 6th); poor, dry, or

TABLE III

RANK ORDER AND FREQUENCY OF OES HEALTH PROBLEMS

Rank	Condition	Number affected	Frequency of diseased (n = 530,%)	Overall frequency (n = 740,%)
1	Diarrhea	142	26.8	19.2
2	Sebaceous cysts	135	25.5	18.0
3	Arthritis	115	21.9	15.7
4	Hip dysplasia	113	21.3	15.3
5	Excitability	100	18.9	13.5
6	Hypothyroidism	99	18.7	13.4
7	Ear infections	97	18.3	13.0
8	Flea allergy	95	17.9	12.7
9	Pseudopregnancy	51(all female)	16.0	11.3
10	Flatulence	76	14.3	10.3
11	Chewing skin or coat	74	14.0	10.0
	Poor, dry, or thin coat	74	14.0	10.0
12	Hot spots	72	13.6	9.7
	Autoimmune thyroiditis	72	13.6	9.7
13	Vaginitis	40(all female)	12.6	8.8
14	Umbilical hernia	63	11.9	8.5
15	Mammary cancer	37(all female)	11.4	8.2
16	Coat color change	58	10.9	7.8
17	Cystitis	55	10.4	7.4
	Passivity	55	10.4	7.4
18	Pruritis (itching)	51	9.6	6.9
19	Pyometra	31(all female)	9.7	6.8
20	Aggressiveness	45	8.5	6.1
21	Infertility, female	27(all female)	8.5	6.0
22	Hair loss	42	7.9	5.7
22	Fearfulness	42	7.9	5.7
23	Dermatitis	41	7.7	5.5
All other conditions		—	≤5	≤5

thin coat and hot spots (ranked 11th and 12th, respectively); umbilical hernia and coat color change (ranked 14th and 16th). Only $\frac{1}{4}$ of the females reported to have mammary cancer were spayed.

These findings are not surprising because, other than umbilical hernia, sex hormonal changes associated with estrus are known to predispose dogs to these conditions. The increased frequency of mammary cancer in intact versus spayed bitches (3:1) is particularly important and emphasizes why retired breeding or pet females should be spayed as soon as possible to reduce their susceptibility to this type of cancer.

TABLE IV

NUMBER AND FREQUENCY OF OES HEALTH PROBLEMS LISTED BY SEX

Rank	Condition	Number and frequency (%)	
		Males	Females
1	Diarrhea	73 (51)	69 (49)
2	Sebaceous cysts	53 (39)	82 (61)
3	Arthritis	46 (40)	69 (60)
4	Hip dysplasia	39 (34)	74 (66)
5	Excitability	35 (35)	65 (65)
6	Hypothyroidism	43 (44)	56 (56)
7	Ear infections	40 (41)	57 (59)
8	Flea allergy	38 (40)	57 (60)
9	Pseudopregnancy	0 (0)	51 (100)
10	Flatulence	34 (45)	42 (55)
11	Chewing skin or coat	26 (35)	48 (65)
	Poor, dry, or thin coat	23 (31)	51 (69)
12	Hot spots	37 (51)	35 (49)
	Autoimmune thyroiditis	30 (42)	42 (58)
13	Vaginitis	0 (0)	40 (100)
14	Umbilical hernia	17 (27)	46 (73)[a]
15	Mammary cancer	0 (0)	37 (100)[b]
16	Coat color change	24 (41)	34 (59)
17	Cystitis	7 (13)	48 (87)[b]
	Passivity	21 (39)	34 (61)

[a] $P < 0.05$.
[b] $P < 0.01$.

At the other end of the spectrum, nearly $\frac{3}{4}$ of females affected with cystitis were spayed and more than $\frac{4}{5}$ of those noted to have passive behavior were spayed. Both of these situations parallel expectations from other studies in a variety of dog breeds.

With respect to the males as a group, fewer were castrated so that neutered males accounted for only $\frac{1}{5}$ to $\frac{1}{3}$ of the dogs having 11 of the 20 most common problems. Only in the case of cystitis (86%) and passive behavior (52%) were most of the dogs castrated.

iv. Age. The age breakdown for dogs affected with the top 17 health problems is shown in Table VI. As expected, the most common diseases were reported more frequently in adult (over 2 years) and geriatric dogs. A striking increase in frequency of diseases associated with aging was also noted in the oldest group for arthritis (70%) and

TABLE V

NUMBER AND FREQUENCY OF NEUTERED OES WITH HEALTH PROBLEMS

Rank	Condition	Total affected			Number and frequency (%)	
		M^a	F^b	$M + F^c$	M/N^d	F/S^e
1	Diarrhea	73	69	142	23(32)	35(51)
2	Sebaceous cysts	53	82	135	23(43)	54(66)
3	Arthritis	46	69	115	17(37)	45(65)
4	Hip dysplasia	39	74	113	12(32)	49(66)
5	Excitability	35	65	100	15(43)	35(59)
6	Hypothyroidism	43	56	99	8(19)	20(36)
7	Ear infections	40	57	97	12(30)	36(63)
8	Flea allergy	38	57	95	10(26)	32(56)
9	Pseudopregnancy	0	51	51	0(0)	51(100)
10	Flatulence	34	42	76	11(32)	28(67)
11	Chewing skin or coat	26	48	74	7(27)	27(57)
	Poor, dry, or thin coat	23	51	74	9(39)	17(33)
12	Hot spots	37	35	72	7(19)	13(37)
	Autoimmune thyroiditis	30	42	72	7(23)	18(43)
13	Vaginitis	0	40	40	0(0)	40(100)
14	Umbilical hernia	17	46	63	6(35)	17(37)
15	Mammary cancer	0	37	37	0(0)	9(24)
16	Coat color change	24	34	58	2(8)	9(27)
17	Cystitis	7	48	55	6(86)	35(73)
	Passivity	21	34	55	11(52)	27

[a] Males.
[b] Females.
[c] Males plus females.
[d] Males, neutered.
[e] Females, spayed.

mammary cancer (73%). Twice as many dogs over 9 years of age had sebaceous cysts (58%) and cystitis (51%) as did dogs with these diseases in the next most frequent age group of 5–9 years (31% and 24%, respectively). Similarly, nearly twice as many of the oldest dogs had flea allergy (47%) when compared to the 5- to 9-year (25%) and 2- to 5-year (23%) groups. These results appear to be significant despite the fact that over 60% of the total population was at least 5 years old, because equal numbers of dogs belonged to the 5–9 years and over 9 years age groups.

v. Genetic influences. Of the 20 conditions ranked among the top 17 health problems, 2 are inherited (hip dysplasia and umbilical hernia)

TABLE VI

NUMBER AND FREQUENCY OF OES HEALTH PROBLEMS BY AGE GROUP

Rank	Condition	Age in years				
		<1	1–2	2–5	5–9	>9
1	Diarrhea	7(5%)	10(7%)	36(25%)	44(31%)	45(32%)
2	Sebaceous cysts	1(1)	0(0)	13(10)	42(31)	79(58)
3	Arthritis	0(0)	1(1)	8(7)	25(22)	81(70)
4	Hip dysplasia	5(4)	6(5)	28(25)	33(29)	41(36)
5	Excitability	0(0)	16(16)	36(36)	25(25)	23(23)
6	Hypothyroidism	0(0)	10(10)	32(32)	37(37)	20(20)
7	Ear infections	2(2)	10(10)	25(26)	24(25)	36(37)
8	Flea allergy	0(0)	4(4)	22(23)	24(25)	45(47)
9	Pseudopregnancy	0(0)	40(78)	11(22)	0(0)	0(0)
10	Flatulence	1(1)	3(4)	17(22)	25(33)	30(40)
11	Chewing skin or coat	1(1)	9(12)	20(27)	23(31)	21(28)
	Poor, dry, or thin coat	2(2)	6(8)	20(27)	20(27)	26(35)
12	Hot spots	1(1)	3(4)	20(28)	23(32)	25(35)
	Autoimmune thyroiditis	0(0)	8(11)	26(36)	24(33)	14(20)
13	Vaginitis	3(7)	16(40)	14(35)	5(13)	2(5)
14	Umbilical hernia	4(6)	8(13)	20(32)	18(29)	13(20)
15	Mammary cancer	0(0)	0(0)	0(0)	10(27)	27(73)
16	Coat color change	2(3)	6(10)	14(24)	18(31)	18(31)
17	Cystitis	2(4)	3(5)	9(16)	13(24)	28(51)
	Passivity	4(7)	5(9)	10(18)	18(33)	18(33)

and another 3 occur in genetically predisposed individuals (hypothyroidism, autoimmune thyroiditis, and flea allergy). All of these 5 established genetically based conditions are believed to be polygenic and multifactorial (Conaway *et al.*, 1985; Corley and Hogan, 1985; Sinha *et al.*, 1990; Davies *et al.*, 1991; Utiger, 1991; Dodds, 1992e; Clark and Stainer, 1994; AVAR, 1994). If conditions known to express a familial pattern that may have a genetic basis are included (sebaceous cysts, arthritis, temperament problems—both excitability and passivity, and mammary cancer), the number of commonly identified health problems having some genetic influence in Old English sheepdogs comes to 10.

d. Specific Diseases in the Old English Sheepdog. For the individual or groups of diseases summarized below, each disease category lists the number and cumulative frequency, sex, and ages of affected dogs in comparison to the total population of 740 dogs profiled. For all diseases examined, 210 (28%) of the total survey group had remained

healthy at the time of reporting. A detailed breakdown of the data is given for those diseases with a frequency equal to or greater than 5%.

i. Joint and bone disorders. The number and frequency of dogs reported to have joint or bone conditions is shown in Table VII. As expected, arthritis and hip dysplasia were much more common than the other joint and bone diseases. Most arthritic dogs were over 9 years of age, whereas hip dysplasia was most prevalent from ages 2 through old age. Thyroid disease was present in 23 or 10.5% of dogs affected with joint or bone diseases, which emphasizes the multifaceted clinical signs associated with hypothyroidism (Dodds, 1992a,d, 1994a,b; Jaggy *et al.,* 1994; Panciera, 1994).

ii. Hip dysplasia. The aggregate data for hip dysplasia are shown in Table VII, and Table VIII describes the severity of dysplasia. While 66% of dogs reported to have hip dysplasia were females, this 2:1 sex ratio in favor of females was similar to the 3:2 ratio found for the

TABLE VII

JOINT AND BONE DISORDERS IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
		A. Rank and sex		
1	Arthritis	116 (16)	46 (40%)	69 (60%)
2	Hip dysplasia	113 (15)	39 (34)	74 (66)
3	Undershot jaw	36 (5)	18 (50)	18 (50)
4	Osteochondritis dissecans	16 (2)		
5	Rheumatoid arthritis	14 (2)		
6	Long bone disease	11 (1.5)		
7	Overshot jaw	8 (1)		
Totals		314 (42.5)[a]		

B. Rank and age

Rank	Condition	Age group (years)				
		<1	1–2	2–5	5–9	>9
1	Arthritis	0(0%)	1(0.9%)	7(6%)	25(22%)	81(70%)
2	Hip dysplasia	5(4)	6(5)	28(25)	33(29)	41(36)
3	Undershot jaw	3(8)	7(19)	13(36)	9(25)	4(11)

[a] When corrected for repeats, these diseases occurred in 217 or 29% of the dogs.

TABLE VIII

DEGREE OF HIP DYSPLASIA IN OES[a]

Number affected	Mild	Moderate	Moderate to severe	Severe
113 (74 females, 39 males)	42(37%)	25(22%)	10(9%)	28(25%)

[a] Severity of disease was not specified for 8 dogs (7%).

population as a whole. It would appear, therefore, that there was only a minor, if any, demonstrable female sex predilection for hip dysplasia. A larger survey of the breed would be needed to determine whether this trend could be substantiated. Data from the Orthopedic Foundation for Animals for the years 1966–1973 indicated that of 2311 Old English sheepdogs evaluated, 29.9% had hip dysplasia, whereas for the years of 1974–1989, 6363 dogs of the breed were examined with 22.5% reported as dysplastic (Corley, 1992). The present survey data for 740 dogs reported only 15.3% as dysplastic.

iii. Skin and coat disorders. While 17 conditions were grouped into this category, only eight had a frequency of more than 5%. Results are shown in Table IX. Hypothyroidism was an underlying factor contributing to the skin diseases of 59 or 21% of these dogs.

iv. Reproductive disorders. The most frequently reported reproductive disorders are listed in Table X. The top 5 reproductive diseases occurred in females and ranged from 6 to 11.3% of the 453 females surveyed. The five next most common disorders of reproduction affected males and ranged from 1.8 to 5.8% of the 284 males surveyed. The last condition, metritis, affected 1.3% of females. As expected, pseudopregnancy occurred primarily in younger females whereas vaginitis and infertility occurred in adults of breeding age (Feldman and Nelson, 1987). Mammary cancer and pyometra occurred in the older adult and geriatric females. Similarly, sterility and lack of libido occurred in the adult group of males, with prostatic disease more common in the geriatric males.

Underlying hypothyroidism was present in a subset of both males and females with reproductive disorders. Specifically, 40 of the 192 (20.8%) reported female reproductive diseases involved bitches that were also hypothyroid (10 of the 51 with pseudopregnancy, 3 of the 6 with metritis, 13 of the 40 with vaginitis, and 10 of the 27 with infertility). Similarly, 6 of the 31 (19.4%) males with reproductive diseases also had hypothyroidism. When combined, thyroid disease contributed

TABLE IX

SKIN AND COAT DISORDERS IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Flea allergy	95 (12.7)	38 (40%)	57 (60%)
2	Chewing skin or coat	74 (10.0)	26 (35)	48 (64)
	Poor, dry, or thin coat	74 (10.0)	23 (31)	51 (69)
3	Hot spots	72 (9.7)	37 (51)	35 (49)
4	Coat color change	58 (7.8)	24 (41)	34 (59)
5	Pruritus (itching)	51 (6.9)	16 (31)	35 (69)
6	Hair loss	42 (5.7)	16 (38)	26 (62)
7	Dermatitis	41 (5.5)	16 (39)	25 (61)
8	Allergic skin disease	32 (4.3)		
9	Dietary allergy	27 (3.6)		
10	Lick granuloma	19 (2.6)		
11	Contact allergy	17 (2.3)		
12	Mange	14 (1.9)		
13	Hypersensitivity	11 (1.5)		
	Atopy (inhalant allergy)	11 (1.5)		
14	Bacterial hypersensitivity	7 (0.9)		
15	Sebaceous adenitis	6 (0.8)		
Totals		651 (88.0)[a]		

[a] When corrected for repeats, these conditions affected 279 or 38% of the dogs.

to the reproductive disorders of 46 or 35.7% of affected dogs and bitches. These findings underscore the importance of thyroid function in maintaining healthy reproductive capacity (Feldman and Nelson, 1987; Dodds, 1992a, 1994a,b; Panciera, 1994).

v. Cancer. The number of animals reported to have various types of cancer is summarized in Table XI. Mammary cancer was the most common and ranked 15th among all the diseases shown in Table III. A total of 89 dogs (12% of the 740) had some form of these cancers.

vi. Eye diseases. A low frequency of eye diseases was reported in the survey. These included: 16 dogs with entropion (7 males, 9 females); 15 with bilateral cataracts (5 males, 10 females); 9 with unilateral cataracts (all females); 8 with third eyelid problems (3 males, 5 females); and 4 each with ectropion and autoimmune uveitis.

vii. Chronic infections. A group of chronic infectious conditions were reported and are summarized in Table XII. The breakdown by age group for the 2 most common (ear and bladder) infections is provided in Table VI. The effect of neutering and age on the incidence of cystitis

TABLE X

REPRODUCTIVE DISORDERS IN OES

Rank	Condition	Number and cumulative frequency (%)	Age in years				
			<1	1–2	2–5	5–9	>9
1	Pseudopregnancy	51 (11.3)	10 (20%)	34 (67%)	6 (12%)	1 (2%)	0 (0%)
2	Vaginitis	40 (8.8)	5 (13)	10 (25)	18 (45)	7 (18)	0 (0)
3	Mammary cancer	37 (8.2)	0 (0)	0 (0)	0 (0)	10 (27)	27 (73)
4	Pyometra	31 (6.8)	0 (0)	1 (3)	1 (3)	4 (45)	15 (48)
5	Infertility, female	27 (6.0)	0 (0)	0 (0)	11 (41)	16 (59)	0 (0)
6	Prostatic disease	14 (4.9)	0 (0)	0 (0)	0 (0)	5 (36)	9 (64)
7	Sterility, male	14 (4.9)	0 (0)	0 (0)	3 (21)	10 (71)	1 (7)
8	Poor libido, male	7 (2.5)					
9	Monorchidism	6 (2.1)					
10	Cryptorchidism	5 (1.8)					
11	Metritis	6 (1.3)					
Totals		238 (32.2)[a]	(42.4% of females; 16.2% of males)				

[a] When corrected for repeats, these conditions affected 211 or 28.5% of the dogs.

TABLE XI

CANCERS IN OES

Rank	Type	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Mammary	37 (8.2)	—	37(100%)
2	Bone	17 (2.3)	8(47%)	9(53)
	Lymph node	17 (2.3)	8(47)	9(53)
3	Liver	9 (1.2)	5(56)	4(44)
	Lung	9 (1.2)	3(33)	6(67)
Totals		89 (12)[a]		

[a] When corrected for repeats, these diseases affected 78 or 10.5% of the dogs.

was discussed above. Dermatitis was more commonly seen in adult (12 were 2–5 years; 16 were 5–9 years) and geriatric animals (11 were over 9 years old). Hypothyroidism was present and probably contributed to the susceptibility to chronic infections in 24 or 13% of affected dogs.

viii. Immune problems. Table XIII summarizes the reported immunologic diseases. The most common by far was autoimmune thyroid disease, although this category was likely to have been grossly underreported because most of the 99 dogs listed as having hypothyroidism presumably had the autoimmune form of thyroid disease (see Section *d*.x).

The true frequency of autoimmune diseases in the Old English sheepdog breed is not reflected by the data from the present survey of

TABLE XII

CHRONIC INFECTIONS IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Ear infections	97 (13)	40(41%)	57(59%)
2	Cystitis	55 (7.4)	7(13)	48(87)
3	Dermatitis	41 (5.5)	16(39)	25(61)
4	Gingivitis	14 (1.9)	8(57)	6(43)
5	Tooth abscess	10 (1.3)	4(40)	6(60)
Totals		217 (29)[a]		

[a] When corrected for repeats, these infections occurred in 182 or 25% of the dogs.

TABLE XIII

IMMUNE PROBLEMS IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Autoimmune thyroiditis	72 (9.7)	30(42%)	42(58%)
2	Thrombocytopenia	12 (1.6)	3(25)	9(75)
3	Hemolytic anemia	11 (1.5)	5(45)	6(55)
4	Immunosuppression	10 (1.3)	4(40)	6(60)
5	Rheumatoid or immune arthritis	6 (0.8)	1(17)	5(83)
	Immune kidney disease	6 (0.8)	1(17)	5(83)
	Uveitis	6 (0.8)	1(17)	5(83)
Totals		123 (16.6)[a]		

[a] When corrected for repeats, these diseases affected 96 or 13% of the dogs.

740 dogs. As documented in a series of earlier articles by this author and others, the Old English sheepdog is predisposed to many types of autoimmune diseases (Boyer *et al.*, 1980; Dodds, 1983, 1990a; Mills *et al.*, 1985; Day and Penhale, 1992; Barker *et al.*, 1993).

Between 1980 and 1990, this author has studied 162 cases of autoimmune blood diseases in Old English sheepdogs. These cases include the first 57 cases reported earlier (Dodds, 1983) and 105 cases seen since then. This time period coincides closely with that encompassing the majority (about 85%) of the animals reported in the present survey. Table XIV provides a descriptive breakdown of the 162 cases compiled previously (Dodds, 1983, 1990a).

Examination of the data presented in Table XIV shows that females outnumbered males as expected by about a 2:1 ratio, regardless of whether they were intact or spayed at the age of onset of their autoimmune disease. Curiously, while cases with both autoimmune hemolytic anemia (AIHA) alone or in combination with idiopathic thrombocytopenic purpura (ITP) had a greater preponderance of females, the number of dogs with ITP alone was equally divided between males and females. Similarly, there were equal numbers of males and females expressing thyroid disease as their primary problem. The age of onset ranged widely, although with several disorders affected animals were younger than considered typical for autoimmune problems (Dodds, 1983).

Thyroid disease was recognized as either a primary or secondary

TABLE XIV

OLD ENGLISH SHEEPDOGS WITH AUTOIMMUNE BLOOD AND ENDOCRINE DISEASES, 1980–1989

Primary disease or problem	Number of animals	Sex		Age of onset (Range)	Other diseases or health problems
		Male[a]	Female[b]		
1980–1982[c]					
AIHA[d] and/or ITP[e]	57	12	45	5 mo–12 yr	Dermatitis, glomerulonephritis, hepatitis, splenomegaly, recent vaccination, bone marrow failure, pemphigus, thyroid and adrenal disease, temperature stress
1983–1989					
AIHA	22	6	16	2–11 yr	Thyroid disease, Addison's disease, vaccination, estrus
ITP	27	14	13	10 mo–11 yr	Thyroid disease, vWD, drug exposure, vitiligo, vaccination
AIHA and ITP	9	1	8	6 mo–13 yr	Thyroid disease, skin disease, allergies, vaccination, drug exposure
Thyroiditis/hypothyroidism	31	15	16	2–9 yr	vWD, urinary tract infection, flea allergy, skin disease, hair loss, vaginitis
Addison's disease	1	—	1	4 yr	AIHA
Polyglandular endocrinopathy	1	—	1	4 yr	Addison's disease, thyroiditis, renal and liver failure
Diabetes	2	—	2	9 mo–1 yr	Epilepsy, ITP
Hypoparathyroidism	1	—	1	5 yr	Hypocalcemia, thyroid disease, renal failure
Rheumatoid arthritis	1	—	1	7.5 yr	Anemia
Vaccine reaction	7	3	4	10 mo–9 yr	Thyroiditis, low vWF, ITP
SLE[f]	3	1	2	3–5.5 yr	Anemia, renal failure, thyroid disease
Total	162	52	110		

[a] 3 neutered.
[b] 15 spayed.
[c] Dodds, 1983.
[d] Autoimmune hemolytic anemia.
[e] Immune-mediated thrombocytopenia.
[f] Systemic lupus erythematosus.

problem in 46 of the 105 cases reported since 1983. This is probably an underestimate, however, because in most of the other cases, thyroid function tests were not run or were inconclusive. Data from 1980 to 1982 with respect to concurrent thyroid dysfunction are incomplete because thyroid testing was not performed as often at that time, and the role of underlying thyroiditis in predisposing to autoimmune blood diseases was not as appreciated (Axelrod and Berman, 1951; Waldmann *et al.,* 1962; Popovic *et al.,* 1977; Hymes *et al.,* 1981; Endo, 1985; Girelli, 1986; Fisher *et al.,* 1987; Dodds, 1987, 1988, 1990a; Sullivan *et al.,* 1993). Other endocrine diseases were involved in seven of the more recent 103 cases, which is consistent with the polyglandular autoimmune syndrome previously reported in dogs (Bowen *et al.,* 1986; Schaer *et al.,* 1986) as well as humans (Trence *et al.,* 1984). Bleeding was a significant clinical problem in 24 of the later cases having ITP or von Willebrand's disease and in 30 of the earlier group with ITP (Dodds, 1983).

Multiple endocrinopathies or autoimmune syndromes occur in human patients with underlying autoimmune thyroid disease (hypo- or hyperthyroidism). These include concurrent Addison's disease, diabetes, reproductive gonadal failure, skin diseases and alopecia, and malabsorption syndrome (Trence *et al.,* 1984; Fisher *et al.,* 1987). The most common nonendocrinologic autoimmune disorders associated with this syndrome are AIHA, ITP, chronic active hepatitis, and immune-complex glomerulonephritis (systemic lupus erythematosus [SLE], or SLE-like kidney disease) (Fisher *et al.,* 1987). The Old English sheepdog is particularly prone to the AIHA, ITP, and thyroid disorders (Boyer *et al.,* 1980; Mills *et al.,* 1985; Dodds, 1983, 1990a; Day and Penhale, 1992; Barker *et al.,* 1993). In some cases the multiorgan pathology is precipitated by drugs or recent viral disease or use of combination modified live virus vaccines (Dodds, 1983, 1990a). Thus, it is not unusual for dogs of breeds prone to thyroid disease to develop a polyglandular endocrinopathy or multisystemic autoimmune syndrome (Mill and Campbell, 1992; Dodds, 1992e).

The disease experience with a particular Old English sheepdog family illustrates this situation (Giger and Dodds, 1992). Two littermate females were admitted at 7.5 months of age to the University of Pennsylvania, School of Veterinary Medicine with reported adverse reactions to polyvalent booster-vaccines. Both had borderline normal levels of von Willebrand factor antigen (51 and 55%), abnormal thyroid function tests with elevated antithyroglobulin antibodies (107 and 121 Relative Antibody Units; <3 is normal), and were biopsy confirmed to have lymphocytic thyroiditis at 10 to 12 months of age. Two other

littermates (one male, one female) had severe vaccine reactions, and the male had low von Willebrand factor antigen (43%) and elevated antithyroglobulin antibody. Thus, four of the five puppies in this litter were affected with immune-mediated disease within the first year of life. Other immediate family members were also affected. The sire and two litterbrothers of the dam also had thyroid disease, and the dam had low von Willebrand factor antigen (31%), abnormal thyroid function tests, and elevated circulating T3 autoantibody and antithyroglobulin antibody (53 Relative Antibody Units). The maternal grandsire had normal won Willebrand factor (75%) but had elevated antithyrogobulin (60 Relative Antibody Units) when tested as a geriatric dog. The maternal great granddam had produced another daughter with thyroid disease that developed into thyroid adenocarcinoma at age 10 years. This female's paternal grandsire was the foundation sire of many dogs affected with AIHA and/or ITP and his littersister had died of AIHA. The recurring pattern of autoimmune disorders in this family was even more remarkable because these dogs represented a closely related subset of the larger group summarized in Table XIV (Giger and Dodds, 1992).

Assessment of the available pedigrees from 108 of these 162 Old English sheepdog cases indicates a close relationship among all but seven of the affected dogs. Two of three pedigrees available from the studies of Day and Penhale (1992) were also related to this large North American study group; the third pedigree was incomplete and so the ancestry could not be traced further.

ix. Temperament and behavioral problems. Temperament is of paramount concern along with soundness for all purebred dog breeders and owners. A breakdown of the temperament problems reported by survey respondents is found in Table XV. The reported temperament or behavioral problems were grouped as either hyperactive and aggressive or passive and lethargic. A total of 171 reports fell into the former category (the sum of rankings 1, 3, 8, and 9), and an equivalent of number of 172 were in the latter (rankings 2 and 4–7). Most of the dogs reported as excitable or aggressive were adults (52 were 2–5 years; 39 were 5–9 years; and 34 were over 9 years old). Similarly, most of those reported as passive, fearful, or shy were adults (36 were 2–5 years; 34 were 5–9 years; and 42 were over 9 years old). Underlying thyroid disease was present in 26 or 12% of dogs with temperament or behavioral abnormalities, thereby supporting the role of thyroid function in affecting behavior (Dodds, 1992c, 1994a; Jaggy *et al.*, 1994).

x. Thyroid disease and related conditions. Hypothyroidism and autoimmune thyroiditis were reported in 99 and 72 dogs, respectively (Table XVI). As the majority of thyroid disease in the dog is caused by

TABLE XV

TEMPERAMENT AND BEHAVIORAL PROBLEMS IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Excitable	100 (13.5)	35(35%)	65(65%)
2	Passive	55 (7.4)	21(39)	34(61)
3	Aggressive	45 (6.1)	23(51)	22(49)
4	Fearful	42 (5.7)	16(38)	26(62)
5	Shy	37 (5.0)	9(24)	28(76)
6	Unstable	20 (2.7)	9(45)	11(55)
7	Lethargic	18 (2.4)	3(17)	15(83)
8	Irritable	17 (2.3)		
9	Hyperexcitable	9 (1.2)		
Totals		343 (46.4)[a]		

[a] When corrected for repeats, these problems affected 214 or 29% of the dogs.

familial autoimmune thyroiditis (Gosselin *et al.*, 1982; Ferguson, 1984; Haines *et al.*, 1984; Beale, 1990; Beale *et al.*, 1990; Chastain, 1990; Dodds, 1992a,d, 1994a), when combined, these two diagnoses accounted for 129 dogs or 17.4% of the total (corrected for 69 repeats). Of the

TABLE XVI

THYROID DISEASE AND RELATED CONDITIONS IN OES

Rank	Condition	Number and cumulative frequency (%)
1	Hypothyroidism	99 (13.4)
2	Autoimmune thyroiditis	72 (9.7)
3	Thyroid + chronic skin disorders	59 (8.0)
4	Thyroid + reproductive disorders	46 (6.2)
5	Thyroid + reproductive + skin disorders	33 (4.4)
6	Thyroid + temperament problems	26 (3.5)
7	Thyroid + chronic infections	24 (3.2)
8	Thyroid + joint or bone disorders	23 (3.1)
9	Thyroid + blood diseases	22 (3.0)
10	Thyroid + skin disorders + chronic infections	21 (2.8)
11	Thyroid + temperament problems + joint or bone disorders	12 (1.6)
Totals		437 (59.0)[a]

[a] When corrected for repeats, 357 or 48% of the dogs were affected with thyroid disease and related conditions.

72 dogs reported to have autoimmune thyroiditis, 30 (42%) were not reported by the respondents as being hypothyroid.

Thyroiditis is an immune-mediated disease process that develops in genetically susceptible individuals and is characterized by the presence of antithyroid antibodies in the blood or tissues (Gosselin *et al.*, 1982; Haines *et al.*, 1984; Conaway *et al.*, 1985; Sakata *et al.*, 1985; Fisher *et al.*, 1987; Rajatanavin *et al.*, 1989; Bethune, 1989; Chastain *et al.*, 1989; Ciampolillo *et al.*, 1989; Beale *et al.*, 1990; Davies *et al.*, 1991; Utiger, 1991; ; Dodds, 1992a, 1994a; Thacker *et al.*, 1992; Tomer and Davies, 1993; Thacker *et al.*, 1995). This condition usually, but not always, progresses eventually to thyroid disease (other commonly used terms for the disease are "lymphocytic thyroiditis" or "Hashimoto's disease"). Thyroiditis is believed to start in most cases around puberty and gradually progresses through midlife and old age to become clinically expressed hypothyroidism once thyroid glandular reserve has been depleted (Fisher *et al.*, 1987; Bethune, 1989; Dodds, 1992a). During this process, the animal or person can become more susceptible to immune-mediated or other diseases affecting various target tissues and organs [e.g., AIHA, ITP (Hymes *et al.*, 1981; Girelli, 1986; Dodds, 1988; Sullivan *et al.*, 1993); von Willebrand's disease (Dalton *et al.*, 1987; Dodds, 1988, 1992d); systemic lupus erythematosus (Fisher *et al.*, 1987); seizure disorders (Dodds, 1992c; Jaggy *et al.*, 1994); chronic infections (Dodds, 1992a,e, 1994a; Tomer and Davies, 1993); immunosuppressive viral infections such as distemper, parvovirus, and retrovirus diseases (Dodds, 1983; Davies *et al.*, 1991; Tomer and Davies, 1993; Dodds, 1995); bone marrow failure (Levin and Bessman, 1983; Fisher *et al.*, 1987; Dodds, 1988; Sullivan *et al.*, 1993); leukemia and lymphoma (Ciampolillo *et al.*, 1989); chronic active hepatitis (Trence *et al.*, 1984; Fisher *et al.*, 1987); immune eye, joint, kidney, and adrenal (Addison's) diseases (Trence *et al.*, 1984); chronic allergic and immune skin and muscle disorders (Trence *et al.*, 1984; Tomer and Davies, 1993; Steinman, 1993)]. The prerequisite genetic basis of this disorder has been established in humans (Sinha *et al.*, 1990; Utiger, 1991; Tomer and Davies, 1993) and dogs (Haines *et al.*, 1984; Conaway *et al.*, 1985), as well as several other species (Davies *et al.*, 1991).

Of the 99 dogs reported to be hypothyroid, 94 or 95% of them were receiving thyroid hormone replacement therapy. For those dogs on therapy, 77 (82%) were medicated twice daily. This is the preferred method of treatment to ensure appropriate clinical response (Panciera *et al.*, 1990; Nachreiner and Refsal, 1992a; Nachreiner *et al.*, 1993) and to regulate endogenous thyroid metabolism by inhibiting further progression of the autoimmune thyroid tissue destruction (Sakata *et al.*, 1985; Dodds, 1992e, 1994a).

TABLE XVII

ENDOCRINE DISEASES IN OES

Rank	Condition	Number and cumulative frequency (%)
1	Hypothyroidism	99 (13.4)
2	Autoimmune thyroiditis	72 (9.7)
3	Addison's disease (hypoadrenocorticism)	5 (0.7)
4	Cushing's disease (hyperadrenocorticism)	3 (0.4)
5	Diabetes	1 (0.1)
Totals		180 (24.3)[a]

[a] When corrected for repeats, 138 or 19% of dogs had endocrine diseases and 94% of these involved the thyroid gland.

Because of the multifaceted clinical signs associated with thyroid dysfunction (see preceding), the survey data were further analyzed by inclusion of the more common related conditions (Table XVI).

xi. Endocrine diseases. With the exception of hypothyroidism and autoimmune thyroiditis, very few animals were reported to have endocrine diseases (Table XVII).

xii. Blood diseases. The most commonly reported blood diseases were ITP and AIHA (Table XVIII). Thyroid disease was also present in half (18 or 52.9%) of the dogs with blood diseases.

xiii. Bleeding disorders. Aural hematomas, which manifest as bleeding into the dependent part of the ears, were the most frequently

TABLE XVIII

BLOOD DISEASES IN OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Female
1	Thrombocytopenia	12 (1.6)	3(25%)	9(75%)
2	Hemolytic anemia	11 (1.5)	5(45)	6(55)
3	von Willebrand's disease	4 (0.5)		
4	Systemic lupus erythematosus	3 (0.4)		
	Bone marrow failure	3 (0.4)		
	Lymphoma	3 (0.4)		
5	Leukemia	2 (0.3)		
Totals		38 (5.1)[a]		

[a] When corrected for repeats, 34 or 5% of dogs had blood diseases and 77% were of autoimmune type.

TABLE XIX

BLEEDING DISORDERS OF OES

Rank	Condition	Number and cumulative frequency (%)	Sex	
			Males	Females
1	Aural hematoma	29 (3.9)	14(48%)	15(52%)
2	Nosebleeds	12 (1.6)	8(67)	4(33)
3	Thrombocytopenia	12 (1.6)	3(25)	9(75)
4	von Willebrand's disease	4 (0.5)		
Totals		57 (7.7)[a]		

[a] When corrected for repeats, 53 or 7% of dogs experienced excessive bleeding.

reported bleeding problem followed by thrombocytopenia and epistaxis. For the aural hematomas, 90% occurred in dogs over 7 years of age and 80% of the affected females were spayed. The data are shown in Table XIX.

xiv. Gastrointestinal disorders. A variety of gastrointestinal problems were reported for the survey population (Table XX). The most prevalent were diarrhea (19.2%) and flatulence (10.3%), which affected a significant number of dogs. For the 142 dogs reported with diarrhea,

TABLE XX

GASTROINTESTINAL DISORDERS OF OES

Rank	Condition	Number and cumulative frequency %	Sex	
			Males	Females
1	Diarrhea	142 (19.2)	73(51%)	69(49%)
2	Flatulence	76 (10.3)	34(45)	42(55)
3	Vomiting	50 (6.7)	19(38)	31(62)
4	Giardiasis	22 (2.9)	9(41)	13(59)
5	Bloat (acute gastric dilatation)	21 (2.8)	8(38)	13(62)
6	Colitis, chronic	20 (2.7)	12(60)	8(40)
7	Coccidiosis	15 (2.0)	3(20)	12(80)
8	Gastritis, chronic	9 (1.2)	6(67)	3(33)
Totals		355 (48.0)[a]		

[a] When corrected for repeats, 237 or 32% of dogs surveyed reported gastrointestinal problems.

it was characterized as acute in 47 (33%) and chronic in 22 (15%). In 71 dogs (50%) the diarrhea was recurrent, and there was no sex difference found for these subcategories. Vomiting was described as persistent in 8 (16%) of the 50 affected dogs and recurrent in 30 (60%). Of the 21 cases of bloat, torsion or volvulus was associated with the gastric dilatation in 9 (43%). There were 37 reports of coccidiosis and/or giardiasis which involved 30 dogs (7 animals had infections with both of these intestinal parasites). As these are opportunistic organisms, clinical signs of mild to severe, watery diarrhea typically occurred in stressed or immunocompromised individuals. Hypothyroidism was also present in 17 (57%) of dogs with either *Giardia* or *Coccidia sp.* infections.

xv. Other diseases. The remaining diseases or conditions were reported with only low frequency. These included: heartworm disease, 6 dogs; drug or toxicity problems, 7; dental cavities, 7; abnormal dental tartar, 24 (3.2%); tooth malformations, 37 (5%) including missing teeth and retained deciduous teeth; liver failure in 10 dogs all over 8 years old; enlarged spleen in 7 all over 7 years old; and bladder stones, 6.

xvi. Summary of health problems in Old English sheepdogs by disease category. The data reported for specific health conditions were grouped by disease or disorder category. These are summarized in rank order in Table XXI.

TABLE XXI

SUMMARY OF HEALTH PROBLEMS IN OES BY DISEASE CATEGORY

Rank	Disease category	Number and cumulative frequency (%)[a]
1	Thyroid disease and related conditions	357 (48.2)
2	Skin and coat disorders	279 (38.0)
3	Gastrointestinal disorders	237 (32.0)
4	Joint and bone disorders	217 (29.0)
5	Temperament and behavior problems	214 (28.9)
6	Reproductive disorders	211 (28.5)
7	Chronic infections	182 (24.6)
8	Endocrine diseases	138 (18.6)
9	Thyroid disease	129 (17.4)
10	Hip dysplasia	113 (15.3)
11	Immune problems	96 (13.0)
12	Cancer	78 (10.5)
13	Bleeding disorders	53 (7.2)
14	Blood diseases	34 (4.6)

[a] Data have been corrected for repeats in each category.

C. Bearded Collies

In 1992, the Bearded Collie Club of America Health Committee conducted a health survey similar to that of Old English sheepdogs (Aronson, 1992). A preliminary report was issued on the responses from 277 surveys involving 707 dogs (Aronson, 1992). The final report included data from 331 surveys and 804 dogs, 384 males (117 neutered, 10 unknown if neutered) and 420 females (203 spayed, 10 unknown if spayed) (Aronson, 1994, 1995). One hundred and thirty-three dogs were 10 years old or more (62 males, 71 females) and 83 dogs were 1 year old or less (41 males, 42 females). Forty-one states were represented, two reports came from Canada, five from the United Kingdom, and one from Australia.

When conditions affecting the initial group of 707 bearded collies were tabulated in rank order (in a manner similar to that shown for the Old English sheepdog in Table III), the following ranking was obtained (Aronson, 1992):

1. diarrhea
2. flea allergy
3. stillborn puppies
4. sebaceous cysts
5. fearfulness
6. cesarean section
7. false pregnancy
8. hot spots
9. umbilical hernia
10. shyness
11. parasitism
12. abnormal estrous cycles
13. arthritis, flatulence, chewing and biting skin
14. pruritis
15. poor, dry, or thin haircoat
16. food hypersensitivity
17. giardiasis
18. food intolerance, hypothyroidism, passivity
19. injury
20. vaginitis
21. cystitis, autoimmune thyroiditis, atopy
22. depigmentation
23. dietary allergy
24. allergic skin disease, missing teeth, vomiting, persistent or chronic
25. hip dysplasia
26. monorchidism
27. nonspecific dermatitis
28. ear infections, excitability, undescended testicles

The incidence of hip dysplasia in this survey (4.5%) was less than the 10.2% reported for this breed by the Orthopedic Foundation for Animals (Corley, 1992; Aronson, 1994). Of the 36 dogs with hip dysplasia (18 of each sex), 18 reported moderate to severe disease although the

severity was not stated for 4 cases. The most commonly reported eye problems were bilateral (17) and juvenile (9) cataracts. Umbilical hernias affected 79 dogs (31 males, 48 females). Two hundred ninety-three of the 800 dogs reported in the final analysis had at least 1 and as many as 16 had skin, hair, or mucous membrane conditions. The dogs most affected in this category were those with hypothyroidism (32 animals), hypothyroidism with other endocrine and autoimmune problems (6 dogs), or other autoimmune and endocrine disease (25 dogs). The latter two groups apparently had a polyglandular autoimmune syndrome (Trence *et al.*, 1984). The most common problems were flea allergy (140 dogs); poor, dry, or thin coat (69); hot spots of severe or chronic type (68); persistent chewing or biting (65); pruritis (62); allergic skin disease (48); dietary allergy (44); nonspecific dermatitis (1); atopy (39); depigmentation (38); and alopecia (33).

Hypothyroidism was the most commonly reported endocrine disease (55 dogs; 22 males, 33 females), and 49 of them were receiving thyroid supplement. Hypothyroidism was reported in several generations of some families and in several members of the same litter, findings which underscore the genetic factors that predispose animals to these conditions (Conaway *et al.*, 1985; Dodds, 1992a,e). Addison's disease, another prevalent autoimmune endocrine disorder of the bearded collie, was reported in 17 dogs (6 males, 11 females). Some of these dogs also were hypothyroid, a combination known as Schmidt's syndrome (Trence *et al.*, 1984; Fisher *et al.*, 1987).

Heart problems were uncommon, whereas muscle problems were primarily trembling (13 dogs) and weakness (15 dogs). Some dogs with muscle problems were likely exhibiting signs of underlying Addison's disease. Various forms of cancer were reported, the most common of which was mammary cancer (6 dogs). A large group of infectious diseases were listed with parasitic (58 dogs), bacterial (310), and opportunistic conditions (giardiasis, 55; coccidiosis, 33) being the most common. Cystitis was by far the most common urinary tract problem, and as expected, 38 cases were in females (11 greater than 10 years of age) and 5 were in males (2 greater than 10 years old).

The primary gastrointestinal disorders were acute diarrhea (96 dogs), recurrent diarrhea (68), flatulence (49), recurrent vomiting (30), and chronic colitis (20), but these numbers often reflected duplicate entries for the same animals. The most common neurological problems were hyperexcitability (17), seizures (10), and vaccine reactions (9). A group of dogs had one or more behavioral problems. Fearful temperaments were more common (89) than shyness (69) or passivity (50), but

37 dogs were abnormally excitable and 33 were aggressive or irritable (26). Another 18 were considered lethargic and 15 were unstable (erratic or unpredictable).

Other than the more commonly reported autoimmune endocrine diseases, 14 dogs had immune suppressive disorders and a few had AIHA, ITP, SLE, uveitis or VKH syndrome, pemphigus, and rheumatoid arthritis. The most common dental or mouth problems were missing teeth (40 dogs), halitosis (35), retained deciduous teeth (26), and gingivitis (21). Sebaceous cysts were commonly reported (119 dogs), and simple sebaceous adenomas affected 31 dogs. Chronic or persistent ear infections were present in 44 animals, and 19 were deaf. Food hypersensitivity (54) and intolerance (47) were also relatively common. Vaccine reactions were noted in 9 dogs and vaccine failure in 10 dogs.

Reproductive problems most commonly reported were false pregnancy (44 bitches), stillborn puppies (29), vaginitis (30), and abnormal estrous cycles (28). Eighteen males had cryptorchidism and 13 were monorchid. These data generated by bearded collie breeders were strikingly similar to those of the Old English sheepdog and are shown in Tables I–XXI.

D. Irish Wolfhounds

The prevalence of von Willebrand's disease and thyroid disease has increased rapidly over the last decade in more than 50 affected breeds despite the collective efforts of conscientious breeders working with veterinarians to test and screen out carriers from their breeding programs. The Irish wolfhound is one of the affected breeds.

The purpose of the present screening study was to determine the prevalence of both disorders in the Irish wolfhound and examine the potential relationship between levels of von Willebrand factor antigen (vWF:Ag) and thyroid hormones in this breed. One hundred seventy-nine dogs participated in the project [the Irish Wolfhound Club of America (IWCA) Physiological Testing Program], which was held at the National Specialty Show in Newport, Rhode Island, May 14–16, 1992. Testing for von Willebrand's disease was performed at the Albany, New York laboratory of the author's associates (Dodds, 1992f); thyroid profiles were measured at the Animal Health Diagnostic Laboratory, Michigan State University, Lansing, Michigan by Dr. Ray Nachreiner and staff (Nachreiner and Refsal, 1992b), and Dr. Neil Harpster of Angell Memorial Animal Hospital in Boston, Massachusetts performed electrocardiograms at the test site. This testing pro-

gram has continued on an annual basis since then, although the aggregate results have yet to be collated and analyzed.

1. Age and Sex

Of the 179 dogs screened, the age of 9 or 5% and sex of 3 or 2% were not provided. Ages were listed for 170 dogs. The youngest dog was 4 months old and the oldest was 10 years old. There were 23 puppies under a year of age, 44 adolescents between 1 and 2 years of age, and 65 adults between 2 and 5 years of age. Another 34 dogs were between 5 and 8 years of age, and 4 dogs were 8 years or older. If the number of adults from 2 to 8 years was combined, the total becomes 99 which represents 58% of those with known age that were screened. Stated another way, most (132 or 74%) of the dogs tested were less than 5 years of age. There were 75 males (43%) and 101 females (57%) among the 176 dogs where the sex was listed. Of these, 3 males were neutered and 9 females were spayed.

2. Familial Influence

As the familial relationship between many of those tested was unknown, the interpretation of test results for vWF:Ag and potentially for thyroid parameters may be biased. For example, if a relatively large number of dogs from the same family, litter, sire, or dam were included, the data base could be skewed as it reflects the influence of a common genotype. If the dogs come from the same owner and environment this could also have some as yet unrecognized influence on results.

3. Health History

Sixty-one dogs (34% of the 179 tested) had a history of various health problems. These included a total of 72 chronic or recurrent conditions, the majority (50 or 69%) of which involved females; only 22 (31%) were males (Table XXII). The remaining 118 dogs (66% of the 179 tested) were presumed to have been healthy throughout their lifetimes, although this is an overestimate because some participants did not complete the health questionnaire.

The most common health problem listed by respondents was chronic infections (48 dogs or 67% of the conditions listed). As was true for the total group with health problems, the majority (41 or 85%) involved females. The next most common disorders involved the reproductive tract and allergies, which affected 28 (39%) and 22 (31%) of the group, respectively. Females were represented more frequently in these cate-

TABLE XXII

HEALTH HISTORY OF IRISH WOLFHOUNDS

Rank	Condition	Number of dogs	Sex[a]		Frequency diseased (%)	Frequency of total (n = 179,%)
			M[b]	F[c]		
1	Presumed healthy	118	50	65	—	66
2	All health problems[d]	72	22	50	100	40
3	Chronic infections	48	7	41	67	27
4	Reproductive disorders	28	3	25	39	16
5	Allergies	22	7	15	31	12
6	Haircoat or pigment change	13	2	11	18	7
7	Diarrhea	8	5	3	11	4
8	Thyroid disease	7	2	5	10	4
9	Arthritis	7	4	3	10	4
10	All other conditions	24	15	9	33	13

[a] Another 3, unknown sex.
[b] Male.
[c] Female.
[d] Includes those with more than one condition.

gories as well (25 or 89% and 15 or 68%, respectively). Chronic problems with hair coat and pigmentation were reported in 13 dogs (18%). Of these, 11 (85%) were females and 2 (15%) were males. Hypothyroidism was reported in 7 dogs (10% of total), and 5 were on thyroid replacement therapy when tested. The other 2 affected dogs were not receiving therapy, for reasons that are unclear. The majority of affected dogs (5 or 71%) were females (Table XXII).

Diarrhea of chronic nature was noted in 8 dogs (11%), 5 males and 3 females. Several had chronic giardiasis. Seven dogs had chronic arthritic disease, and another 24 dogs had miscellaneous conditions including chronic parasitism(8), halitosis(4), cardiac disease(3), vaccine failure and canine kennel cough(3), excessive bleeding(2), inability to maintain weight(1), seizures(1), muscle weakness(1), and liver and kidney disease(1).

Table XXIII describes the specific diseases of the four most common disease categories. Chronic infectious disorders, involving the skin (21) and reproductive tract (17), were most often reported. The most common reproductive disorder was vaginitis (15), followed by sterility or infertility (7), pseudopregnancy (7), and abnormal heat cycles (5), two cases of metritis, and one case of monorchidism. The most commonly reported allergy was to fleas (13). Changes in hair coat texture,

TABLE XXIII

SPECIFIC DISEASES OF IRISH WOLFHOUNDS

Rank	Condition	Number of dogs[a]	Sex		Frequency diseased (%)	Frequency of total (n = 179,%)
			M[b]	F[c]		
1	Chronic infections	48	7	41	67	27
	Skin	21	6	15	29	12
	Reproductive tract	17	0	17	24	9
	Anal glands	7	3	4	10	4
	Ears	6	2	4	8	3
	Urinary tract	5	0	5	7	3
	Pneumonia	3	0	3	4	2
2	Reproductive disorders	28	3	25	39	16
	Vaginitis	15	0	10	21	8
	Sterility or infertility	7	2	5	10	4
	Pseudopregnancy	7	0	5	10	4
	Abnormal cycles	5	0	5	7	3
	Metritis	2	0	2	3	1
	Monorchidism	1	1	0	1	<1
3	Allergies	22	7	15	31	12
	Fleas	13	5	8	18	7
	Diet	4	0	4	6	2
	Atopy	3	0	3	4	2
	Drug (sulfonamides)	2	1	1	3	1
4	Haircoat or pigment change	13	2	11	18	7
	Poor, dry, thin coat	7	2	5	10	4
	Coat color change	4	1	3	6	2
	Vitiligo (loss of pigment)	3	0	3	4	2

[a] Includes those with more than one condition.
[b] Male.
[c] Female.

quality, color, and amount were the fourth most frequently noted category of health problem. Seven dogs had poor, dry, or thin coats, four had a noticeable change in coat color, and three had vitiligo (depigmentation of nose, lips, and eye rims or skin).

4. von Willebrand Factor Antigen Results

Results of plasma vWF:Ag levels for the 179 dogs tested were disappointing because only 11 dogs (6%) had values within the established normal ranges. Another 57 dogs (29%) had borderline normal or equivocal test results (50–69% vWF:Ag), while the majority of Irish wolf-

hounds tested had levels below 50% (117 dogs or 65%) (Table XXIVA). The lowest test reading was 6% and the highest was 120% vWF:Ag.

As discussed earlier, results obtained from members of the same family or litter could potentially bias interpretation of the overall findings. Specifically with respect to vWF:Ag, presence of the congenital inherited form of vWD would be reflected in an increased number of affected animals within that family. As the overall number of animals having abnormal results was high in this population, it is difficult to assess potential influence of the von Willebrand's disease gene in related family members. This would be particularly relevant if an individual vWD carrier sire and several of his offspring were included in the clinic (i.e., the "founder" effect).

Twelve of the dogs tested for vWF:Ag levels had one or more previous tests. Eleven of them had levels below the normal range (less than 50%), and one was in the borderline range (50–69%) in the current study. The previous test results were identical or similar in five dogs. In this group one animal had been tested twice, one three times, and one four times previously. These five dogs have had consistently abnormal vWF:Ag levels within a narrow range of test values. In six animals results of the previous tests were higher than the present ones. In four of them, original results were within the normal range (70–88%), whereas for two dogs values were borderline normal (59–60%). Another animal that tested within the normal range 5 years ago subsequently tested below the normal range and currently had even lower levels of vWF:Ag. None of these six dogs had any significant illness noted on their health history, and none had been neutered or spayed; both sexes were represented. Thyroid function on five of the six was within normal limits, and was borderline normal on the other dog. Thus, no explanation could be found for their drop in vWF:Ag levels. Some genetic or physiological influence had presumably altered the ability of their endothelial cells to produce or release vWF protein (Dodds, 1989, 1992d; Brooks *et al.*, 1992; Panciera and Johnson, 1994). Whether this had any potential clinical significance for the future health of these dogs is unknown.

Table XXIVB provides a breakdown of the vWF:Ag levels by age (170 dogs). Of the 23 dogs less than 1 year of age at the time of testing, 10 or 43% had levels below the normal range. Another 12 or 52% were borderline normal and only 1 animal had normal levels. Of the 44 dogs between 1 and 2 years of age, 29 or 66% had abnormal levels of vWF:Ag, 14 or 32% had borderline levels, and again just 1 animal had normal levels. Of the 65 young adults between 2 and 5 years of age, 41 or 63% had low levels, 20 or 31% had borderline levels, and 4 or 6% had

TABLE XXIV

vWF:Ag Assay Results in Irish Wolfhounds

	Total number	vWF:Ag(%):	Number of Dogs 0–49	50–69	70+
A. Summary					
	179		117 (65%)	51 (21%)	11 (6%)
B. vWF:Ag and Age					
Years	170				
Less than 1	23		13 (57)	9 (39)	1 (4)
1–2	44		29 (66)	14 (32)	1 (2)
2–5	64		41 (64)	19 (30)	4 (6)
5–8	35		26 (74)	7 (20)	2 (6)
8+	4		4 (100)	0 —	0 —
C. vWF:Ag and Sex					
Sex	176				
Males, intact	72		50 (69)	15 (21)	7 (9)
Males, neutered	3		2 —	1 —	0 —
Females, intact	92		54 (61)	35 (35)	3 (4)
Females, spayed	9		8 —	0 —	1 —
D. vWF:Ag and Health Problems					
Condition					
Presumed healthy	118		73 (62)	35 (30)	10 (8)
All health problems[a]	72		52 (72)	18 (25)	2 (3)
Chronic infections	48		38	9	1
Reproductive disorders	28		22	6	0
Allergies	22		21	1	0
Haircoat or pigment change	13		11	2	0
Diarrhea	8		5	3	0
Thyroid disease	7		7	0	0
Arthritis	7		6	1	0
All other conditions	24		16	6	2
E. vWF:Ag and Thyroid Levels					
Thyroid level					
Within normal ranges[b]	95		63 (54)	29 (57)	3 (27)
Borderline normal[c]	61		39 (33)	17 (33)	5 (10)
Below normal[b]	23		15 (13)	5 (10)	3 (27)

[a] Includes those with more than one condition.

[b] For all 6 thyroid analytes tested.

[c] For one or more analytes tested.

normal levels of vWF:Ag. Of the middle aged dogs between 5 and 8 years of age, 26 or 74% had abnormal levels, 7 or 20% had borderline levels, and 2 or 6% had normal levels. All 4 dogs over 8 years of age or higher had abnormal levels of vWF:Ag.

The sex breakdown for vWF:Ag levels is shown in Table XXIVC. For the 176 animals whose sex was known, the data show no sex difference in the test results. Levels of vWF:Ag in the 118 animals presumed to be healthy are compared to those of the 61 dogs exhibiting 72 health problems in Table XXIVD. There was no significant difference between these two population groups. Thus, there did not appear to be any significant influence of age, sex or health problems on test results.

5. Thyroid Profile Results

Complete baseline thyroid profiles were run by Drs. Nachreiner and Refsal at Michigan State University (1992b). As to the question of whether the established thyroid hormone concentrations for normal dogs apply generally to all breeds, the present study assessed values for Irish wolfhounds in comparison to these reference ranges. Of the 179 animals tested at the clinic, 95 or 53% of them had values for all thyroid parameters within the normal ranges for adult dogs established by Michigan State University (Nachreiner and Refsal, 1992b; Dodds, 1992f). Another 61 or 34% of the dogs had one or more test values below these published normal limits. Twenty-three animals or 13% had levels that were all below the established normal ranges for this laboratory. In the latter instance, two of the dogs had elevated levels of T4 and T3 autoantibodies. One dog had elevated T4 and T3 autoantibodies whereas the second dog only had an elevated T3 autoantibody.

When thyroid test profiles were examined by specific analyte, all but the free T3 values appeared to have a somewhat lower distribution than the established canine reference ranges (Nachreiner and Refsal, 1992a; Refsal and Nachreiner, 1993). For example, values for the majority of the tested Irish wolfhound population (i.e., representative of their true normal range) were 15–40 nmol/liter for total T4; 1.0–1.75 nmol/liter for total T3; 6–16 pmol/liter for free T4; 1.75–3.25 pmol/liter for free T3; <20 for T4 autoantibody, and <10 for T3 autoantibody.

To determine whether other variables such as age, sex, health status, and type of heartworm preventive used affected thyroid values in this population group, separate analyses were made for each variable (Nachreiner and Refsal, 1992a). With respect to age, there was no significant population correlation seen with any of the thyroid analytes except for a trend toward lower levels in the few animals over 8

years old. However, when values for all six analytes were assessed for dogs within different age groupings, 32 or 73% of dogs between 1 and 2 years of age had all results with the reference ranges, 29 or 45% of 2–5 year olds had all normal values, and even fewer (12 or 34%) of those between 5 and 8 years of age had normal levels (Dodds, 1992f). Whether this trend represents an influence of aging on thyroid levels and/or the effect of chronic diseases such as reproductive or subclinical thyroid dysfunction is unknown (Reimers *et al.*, 1990). If these results reflected subtle early changes of thyroid disease, one would have expected an increased frequency of lower values as the animals approach midlife.

There was no influence of sex on the results for thyroid profiles whether or not the dogs were intact or neutered. Similarly, no significant differences were found among dogs taking heartworm preventives daily, monthly, or seasonally (Nachreiner and Refsal, 1992a).

Health problems are known to influence thyroid hormone concentrations (Feldman and Nelson, 1987; Ferguson, 1988; Larsson, 1988; Chastain, 1990; Nelson *et al.*, 1991; Torres *et al.*, 1991). For the 118 animals that were presumed healthy, 68 or 58% had all thyroid values within the established reference ranges. Another 32 or 27% had borderline normal levels (i.e., one or more test analyte fell below the normal range), and 18 or 15% animals had all of the analytes below the established ranges. The 61 dogs described as having 72 chronic health problems had thyroid values as follows: 30 or 42% had values in the normal range, an identical number had values in the borderline range, and 12 or 17% were below normal. While this suggests that fewer animals had thyroid values in the normal range when health problems coexisted, no significant differences were found (Dodds, 1992f; Nachreiner and Refsal, 1992a). The reasons probably reflect the relatively small numbers involved and the fact that the presumed healthy dogs included those where no information was given on the health questionnaire.

Lastly, Table XXIVE compares thyroid values and vWF:Ag levels. Of the 117 animals with abnormal levels of vWF:Ag, 63 or 54% had thyroid analytes all within the established normal ranges, 39 or 33% had borderline normal levels of thyroid hormones, and 15 or 13% had levels below the established normal limits. Similar results were obtained for the group of dogs that tested with borderline normal vWF:Ag levels (between 50 and 69%). As only a small number of animals had vWF:Ag levels in the normal ranges (70% or higher), no interpretation can be made about these data in relationship to thyroid hormone levels. These findings fail to show any significant influence of thyroid hormone concentrations on the high proportion of Irish wolfhounds in

this study population found to have abnormal or borderline normal levels of vWF:Ag. This suggested that in the Irish wolfhound breed, congenital and inherited and/or physiological influences on production of von Willebrand factor are responsible for the observed results.

The production, storage, and secretion of von Willebrand factor are known to be under complex physiological control in health and disease (Avgeris *et al.*, 1990; Panciera and Johnson, 1994). Though well established in clinical and experimental settings, the relationship between thyroid function, von Willebrand factor, and von Willebrand's disease is not well understood (Ziegler *et al.*, 1986; Dalton *et al.*, 1987; Dodds, 1988, 1989, 1992d; Brooks *et al.*, 1992; Panciera and Johnson, 1994). It would be important to follow the health status and vWF:Ag levels of this Irish wolfhound cohort as the animals age in order to assess the influence of low levels of vWF:Ag on their health and longevity.

E. Basenjis

Since 1976, breeders and owners of basenjis have become increasingly concerned about the prevalence of Fanconi syndrome (Brown, 1989; Noonan and Kay, 1990; Gonto, 1993). Fanciers of this breed previously had to deal with pyruvate kinase deficiency, another inherited problem which adversely impacted the relatively small basenji gene pool (Searcy *et al.*, 1979; Giger and Noble, 1991).

Fanconi syndrome is an inherited renal tubular dysfunction resulting from a late-acting recessive lethal gene. The onset of clinical signs is typically between 4 and 8 years of age when animals already may have been used for breeding (Noonan and Kay, 1990; Gonto, 1993). This situation has lead to an increasing prevalence of the disorder and is compounded by the nonspecific symptoms of renal failure, which are often misdiagnosed. Clinical signs usually include polydipsia, polyuria, dehydration, weight loss despite normal appetite, decreased activity, and changes in the coat. Laboratory findings include glycosuria, with normal blood glucose, generalized amino aciduria, proteinuria, hyperphosphaturia, and metabolic acidosis. Affected dogs should not be used for breeding (Noonan and Kay, 1990). Recent management and treatment approaches for dogs with Fanconi syndrome have been encouraging and allow most affected dogs to remain clinically stable for years (Gonto, 1993).

Initially, asymptomatic dogs with glycosuria and metabolic acidosis are given plenty of fresh water, high-quality dog food containing canned red meat at least once a week, and daily multivitamin-mineral and phosphorus-calcium supplements. Periodic laboratory testing of

blood chemistry and urine is recommended. For dogs already showing polydipsia and polyuria, the multivitamin and mineral supplements are given twice daily along with a potassium supplement, if hypokalemia is severe. Oral sodium bicarbonate is used to stabilize blood pH based upon results of venous blood gas measurements (total carbon dioxide or bicarbonate levels from a multichannel chemistry analyzer are not sufficient here). Additional amino acid or megavitamin-mineral supplements may be needed on a monthly basis to correct losses of trace minerals. Once the patient is stabilized, follow-up laboratory testing of blood and urine should be performed twice a year or whenever symptoms reoccur (Gonto, 1993).

The prevalence and distribution of Fanconi syndrome and other health problems in basenjis were ascertained by a health survey from the Basenji Club of America (BCA). One thousand questionnaires were sent to breeders and owners throughout the United States (Noonan and Kay, 1990). Six hundred twenty-four (62%) surveys were returned, and these reported on 1,051 basenjis. Of these, 385 (62%) reported on animals that were healthy at the time of survey and had never had Fanconi syndrome or other diseases. Anonymity was assured by having responses submitted to an independent group. One hundred fifty-six of the 386 (40%) owners who indicated an interest in breeding their basenjis at some future point were unfamiliar with Fanconi syndrome before reading about it in the survey questionnaire. The majority of this group had never been informed of this disease by their veterinarians. Of further concern was the fact that 44 of 58 (76%) owners of dogs affected with Fanconi syndrome still intended to use them for breeding, a practice that is clearly ill-advised. Given the midlife late onset of clinical signs in this disease, indiscriminate breeding of affected or untested basenjis will inevitably be detrimental to the future health of the breed (Noonan and Kay, 1990; Gonto, 1993). Hopefully, recent concerted efforts by the BCA, other basenji fanciers, and scientists working with them will convey the urgent need to screen all potential breeding stock. Affected dogs and their carrier parents should be removed from the gene pool (Brown, 1989; Gonto, 1993).

The geographic distribution of affected dogs showed that 40% of cases came from five states; namely, California, Texas, Pennsylvania, New York, and Ohio, with a slightly higher than average incidence noted in areas contiguous to these states (Noonan and Kay, 1990). Diseases other than Fanconi syndrome were reported in 134 responses. These included: hypothyroidism in 38 (28%), diabetes mellitus in 12 (9%), malabsorption syndrome in 12 (9%), and urinary tract infections in 10 (7%).

The number of hypothyroid basenjis was relatively large, a finding previously noted by Brown (1989) and this author. The concurrent presence of thyroid disease appears to confound results of testing for Fanconi syndrome in some cases (Brown, 1989). It can produce both false-positive and -negative results, presumably because of the effects of thyroid hormone on the sodium-potassium pump and induction of glycosuria. However, some dogs exhibiting glycosuria and hypothyroidism become normal once their thyroid dysfunction is corrected by supplementation. The situation is further complicated by the reverse experience, where apparent hypothyroidism disappears once symptoms of Fanconi syndrome have been corrected (Gonto, 1993). Some dogs with Fanconi syndrome also have abnormal liver enzymes even though they may not show symptoms of liver dysfunction. As low-grade chronic active hepatitis can accompany thyroid disease in humans and animals (Trence *et al.,* 1984; Fisher *et al.,* 1987; Dodds, 1992a,e), it is unclear whether these dogs truly have liver disease or some secondary physiological or biochemical imbalance resulting from Fanconi syndrome that has affected both hepatic and thyroid metabolism. Regardless, both Fanconi syndrome and hypothyroidism usually occur in midlife and are known to be prevalent in the basenji breed (Brown, 1989; Noonan and Kay, 1990). It would be prudent, therefore, to screen all adult basenjis for both disorders.

F. Irish Setters

The Irish Setter Club of America (ISCA) is one of several national breed clubs that have established a nonprofit foundation as a mechanism for supporting research studies of diseases and other problems that afflict their particular breeds as well as dogs in general. Another example is the Collie Club of America Foundation, Inc., established in 1986 as "a consequence of the Club's willingness to disclose breed deficiencies" (Sundstrom, 1991). In 1992, the ISCA Health Committee compiled results of a survey questionnaire distributed by the organization to owners and breeders of Irish setters. Results of the survey were analyzed with the help of the Institute for Genetic Disease Control, Davis, California (ISCA, 1992). Three hundred fifty-six breeders and owners of Irish setters completed the questionnaire, which reflected the attitudes and concerns of the respondents but was not intended to rank or estimate the prevalence or importance of a specific disease with respect to the health of the breed as a whole. Nevertheless, useful information was obtained for a wide range of health problems including temperament, dentition, and reproduction. Comments concerning

the need for further study of specific problems had a 35% correlation with the diseases they represented.

The respondents had owned Irish setters for 19.1 years on average, with 50% having dogs for more than 10 but less than 28 years. About half of the people who returned the questionnaire were considered to be novice breeders (produce one or no litters), and the other half were classified as experienced breeders. The average number of litters for the experienced breeders was 5.2. The average lifespan of Irish setters owned by respondents was 11.1 years, but this value excluded dogs that died of accidental causes or disease at a young age. Therefore, this value may have been biased toward a longer life. With respect to diseases recognized in Irish setters and being screened for prior to breeding, only hip dysplasia and progressive retinal atrophy were tested for on a regular basis. Testing for allergies was performed in a smaller number of the respondent's animals. For specific disease states, the most frequent responses applied to: hypothyroidism (46%), various types of cancer (43%), bloat, chronic ear problems, and allergies (each 41%), seizures (38%), hip dysplasia (36%), panosteitis and spondylosis (each 23%), heart disease (20%), hypertrophic osteodystrophy (18%), cataracts (17%), various autoimmune diseases (15%), osteoarthritis (14%), osteochondritis dissecans (13%), megaesophagus (8%), other bone diseases and progressive retinal atrophy (each 4%), and laryngeal paralysis (3%). Of these 19 disease categories, most of the respondents indicated that further studies were needed for the bloat and seizure disorders (152 and 139 responses, respectively). Other diseases for which the group indicated more study was important (22–41 requests) included: hypothyroidism, cancers, allergies, hip dysplasia, hypertrophic osteodystrophy, autoimmune diseases, and progressive retinal atrophy (ISCA, 1992).

The compilation of specific responses offered some generally useful information about the current status of the breed. Regarding reproduction, more than half (54%) of the bitches and 45% of the stud dogs were screened for brucellosis at time of breeding, and about 25% of these breeding animals were also tested for mycoplasma and parasites. About 35% of the bitches were bred artificially and 71% of these females conceived. The average litter size was 9 puppies with an average survival rate per litter of 8.5 puppies. The age of onset of various diseases was noted as follows: progressive retinal atrophy, 9 months of age; entropion and ectropion, both at 6 months with entropion recognized more frequently than ectropion; cataracts, 7.4 years; bloat, 5.8 years; seizures, 3.9 years; hip dysplasia, 2.3 years; osteochondritis dissecans and hypertrophic osteodystrophy, both at 8 months; spondylosis,

6 years; osteoarthritis, 8.4 years; cancer, 8.7 years; heart disease, 4.6 years; allergies, 2.2 years; hypothyroidism, 3 years; laryngeal paralysis, 9.5 years; megaesophagus, 1.3 years; chronic ear problems, 2.5 years; shyness, 1.2 years; aggression, 2.4 years; failure to ovulate, 2.3 years; infertility, 4 years; fetal resorption, 4.4 years; whelping difficulties, 5.2 years; agalactia, 4.3 years; and lack of maternal instinct, 4.3 years (ISCA, 1992).

The results of this health questionnaire provide guidance to the parent ISCA about the attitudes and concerns of its membership. Hopefully, the increased interest in and emphasis on the more prevalent diseases affecting Irish setters will lead to more accurate and practical genetic screening programs. The recent breakthrough by Dr. Gustavo Aguirre and colleagues at Cornell University's Baker Research Institute in developing a molecular genetic screening test for progressive retinal atrophy in Irish setters is a major step forward in this effort (Smith, 1994).

G. Newfoundlands

In 1988, the Newfoundland Club of America (NCA) conducted a national survey of the prevalence of disease in the breed. The study was sponsored by the NCA Health and Longevity Committee and reported in 1989. The survey grew out of concern within the Newfoundland breed for the prevalence of hip dysplasia and subaortic stenosis. While these diseases have had a significant impact on the health and longevity of the breed, it was increasingly apparent that other problems existed and needed to be addressed. The Canine Consumer Report (1994), for which this author supplied much of the scientific literature base, lists 23 hereditary or congenital diseases in the Newfoundland.

The NCA health questionnaire was sent to 1,366 members in March 1988. A copy of the questionnaire was also included in the Summer 1988 issue of *Newf Tide*, the NCA newsletter. With the addition of new members, at least 1512 individuals received the questionnaire. Seven hundred seventy-one responses were received, but 18 of these were duplicates. Thus, 753 responses were analyzed and these reported on the health status of 1853 dogs. The response rate for the survey was 45.9%. Most of the individuals responded to the direct membership mailing as opposed to the newsletter insert (NCA, 1989).

The sex breakdown for the 1752 dogs owned by NCA members was 42% male and 58% female. When these results were further broken down by the number of dogs owned by the respondents, however, as the number of dogs owned increased, the proportion of males decreased.

Thus, as might be expected, those that breed Newfoundlands were more likely to keep females in their kennels than males, although this hypothesis was disputed by some of the membership. With respect to age of the population surveyed, over 75% of the dogs were born in 1982 or later, with 57% of the group being born in or after 1984. The average number of dogs owned by each member respondent was 2.5. Of these, 641 (37%) were healthy at the time of survey, and the remaining 1111 dogs reported an average of 2.1 diseases per dog.

When diseases affecting the Newfoundland were tabulated in rank order (in a manner similar to that shown for the Old English sheepdog in Table III), the following ranking was obtained (NCA, 1989):

1. hip dysplasia
2. dermatitis, allergic and nonspecific
3. hypothyroidism
4. entropion
5. acute infectious disease
6. ruptured cruciate ligament
7. trembling
8. undershot jaw
9. cardiac disease (subaortic stenosis and other forms)
10. panosteitis
11. pyometra
12. other eye diseases
13. other orthopedic diseases
14. osteochondritis dissecans
15. cancer
16. ectropion
17. elbow dysplasia
18. umbilical hernia
19. arthritis
20. undescended testicle
21. everted nictitating membrane
22. injuries
23. bloat
24. endocrine or immune system disease
25. patellar luxation
26. urinary system disorder
27. general allergies
28. gastrointestinal disorder
29. neurologic disorder
30. reproductive disorder
31. overshot jaw
32. nonmalignant tumors and cysts
33. Wobbler syndrome
34. dermoid cyst

By far the most commonly reported problem (392 affected) was hip dysplasia, followed by dermatitis (275 dogs) in which allergic dermatitis accounted for 234 cases. The third most common problem was hypothyroidism (131 dogs), followed by entropion (100 dogs). The frequency of affected animals dropped down from there to 72 dogs with acute infectious disease (ranked 5th) to 2 dogs with dermoid cysts (ranked 34th). When these diseases were further analyzed according to the number of dogs owned by the respondent, similar rankings were obtained for the more common problems, namely hip dysplasia, der-

matitis, and hypothyroidism. As far as specific diseases are concerned, hip dysplasia affected more females (59%) than males (40%). The diagnosis was made at or before 6 months of age in 38% of the dogs, while two-thirds (67%) of all dogs diagnosed with hip dysplasia were identified before 18 months of age. The aggregate data on hip dysplasia from this survey indicated that 21.2% of the dogs were dysplastic, which is less than the 32.5% of Newfoundlands affected as reported by the Orthopedic Foundation for Animals (Corley and Hogan, 1985).

The number of dogs reported to have dermatitis, the second ranked disease, was equal for both sexes, and the major problem was allergic dermatitis. Eighty-nine percent of cases occurred before 5 years of age, although 37% of the skin disease occurred in the first year of life. The average age of onset or diagnosis of dermatitis was 19.7 months. Similar analysis for the frequency of hypothyroidism showed a female sex preponderance (65%) versus males (35%). The average age of onset of hypothyroidism was 38.5 months. As expected, entropion was most frequently observed in the first year of life, with nearly 90% diagnosed before age 2 years. A summary of disease association by gender indicated that several of these diseases were more common in males than would be expected. These included: all forms of dermatitis, trembling, panosteitis, osteochondritis dissecans, cancer, and elbow dysplasia. On the other hand, females had a higher than expected prevalence of hip dysplasia, hypothyroidism, entropion, and cruciate ligament rupture.

The survey reported the deaths of 135 dogs (60 males and 75 females). The average age at death was 6.2 years, which compares closely with that of another giant breed, the Irish wolfhound, with an average age at death of 6.5 years. Unlike the Old English sheepdog (see Table II), there was no significant difference between the mean lifespan for males or females. Cancer was the major cause of death in the dogs reported (21.5%). The average age of death due to cancer was 7.3 years. The second leading cause of death was heart disease, excluding subaortic stenosis. The mean age of death from heart disease was 5.7 years and this affected 9.6% of the 135 reported deaths. Euthanasia, due to the effects of hip dysplasia, was the third leading cause of reported deaths (7.4%). Seven of these deaths occurred in the first year of life. The remaining deaths were attributed to a variety of different causes including "old age."

One interesting conclusion from the study was that three of the top five ranked diseases identified among the 1180 Newfoundlands involved thyroid disease or related conditions (NCA, 1989). In this case, the most frequent related conditions were dermatitis, especially of the allergic type, and acute infectious disease. The number of animals

affected with hypothyroidism and these two other conditions was 478 or 41% of the total number of affected dogs. This number is similar to the 48% of Old English sheepdogs reported to have thyroid disease and related conditions. (see Tables XVI and XXI). If all the other ranked conditions known to be associated with thyroid dysfunction were combined, the percentage of Newfoundlands having thyroid disease and related conditions would be considerably higher. This analysis could not be made from the published information, however, as the raw data were not available to correct for duplications. Nevertheless, the parallels for the health problems of these two large breeds of dogs is striking.

H. Dalmatians

The dalmatian breed suffers from several serious genetic defects including abnormalities of purine metabolism leading to urate urolithiasis, and hereditary deafness, which has been associated with both eye and coat color changes in the breed (Schaible, 1986; Holliday *et al.*, 1992; Strain *et al.*, 1992; Sorenson and Ling, 1993a,b; Bartges *et al.*, 1994; Greibrokk, 1994). The Dalmatian Club of America (DCA) has been actively involved in promoting awareness of and testing for these disorders.

1. Urate Urolithiasis

The prevalence of urate urolithiasis in dalmatians was compiled for a 10-year period from June 1981 through December 1991 at the University of Minnesota Veterinary Teaching Hospital (Bartges *et al.*, 1994). During the study period, dalmatians accounted for 119 (0.5%) of the total number of dogs admitted to the hospital. There were 82 females and 95 males, and gender was not reported for another 13 dogs. Uroliths were retrieved and analyzed from 18 of the 190 (9.5%), and urate was identified in 17 of these. The odds that uroliths from dalmatians were composed of urate were 229 times greater than for dogs of other breeds, and the odds that a dalmatian admitted to the hospital was affected with urate uroliths were 122 times greater than that for other breeds.

In addition to these data for admitted animals, uroliths from another 387 dalmatians (16 females and 360 males, gender not given for 11 dogs) were submitted to the Minnesota Urolith Center for quantitative mineral analysis during this same study decade. Urate was identified in 317 (82%) of the uroliths retrieved from dalmatians. This incidence was striking in comparison to the 293 of the remaining 10,801 (2.7%)

urate uroliths retrieved from dogs of other breeds. The odds that uroliths retrieved from dalmatians were composed of urate was 162 times greater than for uroliths retrieved from other breeds. The mean age of dalmatians with urate uroliths was 4.2 years. These data compare favorably with the admitted case cohort, where the mean age of affected dogs was 4.6 years, and male dalmatians were 16.4 times more likely to be affected than female dalmatians (Bartges *et al.,* 1994).

2. Hereditary Deafness

Screening for congenital hereditary deafness involves assessment of the brainstem auditory-evoked response (BAER) potential. When combined with clinical observations, this testing is used to determine the incidence and sex distribution of bilateral and unilateral BAER abnormalities, and association with heterochromia iridis, a pigmentation anomaly of blue eyes. The deafness defect in dalmatians is presumed to be associated with their white coat color, as it arises from the action of a coat color spotting gene similar to that seen in other breeds of dogs, mice, guinea pigs, and white mink. By contrast, inherited deafness in white cats is associated with a dominant gene for the white coat color rather than the spotting gene (Holliday *et al.,* 1992). The deafness in these dogs is reported to occur as early as 4 weeks or as late as 4 months of age. The incidence of unilateral or bilateral deafness as assessed by BAER was 59% in a group of 46 dogs and 28% in a larger sample of 900 dogs (Holliday *et al.,* 1992). The increased frequency of deafness was associated with the blue eye color and the presence of patches at birth that differ from the characteristic dalmatian spots in size and hair texture. Typical dalmatian spotting does not develop until puppies are 2 to 3 weeks of age, whereas the patches were seen at birth in the American, European, and Norwegian dalmatian study populations (Strain *et al.,* 1992; Greibrokk, 1994). Results of the published population surveys determined that the deafness and eye and coat color phenotypes were most likely the result of an autosomal recessive, multifactorial gene with incomplete penetrance (Greibrokk, 1994). In Norway, the level of bilateral deafness was found to be only 3.6% as compared to the 8–13% reported for the United States. The lower rate in Norway is believed to be due to an intensive campaign by breeders to select against dalmatians with blue eyes, and to the relatively high genetic diversity in the Norwegian dalmatian population (Greibrokk, 1994).

The results alluded to above are based upon data collected from three large population surveys (Holliday *et al.,* 1992; Strain *et al.,* 1992; Greibrokk, 1994). In the first study, BAER was performed on 900 dalmatians. The survey period lasted for 4 years and compared data

from 749 dogs considered to be representative of the breed at large, in comparison to another 151 dogs in which selection of breeding stock was based on results of BAER testing at the beginning of the survey period. Under the conditions of testing, dogs with bilaterally absent BAER were clinically deaf, whereas those with unilaterally absent BAER were not clinically deaf but appeared dependent on their normal ears for their auditory-cued behavior. Dogs with unilaterally absent BAER were frequently misidentified as normal by uninformed observers. Six hundred forty-eight (72%) of the 900 dogs were normal, 189 (21%) had unilateral absence of BAER, and 63 (7%) had bilateral absence of BAER or were clinically deaf. Statistically significant differences were observed between males and females as 24% of females were unilaterally and 8.2% were bilaterally abnormal, whereas only 17.8% of the males were unilaterally and 5.7% were bilaterally abnormal.

With respect to blue eyes (heterochromia iridis) caused by partial or complete lack of pigmentation of the stroma of the iris, dogs with grossly visible blue eyes had a significantly higher incidence of BAER abnormality than did dogs with fully pigmented iridial stromas. This difference was highly statistically significant ($P = 0.0001$). Furthermore, females had a significantly higher incidence of heterochromia iridis than did males. There was also a significantly lower incidence of BAER abnormality in the group of dogs specifically selected for breeding on the basis of previous BAER testing than for the population at large. This latter difference could not be attributed to differences associated with gender or presence of heterochromia iridis, and suggested that the benefits of prior selection by BAER screening were real (Holliday *et al.*, 1992).

The second large population study involved 1,031 dalmatians from three geographically separate areas (Strain *et al.*, 1992). In addition to BAER testing, phenotypic markers thought to be associated with congenital deafness in dalmatians were also assessed. These included sex, haircoat color, pigmentation of different areas of skin (eye rims, nose, and ears), presence of a patch, spot size or density of spotting, BAER status of the sire and dam, and the presence of heterochromia iridis or retinal pigmentation. When data were combined from the three test sites, there was an 8.1% incidence of bilateral deafness (83 dogs), 21.6% unilateral deafness (223 dogs), and an overall incidence of 29% with hearing disorders. Significant phenotypic associations with deafness were found for the presence of patches, sire and dam BAER status, heterochromia iridis, and pigmentation of the retina. When these analyses were segregated according to the individual test sites (Louisiana State University at Baton Rouge; Veterinary Neurological Center,

Phoenix, Arizona; and Cameron Park Veterinary Hospital, Cameron Park, California), results for several of the significant phenotypic markers identified for the total group were not found. These findings suggested that deafness patterns have several population expressions, and indicated that the association of phenotypic markers with deafness may not be functionally significant (Strain *et al.*, 1992). Overall, results for this group of more than 1000 Dalmatians closely matched those of a study performed by Holliday *et al.* (1992).

When the aggregate data for the three test sites were further broken down by specific location, bilateral deafness was seen in 12.7% at the Louisiana site in comparison to 5.9% for Arizona and 4.9% for California. The difference in these percentages reflected the several years of selective breeding against unilateral deafness practiced at the California test site. The phenotypic markers associated with deafness at the individual test sites differed except for patches, spot marking, the BAER status of the sire, and heterochromia iridis. Thus, it would be unwise to select against puppies or adult breeding stock on the basis of phenotypic markers such as heterochromia iridis without concurrent BAER testing. Because of the likely multifactorial inheritance pattern for deafness in the dalmatian and its associated phenotypic markers, the best indicators to permit breeding decisions remain testing of the sire and dam using the BAER potential (Strain *et al.*, 1992).

In comparison to the two studies summarized above in the United States, a population of 1,843 dalmatian puppies was screened in Norway. In this study, deafness was determined not by the more accurate BAER technique but by classical noise-response methodology used by breeders on puppies between the ages of 2.5 and 4 weeks. Results indicated that 4.9% of the puppies were classified by the breeders as being deaf, 4.7% were blue-eyed, and 10.9% had colored skin patches. This overall incidence in deafness and associated phenotypic markers was lower than that reported for the United States and Europe. The most effective means of reducing the deafness rate among offspring was stated to be identification and removal from the breeding pool of all unilateral deaf dogs as well as those with blue eyes (Greibrokk, 1994).

III. Current and Future Trends

A. Physical Characteristics of Disease

In the early days when animal breeders began recognizing recurring symptoms of disease states or physical characteristics, the undesirable features of these traits led them to select away from the problems by

test mating and eliminating affected animals from the breeding pool. While this remains one way to select against inherited and congenital diseases, more reliable approaches have been implemented by screening for biochemical markers and most recently by using molecular genetic techniques. Nevertheless, selection against phenotypic characteristics is still a useful method of controlling the incidence of inherited disease provided that breeders and owners realize that one or both parents and some other relatives are likely to be asymptomatic carriers of the trait.

Examples whereby physical characteristics offer a definitive or potential benchmark to identify the presence of an inherited disease include: lameness in young adult dogs characteristic of various bone and joint diseases, bleeding and anemia seen with coagulation disorders and red cell enzyme deficiencies, acute or chronic renal disease in young dogs; various changes in the eye, sudden death associated with bloat or heart disease, muscular weakness and collapse associated with neuromuscular diseases and other systemic conditions such as Addison's disease, blue eyes associated with deafness in dalmatians or other white breeds, various anatomical anomalies, and variations in coat color or type. For some breeds, newborn puppies exhibiting specific characteristics such as fluffy coats in Pembroke Welsh corgis, grey collies (cyclic neutropenia), dalmatians with dark skin patches, and white or albino dogs of breeds such as the boxer, German shepherd and Doberman pinscher, are undesirable physical characteristics whether or not they are associated with a specific set of clinical signs.

B. Biochemical Markers of Disease

While dog breeders for the most part have endorsed the long-standing genetic screening programs for hip dysplasia and blood and eye diseases, emphasis on other genetic defects has arisen, now that the major infectious, parasitic, nutritional, and traumatic diseases have been addressed and controlled to a large extent by modern veterinary medical practice. Furthermore, most dog fanciers become involved in breeding and showing their animals as a hobby rather than a prosperous enterprise as might apply to livestock or the performance racing industry. The intense commitment to this hobby with its attendant social praise for the successful breeder and exhibiter poses ethical dilemmas when prizewinning animals are identified as carriers of a particular genetic defect.

There have been relatively few experts in veterinary genetics to teach this subject in the veterinary school curriculum or to maintain an active dialogue through seminars and writings with the dog fancy

at large. As pointed out by Patterson *et al.* (1988) "The veterinary profession has arrived at a stage when it should be prepared to provide the diagnostic and genetic counseling services needed to reduce the frequency of genetic defects in companion animals as well as livestock." This can be accomplished only by imparting knowledge about the specific genetic disorders affecting each breed, including their clinical characteristics, modes of inheritance, and specific tests available to detect carrier state. Additionally, individuals qualified in genetics need to assist breed organizations in developing effective and practical programs to apply rigorous selection against abnormal genes in order to eliminate or reduce their frequency to an acceptable level. One consequence of the line breeding and inbreeding frequently practiced with purebred dogs is the failure to rigorously select against those carrying genetic defects, thereby increasing the frequency of a problem within the breed. It is not the inbreeding or line breeding per se that causes the problem, but rather the reluctance to identify and cull affected or carrier stock. Detection of carrier animals is essential for the survival of these breeds in the longterm, because most of the genetic defects are autosomal recessive or polygenic traits (Patterson *et al.*, 1988).

For about three decades, veterinary and comparative geneticists have developed and relied upon biochemical markers of specific genetic traits to identify carrier and affected animals (Dodds *et al.*, 1981; Patterson *et al.*, 1988; Rubin, 1989; Holliday *et al.*, 1992; Padgett, 1992; Strain, 1992; Bell, 1993; Clark and Stainer, 1994). The development of these testing methods varied from relatively simple techniques based upon collection of blood, urine, or hair samples to more sophisticated electroretinograms for eye diseases and brainstem auditory-evoked potential testing for congenital deafness. Geneticists establishing these methods aimed to produce reliable, practical, and affordable tests that would be predictive of the gene product, and therefore the genotype of a particular genetic disease. In order to be considered accurate and predictive, retrospective analyses of data developed from these testing programs were compared to the pedigrees of animals being screened as a means of validating the tests. With such methodology, a genetic screening test would be considered reliable if it correctly identified animals as having the normal and abnormal genotypes at least 80% of the time (Jolly *et al.*, 1981). For the inherited bleeding disorders, retrospective analysis of the results confirmed an accuracy rate of more than 90% in classifying the carrier state (Dodds *et al.*, 1981; Dodds, 1982; Brooks *et al.*, 1992). Similar results have been obtained in screening for the inherited eye diseases where ophthalmological evaluations by specialists were employed (Dodds *et al.*, 1981; Rubin, 1989).

In the last decade, more sophisticated tests have been developed for specific conditions. Examples include the metabolic storage diseases such as GM_1-gangliosidosis in Portuguese water dogs (Greenfield, 1990; Bell, 1993), globoid leukodystrophy of Cairn and West Highland white terriers, mucopolysaccharidosis, tyrosinemia, glycogen storage disease, methylmalonic aciduria of vitamin B12 responsive and nonresponsive types, cystinuria, and renal Fanconi syndrome (Patterson *et al.*, 1988). Many of these latter genetic defects in metabolism were identified by the University of Pennsylvania School of Veterinary Medicine's Inherited Metabolic Disease Laboratory.

The University of Pennsylvania School of Veterinary Medicine has also concentrated on screening dog breeds for congenital heart disease. The frequency of congenital heart malformations was approximately seven per 1,000 hospital cases, similar to the magnitude noted in human infants and children (Patterson *et al.*, 1988). This study also determined that the prevalence of congenital heart disease was significantly higher in purebred than mongrel dogs, and that the five more common anatomic forms of these diseases were not randomly distributed among the affected breeds. Congenital heart diseases having a higher than expected prevalence in some breeds included patent ductus arteriosus, pulmonic stenosis, subaortic stenosis, persistent right aortic arch, and tetralogy of Fallot. For some of these congenital heart defects, breeding experiments and family studies confirmed unequivocally that the malformation was a specific inherited trait. This applied to patent ductus arteriosis in the poodle, pulmonic stenosis in the beagle, subaortic stenosis in the Newfoundland and golden retriever, tetralogy of Fallot in the Keeshond, and persistent right aortic arch in the German shepherd dog (Patterson *et al.*, 1988). With respect to subaortic stenosis, both the Newfoundland Club of America and Golden Retriever Club of America have active programs to promote identification and control of this disorder.

A successful testing program for the copper toxicosis gene of Bedlington and West Highland white terriers has been established by Dr. George Brewer and colleagues at the University of Michigan Medical School and Michigan State University College of Veterinary Medicine (Brewer and Yuzbasiyan-Gurkan, 1989; Smith, 1994). Homozygous affected dogs are eliminated from the breeding pool following their identification after measuring the copper content of a liver biopsy specimen, or less invasively by injecting radioactive copper intravenously and measuring radioactivity in the stools for 48 hours (Brewer *et al.*, 1992). Until recently, the only way to identify carriers of this disorder has been by test matings. While this method of selecting on the basis of phenotype and test mating is effective, it is an expensive and inva-

sive procedure to perform liver biopsies, and test matings are fraught with the ethical dilemma of producing affected unhealthy puppies.

The Genodermatosis Research Foundation was established about 5 years ago with input from standard poodle breeders and veterinary geneticists and pathologists (Drs. Rosser, Dunstan, and Padgett of Michigan State University, and Dr. Hargis of Washington State University). During this period, the Poodle Club of America Foundation and Genodermatosis Research Foundation provided the initial support for the testing program, which involves histopathological examination of skin biopsies from potential affected and carrier animals. The initial disease emphasis was placed on sebaceous adenitis, which was originally identified in the standard poodle breed but has now been recognized in another dozen or so breeds (Laratta, 1991, 1992). While sebaceous adenitis is primarily a cosmetic disorder, the moth-eaten appearance and offensive odor that frequently occur are distasteful for many owners. There is presently no cure for the problem in most breeds with the exception of the vizsla, where treatment with synthetic retinoids has produced some remissions. The other extensive testing program based on employing sophisticated diagnostic techniques is the screening for congenital deafness in dalmatians (discussed above in Section IIH).

Effective genetic screening programs for the inherited bleeding disorders have been developed and implemented by this author over the past 30 years (Dodds *et al.,* 1981, Dodds, 1982, 1988). The principles utilized here parallel those applied in human medicine for screening of analogous hemostatic defects. The most successful of these included the virtual elimination of factor X deficiency in the American cocker spaniel; inherited thrombasthenic-thrombopathia in the otterhound (although this disorder surfaced again recently from current descendants of original stock that were inadequately screened); detection and elimination of hemophilic carriers in many affected breeds, where cooperation between the breeders permitted effective screening; and significant reduction in the prevalence of von Willebrand's disease, the most common inherited canine bleeding disorder, in several breeds such as the Scottish terrier, golden retriever, miniature schnauzer, Pembroke Welsh corgi, and Manchester terrier (Dodds, 1988, 1989; Brooks *et al.,* 1992). On the other hand, despite intensive efforts to screen for von Willebrand's disease in breeds such as the Doberman pinscher and Shetland sheepdog, the prevalence has been going up, perhaps because of the concomitant increase in hypothyroidism in those breeds, which has the potential to express an acquired form of von Willebrand's disease (Zeigler *et al.,* 1986; Avgeris *et al.,* 1990;

Brooks *et al.*, 1992; Panciera and Johnson, 1994). Despite these documented successes from measuring various biochemical markers as predictive of genotype, the most accurate methodology awaits the development of genetic molecular techniques that probe DNA linkage markers or the actual genes involved. Early achievements applying this technology are discussed below.

C. Molecular Genetic Markers of Disease

Recent advances in molecular genetics have focused on mapping the human genome, and this has stimulated interest in developing parallel genetic maps for animals (Ostrander *et al.*, 1993). Once developed, a genetic map provides information about the relative order and placement of genes or specific DNA markers of specific chromosomes. This allows one to locate specific regions on chromosomes where genes of interest are likely to be formed. Once a molecular marker is identified close to a specific gene of interest, screening tests for this particular marker can be used to identify individuals carrying or expressing the trait. As far as the dog is concerned, it would be very helpful to use molecular technology to identify the specific defects associated with the inherited bleeding disorders, eye disorders, and hip dysplasia. Of course, other genetic defects of current concern could also be probed by this technique. According to Ostrander *et al.* (1993), at least 500 genetic markers would have to be identified and characterized to provide a useful genetic map for canines.

Once genetic markers have been identified, applying them to the diagnosis of specific diseases may require developing a specific genetic probe for each of the different breeds that are affected. For example, at least ten breeds of dogs have progressive retinal atrophy, but a different gene is involved in most breeds (Smith, 1994). As stated by Dr. Gustavo Aguirre of Cornell University's Baker Institute "Some breeds have an early onset and others have a low onset of signs. Since many dogs, such as the English cocker spaniel, don't go blind until late in life, it is difficult to know the numbers affected. The disease is inherited as a single gene defect" (Smith, 1994). Current research by this team of investigators has focused on cloning the progressive rod-cone degeneration gene to develop a molecular genetic screening test for progressive retinal atrophy. One of the first successful molecular genetic markers for an inherited canine disease was developed by Drs. Urs Giger, Bruce Smith, and colleagues at the University of Pennsylvania, School of Veterinary Medicine. Their marker screens for the single base pair mutation of the phosphofructokinase enzyme gene of

English springer spaniels. By using this marker test for phosphofructokinase deficiency, carrier and affected dogs can be identified and eliminated from the gene pool (Giger *et al.,* 1993).

In studying the normal dog genome, various DNA markers are being identified (Ostrander *et al.,* 1993). When DNA from dogs affected with a specific genetic disease is examined, researchers must search for a specific DNA marker linked to that diseased gene. The inheritance of the marker can then be used to predict the inheritance of the linked disease gene. In an ideal situation, the distance between the DNA marker and diseased gene is small so that there is a low statistical chance that cleavage and recombination will occur between them. As an example, Dr. Brewer and colleagues in Michigan are working on linkage tests for copper toxicosis of Bedlington terriers (Brewer and Yuzbasiyan-Gurkan, 1989). The copper toxicosis gene in Bedlington terriers is a model for the Wilson's disease gene of humans. In the human condition, the specific gene involved has been linked to a marker enzyme, esterase D, located on the long arm of chromosome 13. As the Wilson's disease and esterase D genes are located close together on this chromosome, esterase D can act as a linked marker gene with which to study Wilson's disease.

It is clear that we have entered an era of much promise with respect to developing specific tests for inherited and other diseases (Headon, 1994; Jackwood, 1994). When a disease is caused by a single gene defect, like some of the examples discussed above, it is much easier to develop DNA marker tests. With more complex diseases having polygenic inheritance, such as hip dysplasia, it will take longer to develop tests because several genes are involved that contribute to the expression of the trait. Also many diseases have incomplete expression or involve a genetic component that requires an environmental trigger to express the disease. An example of the latter situation is the autoimmune diseases (Sinha *et al.,* 1990; Dodds, 1992b,e). Finally, some diseases have late onset expression such as Fanconi syndrome in the basenji, or the neuromuscular diseases such as those seen in the Labrador retriever, rottweiler, and Gordon setter. (Additional details and examples of this molecular approach to studying genetic disease are contained in the chapter by Lothrop in this volume.)

IV. Recommendations

A. Molecular Probes and Analysis

It is estimated to take a minimum of 10 years and several million dollars to map the canine genome. In the meantime, molecular geneti-

cists are searching for the DNA markers for single gene defects in animals. This will take a relatively long time because the genes involved for many diseases have not yet been identified. Ostrander *et al.* (1993) at the University of Washington in Seattle have undertaken a cooperative effort to identify the DNA markers on the normal canine genome. One goal of this project is to map the genes responsible for some of the inherited behavior patterns that are characteristic of different breeds of dogs (Smith, 1994). Once this database of DNA markers is available, it can be used to identify specific genes by linkage that are responsible for inherited traits. As far as the dog fancy is concerned, breeders are most interested in accurately identifying the genes causing the more common genetic disorders. These include those affecting structure and function (bones and joints), vision and hearing (eyes and ears), neuromuscular and cardiac functions, skin and hair coat, and the frequently encountered metabolic and endocrine diseases, many of which have an autoimmune basis. (Additional information about the specific probes developed to date and their use is contained in the chapter by Lothrop in this volume, and will be covered in-depth in a subsequent volume in this series.)

B. Genetic Disease Registries

Establishment of genetic disease registries can be another effective mechanism for control of genetic disease once a mass screening program for detection of heterozygotes is in place (Jolly *et al.*, 1981; Dodds, 1982; Corley, 1992). Typically, only those animals certified free of the genetic defect(s) in question are registered. The identification of affected or carrier animals is best kept confidential, because breeder cooperation and self-esteem are important factors in promoting these screening programs. Strong support from local, regional, national, and even international breed organizations is another prerequisite and depends upon proper dissemination of correct information, as well as breeder education and integrity. Certification of normal animals works well in these situations because it influences the breeding policy of serious breeders and brings selection pressure to bear against affected animals and their close relatives (Dodds, 1982; Corley, 1992). An alternative approach currently receiving increasing support advocates an "open" registry whereby affected and heterozygous carrier animals are identified by the registry and on pedigrees (Laratta, 1992; Padgett, 1992; Smith, 1994).

Before a new or established genetic defect should be considered for widespread certification or registration, a reliable, cost-effective het-

erozygote (carrier) detection program must be available. Requirements of such a program are (Jolly *et al.*, 1981; Dodds, 1982):

1. that the disease in question occur in a defined population or breed with a gene frequency sufficiently high to be of social and/or health importance;
2. that a simple, relatively inexpensive test be available that identifies heterozygotes with a high degree of accuracy (preferably 90%, but at least 80%);
3. that control by culling of heterozygotes not have a deleterious effect on the overall gene pool of the breed or seriously deplete available breeding stock;
4. that the test used and recommended control program be acceptable to breeders and be amplified by adequate educational and public relations programs;
5. that the program be integrated where possible with other disease control programs for the breed to simplify specimen collection or testing procedures; and
6. that adequate genetic counseling and breed club guidelines be provided to ensure that control programs are initiated and implemented effectively. With respect to the latter point, the most expedient way to reduce the gene frequency of an undesirable trait within a breed is to ensure that those animals of superior conformation (phenotype) and genetic potential are screened for the defect.

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Thyroiditis—A Model Canine Autoimmune Disease

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I. Introduction and Background

Although low thyroid function has been recognized for over 100 years and hypothyroidism is the most common endocrinopathy of dogs (Feldman and Nelson, 1987; Dunn, 1989; Chastain, 1990; Jeffers, 1990; Panciera, 1990a), definitive diagnosis can be uncertain, especially when clinical signs are vague and results of diagnostic tests are equivocal. Therefore the true incidence of canine thyroiditis remains difficult to establish. Thyroiditis is representative of a large number of autoimmune diseases in which immunological surveillance falters so that self and nonself become confused and the body attacks itself.

In human medicine, autoimmunity is the cause of most common endocrine disorders (Baker, 1992). This appears to be true for dogs as well. A significant measure of the diagnostic ambiguity about canine hypothyroidism stems from our ignorance of the fundamental causes and contributing mechanisms underlying the autoimmune responses of dogs. The development of autoimmune disease generally involves a multistep process: an initiating event produces nonspecific trauma or inflammation that triggers a secondary immune reaction in a genetically predisposed individual. The endocrine gland is progressively destroyed by the autoimmune attack, and as the hormone titers change, clinical symptoms appear (Baker, 1992). This chapter reviews thyroiditis as a model for research on the general features of canine autoimmunity. One aim is to screen for canine alleles of the major histocompatibility complex (MHC) like those that have been associated with autoimmune disease in people (Sinha *et al.*, 1990; Farid, 1991, 1992; Volpé, 1991).

Many clinical symptoms suggest a deficit in the level of circulating thyroxine (T_4) or triiodothyronine (T_3), but no single symptom or combination of symptoms is generally accepted as definitive. Common presentations include impaired growth of the epidermis or its derivatives, lethargy, exercise intolerance, cold intolerance, mental dullness, reproductive inadequacies, myopathy, neuropathy, cardiovascular abnormalities, and behavioral anomalies (Feldman and Nelson, 1987; Dunn, 1989; Jeffers, 1990; Panciera, 1990a). The disease is most frequent in 4–10 year old dogs, and certain breeds appear genetically predisposed (Feldman and Nelson, 1987). The most common early symptom in dogs is a generally poor skin and hair coat with bilateral alopecia, particularly of the flanks and tailhead. In working dogs, there is commonly loss of energy, reduced concentration, and thinness of the footpads, leading to lameness. In greyhounds, low thyroid hormone levels have been correlated with poor racing performance (Taylor and Hauler, 1983), but this initial suggestion was not confirmed in a follow-up study with larger sample sizes (Beale *et al.*, 1992).

The usual thyroid function tests involve a panel of measurements including total (bound and unbound) and free (unbound) T_3 and T_4 concentrations in the blood. While some experts accept serum total T_4 as the most reliable single diagnostic indicator (Panciera, 1990b; Nelson *et al.*, 1991), others believe that accurately measured free T_4 levels are most predictive of thyroid dysfunction (Larsson, 1988; Refsal and Nachreiner, 1993).

More information helps in understanding difficult presentations. Thus, most investigators recommend a complete panel of thyroid measurements including concentrations of circulating autoantibodies to T_3

and T_4 for assessment of clinical patients and genetic screening of breeds or individual dog families at risk for thyroid disease. If the results are unclear, the panel of hormone measurements is followed by a thyroid stimulating hormone (TSH) stimulation test (Ferguson, 1984; Beale, 1990; Jeffers, 1990) and confirmed by rescue with oral administration of levothyroxine given twice daily (synthetic L-thyroxine). Discussions of these tests are available elsewhere (Panciera, 1990b) and are beyond the scope of the present review. Other factors, including the female reproductive cycle (Reimers *et al.*, 1984), age, sex, body size and illness (Jeffers, 1990; Panciera, 1990b; Reimers *et al.*, 1990), glucocorticoids (Panciera, 1990b; Kaptein *et al.*, 1992), and the genetics of the dog, can influence these values. As in any disease of a regulatory system, the problem could lie in signal production, signal transmission, signal reception, or the effector response downstream. The present chapter will focus on primary hypothyroidism, which is due to insufficient production of T_3 or T_4 in the thyroid gland.

Canine hypothyroidism has a strong genetic component (Haines *et al,* 1984; Conaway *et al.*, 1985a). This is especially apparent in closely bred lines (Musser and Graham, 1968). In a classic example within the beagle colony of the Argonne National Laboratory near Chicago, 401 animals from two partially inbred lines were examined and scored positive for thyroiditis if there were inflammatory cells in sections of the thyroid gland (Fritz *et al.*, 1970). There was a total of 63 cases of the disease in the laboratory colony. When the pedigrees of the afflicted and healthy dogs were compared, a statistically significant genetic component was obvious. In fact, the eight litters of one line showed 23 of the 30 pups with histological evidence of hypothyroidism. The authors also suggest that thyroiditis was less common in the founder dogs of the colony, which were certainly less inbred.

The incidence of thyroiditis differs according to the breed of dogs. In certain breeds, notably borzois (Conaway *et al.*, 1985a), giant schnauzers (Greco *et al.*, 1991), Doberman pinschers, akitas, cocker spaniels, golden retrievers, Irish setters, Old English sheepdogs, Skye terriers, and Shetland sheepdogs (see Jeffers, 1990; Panciera, 1990a), thyroid disease apparently occurs at high frequency in many lines. It is generally believed that thyroiditis is less common in mongrels and outbred dogs like Alaskan huskies.

We are in the midst of a diagnostic revolution—the application of the techniques of molecular biology to the unequivocal detection of carriers of genetic disease. As this review was in its final draft in late 1993, there appeared a much-heralded report of the identification of the human colon cancer gene. This colon cancer gene is, in fact, a homolog of a previously characterized gene of bacteria and yeast (Fish-

el *et al.*, 1993); its identification illustrates the increasing convergence of human medical genetics with basic research in molecular biology.

Within the past 2 years, candidate genes responsible for leukocyte adhesion deficiency in Holstein cattle (Shuster *et al.*, 1992), hyperkalemic periodic paralysis in quarter horses (Rudolph *et al.*, 1992), porcine malignant hyperthermia (Fuji *et al.*, 1992), and early onset progressive retinal atrophy in Irish setters (Farber *et al.*, 1992) have been identified. Veterinary applications of molecular diagnostics usually employ a human or mouse probe (DNA sequence) derived from genes for clinically similar diseases in humans or mice. These four papers emphasize the importance of a comparative approach to animal medicine and the many commonalities that emerge from studies at the molecular level. In addition, genetic marker bands on DNA fingerprints have been closely linked with the Weaver syndrome (Georges *et al.*, 1993) and with the polled (hornless) gene in cattle and should allow marker-assisted selection to breed out these diseases (Womack *et al.*, 1992). When the carriers of these predisposing genes are identified, it is possible to trace the genes back through ancestors to their origins. For example, the leukocyte adhesion deficiency in Holsteins apparently come mostly from one outstanding bull.

Are there analogous candidate genes to account for genetic canine thyroiditis? One might guess that thyroid dysfunction would be associated with structural abnormalities of thyroid-specific proteins.

The coding sequence for the canine thyrotropin receptor has been determined (Parmentier *et al.*, 1989) and the sequence for a variant has also been reported (Libert *et al.*, 1990). A genetic variant of the TSH receptors was reported in neoplastic canine thyroid tissue (Verschueren *et al.*, 1992). However, this receptor does not seem to be involved in typical cases of canine thyroiditis.

Restriction fragment length polymorphism (RFLP) has been reported in the human thyroid peroxidase gene, but the variants are of unknown physiological or pathological significance (Rose *et al.*, 1991). Rare mutant forms of human thyroid peroxidase are associated with abnormal or absent enzyme function (Mangklabruks *et al.*, 1991; McLachlan and Rapoport, 1992). The wild type thyroid peroxidase gene has been substantially sequenced (Kimura *et al.*, 1987), but no sequence information is available on any mutants. Even when the gene is wild type, the cloned cDNAs for human thyroid peroxidase are of several sizes, apparently reflecting the presence of alternative splicing sites during the maturation of the primary RNA transcript into the final messenger RNA. The physiological or pathological significance of these differences in size of message, at least one of which produces a truncated protein apparently

lacking the 5′ sequence for insertion into membranes, has not been evaluated (Kimura *et al.*, 1987; Nagayama *et al.*, 1990; McLachlan and Rapoport, 1992).

The ability to produce autoantibodies to thyroid peroxidase is inherited as an autosomal dominant (Phillips *et al.*, 1990, 1991). The search for a candidate gene for that relatively rare human condition is under way. The significance of such a gene for canine disease is unclear, since thyroid peroxidase is not a pathogenic antigen in dogs affected with autoimmune thyroiditis (Thacker *et al.*, 1994).

Defective alleles of the human gene coding for the thyroid hormone receptor have been cloned. The defect produces an autosomal dominant disease termed "generalized resistance to thyroid hormone" (Nagaya *et al.*, 1992). To our knowledge, an analogous condition has not been reported in dogs.

RFLP analysis on human DNA, probing with a repetitive sequence from the 5′ end of the thyroglobulin gene, has identified a complex DNA fingerprint in man that is of little significance in detecting polymorphisms in thyroglobulin itself (Gérard *et al.*, 1990). We subjected the DNA of hypothyroid and euthyroid dogs to RFLP with human probes from within the thyroglobulin coding region. Although there was variation among individuals, we found no consistent correlation of RFLP pattern and thyroid health (Happ, unpublished observations).

The sequence of the canine thyroglobulin gene promoter has been reported (Donda *et al.*, 1991), and the effects of mutations in this region of human, canine, bovine, and rat promoters have been tested in a transient expression assay in primary cultures of dog thyrocytes (Donda *et al.*, 1993). No variants of this promoter region have been clearly linked to thryoid disease in dogs.

Since physiologically important defects in human and animal thyroid structural genes appear to be quite rare, it seems unlikely that a single candidate gene for a thyroid-specific protein, like thyroid peroxidase or thyroglobulin, would account for genetic canine thyroiditis. In the search for alleles correlated with thyroiditis, we believe that the emphasis should be placed on canine *immuno*genetics.

Autoimmune thyroiditis is but one of many canine autoimmune diseases (Halliwell, 1978; Bennett, 1984; Gorman and Werner, 1986a,b,c). These diseases include autoimmune hemolytic anemia, thrombocytopenia, von Willebrand's disease, pemphigus vulgaris, systemic lupus erythematosus, rheumatoid arthritis, Addison's disease, myositis, and many others. There is widespread suspicion that some conditions may be linked, due to common underlying defects in the immune system. In human medicine, commonality is designated as autoimmune diathesis

(Rose and Burek, 1991) or polyglandular autoimmunity (Fisher *et al.*, 1987). In dogs, von Willebrand's disease has been associated with hypothyroidism (Dodds, 1988) and a cluster of immune-related diseases have been reported in Old English sheepdogs in North America (Dodds, 1988) and Western Australia (Day and Penhale, 1992). In dogs as in people, autoimmune reactions are likely to be linked with particular genes of the immune system (Day and Penhale, 1987). These strong parallels suggest many experimental approaches to analyze disease mechanisms, to develop screens for genetic predisposition, and to devise strategies for immunotherapy.

This chapter reviews the molecular biology underlying autoimmune responses, discusses briefly animal models of autoimmune disease, evaluates the techniques that might detect canine genes predisposing toward autoimmune thyroiditis, and summarizes the causes and development of autoimmune thyroiditis in human and dogs. Finally, it suggests future trends and priorities for research on autoimmune diseases.

II. Molecular Basis of Autoimmunity—The Failure of Self-Tolerance

Autoimmunity is a family of complex phenomena with multiple causes (Sinha *et al.*, 1990; Carson,1992; Rose and Mackay, 1992). It can involve both T-lymphocyte and B-lymphocyte effector mechanisms. When self-tolerance breaks down, the immune system confuses self-antigens and foreign antigens and turns upon the body. An understanding of autoimmunity must begin with a brief review of the genes involved in self-recognition.

A. The MHC and T-Cell Receptor Complexes

The key proteins involved in recognition and thus the tolerance of self are: (1) the *immunoglobulin receptors* on B-lymphocytes, (2) the *T-cell receptors* (TCR) on T-lymphocytes, and (3) the protein *products of the major histocompatibility complex* (MHC) *genes*. The B-cell immunoglobulin receptors, unlike the TCR and the MHC products, can exist in soluble form as circulating antibodies. Both immunoglobulins and TCR proteins are coded for by gene sequences that have undergone somatic DNA rearrangement and mutation. The MHC antigens are of two groups: MHC class I receptors, found on the surfaces of all nucleated cells, and MHC class II receptors, found on cells that take up

extracellular antigens, partially digest them, and then present the fragments from digestion on their surface receptors. Fragments from *intra*cellular antigens are bound to MHC class I molecules, and fragments from *extra*cellular antigens are bound to MHC class II molecules. The MHC receptors and the antigenic fragments they bear are recognized by the TCRs (Fig. 1).

Since the MHC class I molecules bind fragments from the digestion of proteins made inside cells, they can reveal the presence of intracellular viruses and thus alert the T-cells to the presence of infection. Although some autoimmune diseases have been linked to the class I

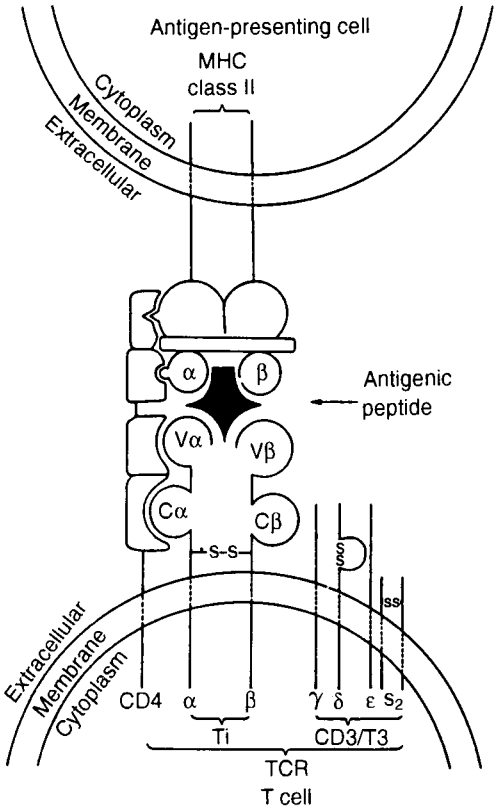

FIG. 1. TCR-antigenic peptide–MHC complex. Antigen is processed within the antigen-presenting cell. The resulting peptide fragments are attached to the MHC and transported to the cell surface. If a TCR (with its accessory CD3 and CD4 proteins) recognizes that complex, the T-cell is activated (from McGregor, 1992). Reprinted with permission of Oxford University Press.

genes, most of the strongest associations are with the class II genes (Nepom and Erlich, 1991). As a general rule, class II molecules are found on cells specialized for the presentation of antigen, such as B-cells, macrophages, and dendritic cells. However, cytokines, such as γ-interferon, can induce other cells, including thyroid epithelia, to produce class II molecules (Todd *et al.*, 1985). Every antigen-presenting cell contains about 200,000 class II molecules. Only 1% (200–300) of the possible MHC-peptide complexes are sufficient to activate a T-cell.

Because naturally occurring autoantibodies occur in the sera of normal individuals, it is clear that B-cells that react to self-antigens can exist without pathogenic consequences. Yet often these B-cells are not activated to become plasma cells that secrete large amounts of antibody. B-cells will become activated only under the influence of helper-T-cells. The critical actors in self-tolerance are the T-cells that regulate the B-cells.

T-cells comprise 70–80% of peripheral blood lymphocytes. The surface of each T-cell is studded with 10,000 to 20,000 TCRs. Every mature TCR is a heterodimer, consisting of two peptide chains, linked to one another by a single disulphide bond and embedded in the plasma membrane. The peptides have four domains: two extracellular, one transmembrane, and one cytoplasmic. Like the immunoglobulins, the TCR peptide has constant and variable regions. The variable regions come close together at the apex of the molecule, farthest from the surface of the cell, to form a shallow groove that binds antigen.

Like B-cells, T-cells originate in the bone marrow, but T-cells undergo an additional maturation stage in the thymus. All T-cells enter the thymus with the same genetic information for making T-cell receptors. Within the thymus, the variability of the TCRs is generated by the rearrangement of the variable (V), diversity (D), and joining (J) elements and by random events that occur at the Vβ–Dβ–Jβ junctions so that each clone produces a TCR with unique specificity (Toyonaga *et al.*, 1985). Once their receptors are functional, the T-cells undergo a two-stage selection. The first stage is a positive selection—T-cells that recognize the particular MHC receptors in that individual survive; those that fail this recognition test would be unproductive and are eliminated. The second stage is a negative selection—T-cells that recognize self-antigens are deleted. The T-cells in the final repertoire have gone through both positive and negative selection. This adaptive process accomplishes tolerance of self.

Organ-specific self-antigens are not present in the thymus, and they may fail to participate in the selection process. For thyroglobulin, which is normally confined to the thyroid follicle, or for myelin-basic

protein, which does not pass the blood-brain barrier, negative selection for T-lymphocytes is often incomplete. One consequence of that incomplete process can be an autoimmune reaction and autoimmune disease. For more complete discussions of the breakdown of tolerance, see Rose and Mackay (1992).

The T-cells that emerge from the thymus are of several varieties, distinguishable by their behavior and by the proteins they carry on their surfaces. Each TCR is associated with other membrane-bound glycoproteins, either CD3 and CD4 (Fig. 1) or CD3 and CD8. Helper T-cells, which bear a CD4 surface marker, secrete lymphokines, promote the differentiation of B-cells into plasma cells that secrete large quantities of soluble antibody, and trigger the maturation of CD8-positive T-cells into cytolytic or suppressor T-cells. T-cells are the gatekeepers. The efficiency and accuracy of recognition of self depends upon both the presentation system (the MHC molecule) and the recognition of peptide-MHC complex by the complementary TCR. The search for genes predisposing toward autoimmune disease has focused on those of the MHC complex and those coding for TCRs (e.g., Marcadet *et al.*, 1985; Todd *et al.*, 1987; Beall *et al.*, 1989, 1993; Nepom and Concannon, 1992; Roman *et al.*, 1992).

B. Polymorphism in HLA and TCR Genes

The human MHC class I molecules (termed HLA-A , B, and C) consist of a heavy chain and a β_2-microglobulin molecule (Nepom and Concannon, 1992). These are major transplantation antigens. They are highly polymorphic, and multiple allelic variants exist for each of the three loci. There are 14 MHC class II loci, clustered in three regions termed HLA-DR, DQ, and DP. The genes are tightly linked, and thus the antigens within the MHC are usually inherited as a block, called the haplotype. Each locus codes for at least one α- and one β-chain. The α-chains are polymorphic and the β-chains are highly polymorphic. For example, the DQA1 gene has 13 known alleles, DQB1 has at least 19 alleles, and the DRB1 gene has at least 68 alleles (Marsh and Bodmer, 1993).

Linking particular alleles with etiologically complex diseases is challenging. A variety of techniques have nonetheless drawn such relationships. At least 76 diseases are associated with particular serologically defined HLA specificities (Tiwari and Terasaki, 1985). Molecular techniques have demonstrated the existence of several alleles within a single HLA serological specificity, and thus the genetic analysis must proceed to a more detailed level. The usual analysis of HLA-

disease connections proceeds in three steps: (1) a serological specificity or a particular restriction fragment on a DNA fingerprint is associated with a disease; (2) haplotypes that carry that marker are defined; and (3) individual genes within that haplotype are evaluated (Nepom and Concannon, 1992). There is a high concordance for multiple sclerosis in monozygotic twins (McFarland *et al.*, 1984), suggesting genetic predisposition is an important factor in this disease. The disease incidence is correlated with an increased frequency of HLA class II haplotypes DRw15, DQw6, Dw2 in Caucasians (Marcadet *et al.*, 1985; Olerup *et al.*, 1989). Likewise, the HLA-DR3(Dw3) specificities, made up of the linked DQ alleles DQB1*0201, DQA1*0501, and DRB1*0301, are linked to autoimmune diseases such as Graves' disease, myesthenia gravis, and type I diabetes. It is not clear which of these genes is responsible for any of the diseases. In fact, the responsible gene might be elsewhere within the haplotype, linked to the DQ-DR cluster (Nepom and Concannon, 1992).

It is useful to define as many loci as possible in the MHC haplotypes on both chromosomes because of the linkages and also because their products might interact in the phenotype. There are intriguing recent reports of MHC class II heterodimers that form between the α chain of one locus and the β chain of another. NZB $\times$ NZW hybrid mice make such heterodimers, and the F_1 animals show a lupuslike syndrome. The heterodimer, the product of a mixed haplotype, plays a key role in the development of autoimmunity (Nygard *et al.*, 1993).

Each HLA receptor accepts a particular set of antigen fragments in its peptide-binding cleft at the end of the molecule. The alleles that predispose to autoimmune disease often include specific amino acid polymorphisms for autoantigenic peptides. The MHC-antigen complex then triggers autoreactive T-cells to initiate an autoimmune reaction. Critical amino acid sequences in the MHC receptor have been identified in rheumatoid arthritis. Several different DRB alleles are linked to rheumatoid arthritis and all code for DRβ molecules, which carry a specific amino acid sequence in the region of amino acids 67 to 71. That sequence is a T-cell recognition element (Nepom and Concannon, 1992).

Polymorphism in the TCR genes has also been linked to autoimmune disease. They encode two forms of receptor—an $\alpha\beta$ form on over 90% of the T-cells and a $\gamma\delta$ form on the remainder. There is immense diversity in the antigen-binding sites of the TCRs for two reasons. First, there is the genomic diversity in T-cell genes, and second, there are many combinational variants introduced as the V, D, J, and constant (C) gene segments are spliced together in a process like that used for the pro-

duction of antibodies in the B-cells. For humans, with an estimated 100 alternative Vα segments and 61 Jα segments for the TCR-α chain, there are 6100 combinations (Roman-Roman *et al.,* 1991). The adjacent region, that coding for TCRβ, has an estimated 80 Vβ gene segments, two Dβ segments, and 13 functional Jβ segments (Toyonaga *et al.,* 1985). Additional variation is introduced by the variations and insertions at the splice sites. For people, there is the theoretical potential for more than 2×10^{20} different combinations of the αβ peptide chains of a TCR.

Polymorphism in the germline TCR repertoire is thought to affect susceptibility to autoimmune disease. The sequencing of all the bases in the human TCR complex is still in progress. The available data indicate that there are many subfamilies of the gene segments. Eighty different Vβ segments fall into 24 subfamilies, while the Vα segments are divisible into 29 subfamilies (references in Nepom and Concannon, 1992). Most of that polymorphism has been detected by RFLP. Beall and coworkers (1989) used RFLP of genomic DNA to survey TCR-Vβ alleles in 40 patients with multiple sclerosis and compared the results with 100 normal individuals. The authors concluded that a multiple sclerosis susceptibility gene may be located in the TCR β-chain gene complex. Seboun *et al.* (1989) used sibling pairs to show TCR-Vβ haplotypes were more likely to be similar in siblings with multiple sclerosis than between afflicted and unafflicted siblings. However, other studies have failed to confirm that association with multiple sclerosis (Hillert *et al.,* 1992). A similar set of conflicting data exists for TCR alleles and insulin-dependent diabetes mellitus (IDDM); the conflicts may be due to genetic heterogeneity of the patient populations (Hibberd *et al.,* 1992).

The lack of consistency in careful studies in different research laboratories may reflect the complexity of the interactions between the MHC products and TCR. Recently, Beall and coworkers (1993) reported that the association of multiple sclerosis with particular TCR Vβ haplotypes is significant only in HLA-DR2+ individuals. If confirmed, this result suggests that gene complementation between HLA class II and TCR Vβ genes predisposes to multiple sclerosis. The important event could be either the positive selections in the thymus for the T-cell repertoire, or much later the actual presentation of the antigen fragment to the TCR. In experimental allergic encephalitis of rodents, an animal model of multiple sclerosis, the response is T-cell mediated and the early determinants in myelin basic protein have been identified. However, additional determinants become immunogenic in later phases of the disease, suggesting that T-cell autoimmune response

spreads from one determinant to others. (Lehmann *et al.*, 1992). As noted by Nepom and Concannon (1992) "the study of TcR gene polymorphism and its impact on autoimmunity is in its infancy."

III. Screening for Canine Genes That Might Predispose toward Thyroiditis

A. Canine Molecular Immunogenetics

The extensive use of dogs as experimental subjects in organ transplantation experiments has sustained research in canine histocompatibility typing. The nomenclature for the canine MHC complex is summarized well in the reports of the Third International Workshop on Canine Immunogenetics. Class I alleles, designated as types DLA-A, B, C, code for serologically defined antigens (Bull *et al.*, 1987). Class II alleles, designated as types DLA-DR, DQ, DP, and DO, code for antigens defined by reactivity in a microlymphotoxicity test (Deeg *et al.*, 1986). As of the Third International Workshop, there were 5 DLA-A antigenic specificities, 4 DLA-B antigenic specificities, 3 DLA-D antigenic specificites, and 10 DLA-D homozygous typing cell specificities. Depending on whether it is homozygous or heterozygous at these loci, any given dog expresses one or two alleles of the α-chain of each class I gene and β-microglobulin and one or two alleles for the α- and β-chains of the class II alleles. This information can also be used for canine paternity testing (Bull and Gerlach, 1992).

An important series of recent papers by Ulla Sarmiento, Rainer Storb, and their coworkers at the University of Washington, Seattle, has demonstrated the allelic polymorphism of the DLA genes and begun to specify the base sequences of the individual alleles. By RFLP analysis, with dog DNA exposed to a human HLA probe, Sarmiento and Storb (1989) report that there are at least eight class I genes including the canine homologs of HLA-A, B, and E genes. When RFLP analysis was applied to canine MHC class II genes, they too were demonstrated to be polymorphic (Sarmiento and Storb, 1988b).

Nine restriction endonucleases were used to digest the DNA from peripheral blood leukocytes of 23 dogs known to be homozygous for the nine DLA-D types, as defined by leukocyte reactions. After separation of the fragments by agarose gel electrophoresis, they were exposed to human HLA probes for DRB, DQB, DPB, and DOB gene sequences. It was possible to discriminate among the nine homozygotes using only two restriction enzymes and a probe for a gene coding for a HLA-DPβ

chain. When a dog DLA-DRB probe is applied to the DNA from dog families, the RFLP patterns assort with the DLA-DRB specificities assigned by mixed leukocyte cultures (Burnett *et al.*, 1994). In a parallel study, Sarmiento and Storb (1988a) detected five α-chain genes.

Starting with dogs known to be homozygous for the DLA-DRBs, the Seattle group sequenced the cDNAs for nine DRB alleles. According to cluster analysis, the nine alleles are subdivided into three major groups that resemble the canine analogs of the human supertypic groups (Sarmiento and Storb, 1990; Sarmiento *et al.*, 1990). Sequencing of DQA and DQB genes revealed more alleles than previously reported by DNA fingerprinting. There now appear to be at least four DQA alleles and four distinct DQB alleles (Sarmiento *et al.*, 1992, 1993). It would not be surprising to have more loci and alleles discovered with increasing study of the canine genome.

In all mammals, the TCR on the cell surface is complexed with the membrane protein CD3, composed of five constant chains. The sequence of the canine CD3ε subunit has been determined (Nash *et al.*, 1991). Preliminary work with human TCR probes suggested that the canine TCRB gene, like its human homolog, has two constant regions (Chaganati *et al.*, 1992). A more recent paper provides the first sequence information on the dog T-cell receptor itself (Ito *et al.*, 1993) The investigators isolated messenger RNA from peripheral leukocytes of a dog, synthesized the corresponding cDNAs, and preferentially amplified the cDNAs for the TCRα and TCRβ chains by adding V gene universal forward primers and reverse primers for either the TCRA or the TCRB gene. From the clones isolated, only one α-chain and one β-chain sequence were found, both of which showed strong similarity to other mammalian TCRs.

B. Screening for MHC Genes

The keen interest in the HLA complex has led to development of many protocols for detecting histocompatibility types. The molecular techniques developed for human HLA typing should be quite applicable to DLA typing (Bull and Gerlach, 1992). These powerful methods will complement classical typing techniques using serology or leukocyte reactions, as described by Bull *et al.* (1987) and Deeg *et al.* (1986) for DLA class I and DLA class II, respectively. They were developed in part as supplements to the serological typings. In a well-characterized and ethnically homogenous population like the Swedes, the assignments are fairly reliable, but much less so in more heterogeneous populations (Olerup *et al.*, 1993).

1. Restriction Fragment Length Polymorphism (RFLP)

Southern blotting is a powerful technique to study target sequences of DNA in a mixture of many fragments that are separated by size. Using multilocus probes, RFLP is widely applied to identify individuals; in that context, it is known as DNA fingerprinting (Jeffreys *et al.*, 1985). It was first used for investigation of HLA class II polymorphism by Wake and coworkers (1982). For MHC typing, RFLP begins with isolation of DNA (generally from blood cells), digestion with one or more restriction endonucleases, and separation of the fragments by size on agarose gels. The DNA fragments are transferred to a solid support (usually nitrocellulose or nylon) by capillary or vacuum blotting, and finally, the blot is immersed in a solution containing single-stranded-DNA sequences (probes) that bind to complementary target sequences in the DNA. The bound probes are usually visualized on X-ray film blackened by their radioactivity or chemiluminescence. The usual result is a stack of short bands, much like a bar code.

With application of many restriction enzymes and probes, it is possible to demonstrate MHC gene polymorphism, much as Sarmiento and Storb (1988b, 1989) have done for DLAs. Meticulous attention to technical details is required for consistency. For the results to be comprehensible and unambiguous, the RFLP patterns must not be too complex and the probes must not cross-react with several loci, or else the alleles become difficult to distinguish from one another. With a very precise choice of restriction enzyme and probe, it is possible to accurately determine the genotype of many HLA-DR and DQ specificities. To minimize cross-reactions with unrelated sequences, short exon-specific probes are now utilized (Bidwell *et al.*, 1993.

Two advantages of this technique are: (1) that many alternative heterologous probes can be used by adjusting the stringency of the hybridization reactions, and (2) that one can demonstrate polymorphism even if one knows relatively little of the actual base sequences in the target DNA. Since the restriction enzyme cuts are often outside the actual coding region of the MHC genes, polymorphism may be found to be of no functional relevance, and furthermore, some polymorphism within the MHC exons is likely to be missed. Great care must be taken in applying specific protocols developed for Caucasian populations to non-Caucasians (Bidwell *et al.*, 1993).

2. Polymerase Chain Reaction / Sequence-Specific Oligonucelotide (PCR-SSO)

The technique of amplification of specific genes or portions of genes with the polymerase chain reaction (PCR) has led to enormous innova-

tions in all subfields of molecular biology, including histotyping. HLA types can be determined without separation of restriction fragments by using probes that are specific for particular alleles. DNA is purified from blood and the HLA genes are amplified by PCR. Aliquots from the reaction mixture are spotted on nitrocellulose or nylon supports, either in a slot blot or a dot blot, and the membrane is immersed in a solution of labeled oligonucleotides. The sites of nucleotide binding are visualized by the usual techniques.

The first stage of discrimination is the PCR, where judicious choice of primers will determine the possible pool of products. As a general rule, the primers are chosen to amplify a sequence of about 300 bases coding for the NH_2-end of the B gene, the highly variable segment that codes for the terminal antigen-binding portion of the receptor's β chain. In a typical set of protocols for human DRB/DQB/DPB typing, one set of generic DR primers and six more sets of specific primers are utilized (Tiercy *et al.*, 1993) to resolve 53 DRB1, 3 DRB3, 3 DRB5, 17 DQB1, and 22 DPB1 alleles with a total of 67 SSO probes. With the first set of primers and 15 oligonucleotide probes, HLA-DQB1 alleles can be distinguished; a second set of primers and 18 probes allow identification of the HLA-DPB1 alleles, and a third set of amplifications and 14 probes allow the identification of all major HLA-DR groups and some specific alleles within them. When the HLA-DR assignments are ambiguous at this point, more specific primers are utilized to distinguish among the DR alleles.

The PCR-SSO techniques are very effectively exploited in human clinical laboratories for typing of leukemic or kidney transplant patients and for volunteer bone marrow donors. The power of this method is impressive. Its principal disadvantages are: (1) the requirement for a large number of SSO probes and very precise optimization of the hybridization and posthybridization washing conditions for each, (2) the considerable investment in time and facilities to be efficient about typing, and (3) the fact that the protocols, as developed for Caucasian patients, are sometimes ambiguous with non-Caucasians, requiring the application of additional probes. Considerable new information about canine base sequences followed by judicious evaluation of the many alternative SSO probes will be required before PCR-SSO can be useful for canine DLAs.

3. Polymerase Chain Reaction / Restriction Fragment Length Polymorphism (PCR-RFLP)

Like PCR-SSO, PCR-RFLP begins with amplification of the base sequence coding for the highly variable amino terminal region of the β-chain. Following amplification, the aliquots of the PCR reaction mix-

ture are digested with a panel of restriction enzymes and subjected to electrophoresis on acrylamide or agarose. The proper choice of allele-specific endonucleases is critical and is made much easier by evaluating the target sequence for each enzyme against the banks of sequenced HLA alleles. Alleles are differentiated from one another on the basis of the size of the digestion fragments.

The PCR-RFLP techniques were originally developed for homozygotes, with distinct primers for each HLA class (Maeda *et al.,* 1990; Uryu *et al.,* 1990; Salazar *et al.,* 1992). As originally proposed for HLA-DRB and -DQB typing, this technique employed five restriction enzymes to distinguish 16 patterns characteristic of HLA-DR and -Dw homozygous serotypes (Maeda *et al.,* 1990). The addition of heterozygotes to the test pool clouded the results with incomplete digestion products (Olerup, 1990). Various modifications of the techniques have been proposed to address these difficulties. Codigestion with two restriction enzymes improves the discrimination among some alleles (Sawitzke *et al.,* 1992). Addition of a constant restriction site in one of the primers offers an internal digestion control (Mercier *et al.,* 1992). A recent improvement is the use of "more informative enzymes," which have a single recognition site in some alleles and none in other alleles in the amplified regions (Inoko and Ota, 1993). The use of 29 enzymes allows 93 different alleles to be distinguished in homozygotes or heterozygotes in a Japanese population, such that genotypes can be defined simply by determining whether the amplified DNA is digested. Additional information can be obtained by amplifying and digesting noncoding as well as coding sequences (Limm *et al.,* 1993; Simons *et al.,* 1993).

Inoko and Ota (1993) argue forcefully for the superiority of PCR-RFLP as compared with PCR-SSO. The advantages include: (1) simplicity (29 commercially available enzymes and no radioisotopes for PCR-RFLP vs 100 PCR-SSO probes, radioisotopes, and adjustment of washing temperatures for PCR-SSO); (2) discrimination (a change in only one is detectable with restriction enzymes while achieving that high stringency with SSO is very time consuming); (3) cost (PCR is less costly when small samples are typed); and (4) linkage information that is provided by PCR-RFLP and not by PCR-SSO. It will be important to determine if these newest PCR-RFLP protocols work well in other laboratories and in the present context, to see whether they can discriminate the DLA alleles.

4. Polymerase Chain Reaction—Sequence-Specific Primers (PCR-SSP)

The PCR-SSP technique is based on the principle that a perfectly matched primer will be more specific in the PCR reaction than a prim-

er with one or several mismatches, especially in the first critical cycles (Olerup and Zetterquist, 1993; Olerup *et al.*, 1993). The specificity of the PCR is the discriminator, with assignment of alleles based on the mere presence or absence of the amplified product. A series of PCR amplifications is performed in parallel, each one of which contains a pair of primers that bind very specifically to only one or a very few alleles. Often the experiments are organized in two stages of increasing resolution. DR "low resolution" PCR-SSP typing employs 40 primers and is followed, if necessary, by DR4 and DR1 subtyping with a panel of additional primers. With carefully purified primers of the correct specificities, the results look very convincing (Olerup and Zetterquist, 1993). This new method seems powerful and is very fast; typical PCR-SSP typings can be performed in less than 2 hours. The disadvantage is the requirement for a large panel of very high quality primers.

IV. Models of Autoimmune Disease

Both genetic and environmental factors contribute to the initial autoimmune responses and the transformation of a benign autoimmune response into a pathological autoimmune condition (Sinha *et al.*, 1990). The reductionist dissection of the contributing factors from one another and the evaluation of the importance of each are necessary for a complete understanding of these very complex phenomena. Animal models that are especially instructive are of two classes: (1) those autoimmune reactions *induced* by injection of antigens and (2) those *spontaneous* autoimmune reactions that occur at high frequency in inbred strains (Bernard *et al.*, 1992). The best-understood model of spontaneous autoimmune disease is IDDM in the nonobese diabetic (NOD) mouse.

The NOD mouse strain was developed in the early 1980s in Japan (Makino *et al.*, 1980). Most young NOD mice spontaneously develop insulitis—lymphocytic infiltration of pancreatic islets and the destruction of many of the insulin-producing β-cells. When all β-cells are destroyed, the afflicted mice develop glycosuria and an IDDM. The full-blown disease, which is rapidly fatal without insulin treatment, is much more common in older females. Over the past decade, the NOD strain has been established in many laboratories in Japan, North America, and Europe. Individual laboratory colonies differ in the frequency of disease but all show insulitis in both sexes and a preponderance of IDDM in older females (Bernard *et al.*, 1992). Some individuals survive for over a year, and many of these older mice become

severly cachetic and jaundiced due to autoimmune hemolytic anemia (Baxter and Mandel, 1991).

Impressive progress has been made in identifying the genes that predispose NOD mice to IDDM. The initial genetic analyses suggested that there were two recessive genes on two different autosomes (Makino *et al.,* 1985). In the next year, Hattori *et al.* (1986) linked the IDDM to a MHC class II complex that was apparently unique to the NOD strain. By crossing NOD mice with the nondiabetic C3H strain, Hattori and coworkers found no diabetes in the F_1 mice, but it was present in a small fraction of the backcrosses and intercrosses. All backcross and intercross mice showing diabetes mellitus were homozygous for a 9.5-kb band on Southern blots, but some mice that were homozygous for the 9.5-kb band were disease-free. Since the 9.5-kb band was necessary but not sufficient, the investigators suggested the existence of one or more additional susceptibility genes not linked to MHC.

Molecular analysis of the responsible MHC in the NOD mice gene revealed the presence of a five-nucleotide substitution in the I-Aβ gene that alters two amino acids of the β chain from proline–aspartic acid in IA^d (wild type) to histidine-serine in the class II $I\text{-}A^{NOD}$ gene (Acha-Orbea and McDevitt, 1987). Transgenic mouse experiments support the conclusion that the $I\text{-}A^{NOD}$ gene, now designated as $I\text{-}A^{g7}$, is involved in disease development (Lund *et al.,* 1990; Miyazaki *et al.,* 1990; Slattery *et al.,* 1990). Two related sister strains of mice, ILI and CTS, which share the IA^{g7} allele but do not develop diabetes, confirm the suggestion that there are non-MHC susceptibility genes. This general pattern seems to apply for human disease as well. The HLA-DQB1*0302 allele with a homologous amino acid substitution is associated with diabetes mellitus in Caucasians (Todd *et al.,* 1987; Morel *et al.,* 1988) but not in similarly afflicted Japanese (Awata *et al.,* 1990). This striking parallel between human and mouse studies argues strongly that multiple genes, MHC and others, are involved in the development of IDDM in diverse mammalian species.

Several of the predisposing mouse genes have been mapped to specific chromosomes. The $I\text{-}A^{g7}$ gene, also known as *Idd-1*, is found in the murine MHC complex on chromosome 17 (Lund *et al.,* 1990; Miyazaki *et al.,* 1990; Slattery *et al.,* 1990). A second locus on chromosome 9, designated *Idd-2,* is associated with diabetes in backcross progeny (Prochazka *et al.,* 1987). Recent work by Todd and colleagues (1991) has mapped two more non-MHC genes, designated *Idd-3* and *Idd-4* to chromosomes 3 and 11, respectively, of the NOD mouse. *Idd-3* affects both the frequency of insulitis and its likelihood to progress to full diabetes.

Idd-4 was associated with diabetes in younger animals but not in those over 144 days.

Insulitis probably begins with infiltration of the pancreatic islets by macrophages (Lee *et al.*, 1988), which present β-cell antigens to T-cells. The activated T-cells predominate in the lesions (Miyazaki *et al.*, 1985). Diabetes can be prematurely induced in newborn NOD mice by transfer of T-cells from sick animals (Yagi *et al.*, 1992 and references therein). An understanding of the disease mechanisms and the steps in the process is emerging from many recent experiments using transgenic mice. For example, Katz *et al.*, (1993) produced NOD lines transgenic for TCR α and β genes from a diabetogenic T-cell clone. In these transgenic strains, the large populations of diabetogenic T-cells at birth led to "rampant" insulitis, but not immediately. Two interesting checkpoints in disease progression were revealed: 1) T-cell infiltration at 2–3 weeks, which may be related to the lag in maturation of the antigen-presenting macrophagesm, and 2) full diabetes at 4.5 months, suggesting that more than massive insulitis is required for disease appearance.

In spite of the extensive studies of IDDM, the nature of the autoantigen presented to the T-cells has remained controversial. Two important recent papers (Kaufman *et al.*, 1993; Tisch *et al.*, 1993) identify the triggering autoantigen as a secreted form of the enzyme glutamic acid decarboxylase (GAD). GAD synthesizes γ-aminobutyric acid, a neurotransmitter in the brain and a putative paracrine signal molecule in pancreatic islets. The T-cell response to the 65-kDa isoform of GAD develops at 4 weeks of age, at the same time as insulitis. The T-cell response is consistent with a presentation of GAD peptides on MHC class II molecules and involvement of T-helper cells. T-cell reactivity begins with the C-terminal regions of GAD65, and over the following weeks it spreads to other parts of the antigen, including a region with similarity to a protein of the Coxsackie virus. The sequence of reactivity argues against molecular mimicry (Sinha *et al.*, 1990; Barnett and Fujinami, 1992) between a viral antigen and GAD65 in triggering the autoimmune response. Subsequently, the T-cells gain reactivity to other β-cell antigens.

V. Autoimmune Thyroiditis in Humans and Animals

A. The Normal Thyroid

The thyroid gland is a loose aggregate of independently functioning thyroid follicles, each of which contains gelatinous colloid. The center

of each follicle is a reaction compartment, sealed from the rest of the tissues of the body by the spheroid ball of epithelial follicular cells. Within the reaction compartment are relatively high levels of iodide, hydrogen peroxide, protein substrates, and enzymatic catalysts. The critical biochemical reaction, iodination of protein-bound tyrosine, leads to thyroxine.

The follicular epithelial cells are much more than a boundary layer of the follicle. They create the reaction environment within the follicle, importing and processing precursors from the blood, passing reactants to the interior space, recovering intermediate products, performing the final steps in manufacture, and liberating the final product. At their basal surfaces, the follicular epithelial cells absorb amino acids from the blood, and within their ribosome-studded endoplasmic reticulum the amino acids are incorporated into thyroglobulin (2748 amino acids, molecular weight 660 kDa in humans) and thyroid peroxidase. Iodide, preferentially absorbed from the blood by a specific carrier molecule in the basal plasma membrane, is transported across the follicular cell, and along with hydrogen peroxide and thyroglobulin is secreted from the apical surface of the cell into the thyroid follicle.

The H_2O_2 generation in the thyroid cells is controlled by thyrotropin, acting through the second messengers cAMP and the calcium-phosphatidylinositol cascade. H_2O_2 generation appears to be the limiting factor in the iodination of thyroglobulin (Corvilain *et al.*, 1991; Raspé *et al.*, 1991).

Thyroid peroxidase is an integral hemoprotein inserted into the apical membrane of the follicle cell with its catalytic domains pointing into the follicular space (Gruffat *et al.*, 1991). Human thyroid peroxidase has 933 amino acids: 61 in the putative cytoplasmic domain, 24 within the membrane, and 848 in the extracellular domain (McLachlan and Rapoport, 1992). The peroxidase oxidizes the hydroxyl groups on tyrosine residues in thyroglobulin. The activated tyrosines readily iodinate and form dimers, yielding finally two or more thyroid hormone residues per molecule of thyroglobulin. Once iodinated, the thyroglobulin is recaptured, apparently by both receptor-mediated and fluid phase processes, and enclosed within the endosomes of the epithelial cells (Rousset and Mornex, 1991). These endosomes constitute intracellular reaction compartments where iodothyroglobulin is enzymatically degraded by lysosomal enzymes to yield peptide fragments and iodinated tyrosine dimers—T_3 or T_4. It is not clear how the two hormones leave the epithelial cell, but a specific transport system, either active or facilitated, at the baso-lateral surface seems quite likely.

When passed into the blood, 99% of the T_4 and T_3 (in a ratio of *ca.* 5:1) is rapidly adsorbed to plasma proteins so that the effective unbound fraction of the hormones is low. About 0.1% of serum T_4 and 1% of serum T_3 are free (Dunn, 1989). The deiodination of T_4 to T_3 occurs readily in peripheral target tissues by a selenium-containing deiodinase (Berry and Larsen, 1993). T_3 binds more readily to the nuclear receptors for thyroid hormone and is three to five times more active physiologically than T_4. There is a fairly wide range of values for physiologically normal animals (Jeffers, 1990). Sudden fluctuations in the effective concentrations of thyroid hormones are largely offset by the high capacity of serum proteins to bind large amounts of T_3 and T_4. A consistent serious disruption of the complex equilibria between bound and free iodotyrosine derivatives and between the T_3 product and its immediate precursor T_4 would significantly affect the health of an animal, but such disruptions seem very rare.

B. The Disease Process

Autoimmune thyroid diseases are characterized by circulating antibodies to thyroid antigens, activated T-cells, and lymphocytic infiltration of the thyroid gland (Utiger, 1990; Wilkin, 1991; McGregor, 1992). The major human diseases are: (1) lymphocytic thyroiditis (Hashimoto's disease), in which there are low thyroid hormone titers and detectable circulating autoantibodies to thyroid peroxidase and thyroglobulin, and (2) Graves' disease, characterized by high levels of circulating thyroid hormones and circulating antibodies to the TSH receptor.

Primary hypothyroidism in dogs has been classified on the basis of its histopathology as either lymphocytic thyroiditis or idiopathic thyroid atrophy. At the histological level, lymphocytic thyroiditis shows many collapsed thyroid follicles, abundant macrophages, atrophy of the thyroid epithelium, infiltration of large numbers of lymphocytes, and partial or total collapse of follicles. Eventually, there is replacement of the thyroid follicles by fibrous connective tissue with a few scattered foci of inflammatory cells (Gosselin *et al.*, 1981, 1982). Idiopathic follicular atrophy, the second histopathologic class of primary hypothyroid disease, is characterized by replacement of thyroid follicular tissue by fatty tissue. Its pathogenesis is not well understood although it may simply be the result of end-stage thyroiditis (Conaway *et al.*, 1985b; Chastain and Ganjam, 1986; Dunn, 1989; Chastain, 1990; Wilkin, 1990).

Canine autoimmune thyroiditis is analogous to human Hashimoto's

disease (Lucke *et al.*, 1983). In the human disease, the infiltrating lymphocytes include both T-cells and B-cell, but T-cells predominate. Both helper ($CD4^+$) and cytotoxic ($CD8^+$) T-cells are present. The death of the thyroid cells occurs through antibody-dependent complement-mediated mechanisms as well as due to the action of killer T-cells. The exact sequence of events and the detailed mechanisms governing each are not well specified, but our knowledge of these stages of thyroiditis will certainly improve as there is increasing ability to establish antigen-specific T-cell clones *in vitro* (Utiger, 1991; Champion *et al.*, 1992).

An important animal model of spontaneous autoimmune thyroiditis is the Obese strain (OS) chicken, a closed flock developed at Cornell University by R. K. Cole (Cole, 1966). The young chicks become hypothyroid within a few weeks of hatching. Iodination is crucial to the development of the spontaneous autoimmune disease, since treatment with compounds that interfere with iodination *in ovo* and in the first few weeks posthatching inhibits both the onset of thyroiditis and the appearance of thyroglobulin autoantibodies (Bagchi *et al.*, 1985, 1990). According to classical genetics, there are three independent lesions: a strong autoimmune response to thyroglobulin affected by a MHC class II gene and two other genes (Bigazzi and Rose, 1985). The autoantibodies appear to be produced by the B-cells that are located in the thyroid glands and not at other sites, like the bone marrow (Maczek *et al.*, 1992). When T-cells from OS chickens were transferred into Cornell strain chickens, the disease was also transferred (Kromer *et al.*, 1985). Recent reports indicate iodine supplementation is necessary for efficient induction of thyroiditis (Brown *et al.*, 1991).

Both Graves' disease and atrophic thyroiditis involve antibodies that affect TSH stimulation of the thyroid cells (Wilkin, 1990). In Graves' disease, a circulating antibody stimulates the receptor and this creates a hyperthyroid condition. In atrophic thyroiditis, antibodies bind to the TSH receptor without stimulating it, leading to hypothyroidism. Neither of these diseases have common canine analogues.

Autoimmune thyroid disease occurs more often in women than in men (Baker, 1992) and it is especially common as a transient postpartum episode that lasts less than a year (Amino *et al.*, 1982; Jansson *et al.*, 1988). It is not yet clear whether physiological stress triggers the episodes, as careful studies have come to opposite conclusions (Gorman, 1990; Winsa *et al.*, 1991). In one study with dogs, females were reported to be more affected than males (Milne and Hayes, 1981). Alaskan husky females on racing sled dog teams occasionally become transiently hypothyroid during heavy fall training following a sum-

mer pregnancy (L. Lowry, personal communication). It has been argued that female hormones tend to be associated with the induction of autoimmune disease and that male hormones are somehow protective (Ahmed *et al.*, 1985).

C. The Thyroid Autoantigens

The four principal targets of the thyroid autoantibodies in people are: (1) thyroglobulin, which is secreted into the follicle, (2) thyroid peroxidase, an integral protein on the apical plasma membrane, (3) the receptor for TSH, facing the blood on the basal membrane, and (4) a recently described 64 kDa autoantigen. The autoantibody against TSH receptor is found in hyperthyroid Graves' patients, and will not be discussed further since autoimmune *hyper*thyroid disease is not common in dogs. The 64-kDa antigen is common to both Graves' and Hashimoto's diseases, but it has not yet been shown to be pathogenic (Dong *et al.*, 1991) and it is expressed in multiple types of cells (Ross *et al.*, 1993). The first two of these autoantigens will be discussed in more detail as they have been strongly associated with hypothyroid disease.

In dogs, the principal circulating autoantibodies are against thyroglobulin (Haines *et al.*, 1984; Beale *et al.*, 1990; Thacker *et al.*, 1992, 1994; Gaschen *et al.*, 1993). Of 1057 dogs hospitalized at the Auburn University veterinary clinic with no clinically evident endocrine disorders, 13.2% showed antithyroglobulin autoantibodies by an ELISA test (Haines *et al.*, 1984). The incidence of such antibodies is higher in the patients with thyroid disorders; in several studies, approximately half of the hypothyroid dogs had autoantibodies to thyroglobulin (Haines *et al.*, 1984; Beale *et al.*, 1990; Thacker *et al.*, 1992; Gaschen *et al.*, 1993).

Antibodies to thyroglobulin in dogs commonly react also with T_3 and to a lesser extent T_4, producing spurious immunoassay results in clinical tests. The assays may overestimate or underestimate the true hormone concentrations, depending on the particular techniques used (Young *et al.*, 1985, 1991; Rajatanavin *et al.*, 1989; Thacker *et al.*, 1992).

Are most antibodies directed against epitopes in thyroglobulin that include iodinated tyrosines? Several studies suggest that iodination of thyroglobulin is required for the onset of autoimmune thyroid disease in many species (Brown *et al.*, 1991; Rayner *et al.*, 1993). Antithyroglobulin production seemed to be stimulated by the iodine supplementation given to children exposed to radiation in Chernobyl (Kinalska *et al.*, 1991). In contrast, recent evidence from a mouse model suggests that thyroglobulin epitopes need not be iodinated in this species (Carayanniotis *et al.*, 1994). Finally, nonhormogenic epitopes of thyro-

globulin must exist in dogs because some develop thyroglobulin autoantibodies without concurrent production of T_3 autoantibodies (Gaschen *et al.*, 1993). When all the conflicting evidence is considered, it remains to be demonstrated that thyroglobulin is strongly pathogenic in mammals or that the thyroglobulin autoantibodies produced by the B-cells are actually destructive to the thyroid gland; they may simply be consequences of the circulating debris produced by the attacks of the T-cells on the thyroid follicles.

The second principal autoantigen from the thyroid gland is thyroid peroxidase, which is present in the healthy thyroid as an integral protein on the basal membrane (toward the follicle). It is in fact the "microsomal antigen" that has been reported for many years in the blood of Hashimoto's patients (Baker, 1992) and is recognized by complement-fixing autoantibodies (Champion *et al.*, 1992). Injection of thyroid peroxidase can induce experimental autoimmune thyroiditis in mice. A pathogenic T-cell epitope on porcine thyroid peroxidase induces an autoimmune response in mice (Kotani *et al.*, 1992.). To date, two linear epitopes for B-cell recognition on human thyroid peroxidase autoantibodies have been reported (references in McLachalan and Rapoport, 1992). However, these are low affinity epitopes and not likely to be factors invoking the autoimmune disease. The true pathogenic epitopes are likely to be conformational and discontinuous, making their identification very difficult with current technology (McLachlan and Rapoport, 1992). The putative pathogenic role of antibodies to thyroid peroxidase remains in dispute (Bogner *et al.*, 1990).

McGregor (1992) states the antibodies to thyroid peroxidase are never significant in animals with *experimental* autoimmune thyroiditis (EAT). Furthermore, autoantibodies to thyroid peroxidase have not been demonstrated in a large group of dogs with spontaneous autoimmune thyroiditis (Thacker *et al.*, 1994).

Like most nucleated cells, thyroid epithelial cells produce MHC class I antigens, and in addition, can be induced to synthesize MHC class II antigens (Bottazzo *et al.*, 1983). Thus thyroid epithelial cells can present antigens not only to $CD4^+$ (helper) T-cells to promote differentiation of B-cells into plasma cells, but also to $CD8^+$ (cytotoxic) T-cells (Feldmann *et al.*, 1992; Weetman, 1992). The synthesis of HLA-DR class II antigens in thyroid cells is induced by the cytokine interferon-γ (IFN-γ) and enhanced by tumor necrosis factor (TNF-α) (Hanafusa *et al.*, 1983; Champion *et al.*, 1991; Asakawa *et al.*, 1992). The IFN-γ and TNF-α also induce production of cell adhesion molecules to which T-cells bind (Tolosa *et al.*, 1992). Since thyroid epithelial cells are themselves capable of synthesizing both IFN-γ and TNF-α (Zheng *et al.*,

1991, 1992), there may be autocrine regulation of adhesion of T-cells to the thyroid epithelium. Transforming growth factor-β (TGF-β) is a negative signal. When TGF-β is applied *in vitro,* it suppresses proliferation of T-cells from the thyroids of Graves' patients and inhibits class II expression by the cells of the thyroid epithelium (Widder *et al.,* 1992).

D. The Significance of MHC and TCR Polymorphism for Thyroid Disease

HLA associations with human thyroid disease have been reported by several laboratories. HLA-DRBI, -DRBII, -DQAI, and -DQB gene products may be involved in the onset or development of Hashimoto's disease (Farıd, 1991, 1992), but no associations appear to be very strong. Badenhoop and coworkers (1990) used RFLP to look for the association of HLA-DQB1 alleles with Hashimoto's disease and found a positive correlation with DQw7 and some correlation also with the adjacent DQA1. However, this association was not confirmed by the later work of Roman *et al.* (1992). The HLA-DR3 loci have often been implicated in the development of Graves' disease in Caucasians, but the tight linkage between haplotypes A1, B8, DR3, DRw52, and DQw2 makes it difficult to assess the true significance of each (Farid, 1992). In spite of these reports, several authors have argued that the expression of class II antigens does not initiate the autoimmune attack on the thyroid, but rather it amplifies an ongoing process (DeGroot and Quintans, 1989; McGregor, 1992).

T-cells are clearly involved in the pathogenesis of autoimmune thyroid disease (Davies *et al.,* 1991, 1992), and unusual lymphocyte subsets, including $CD4^+CD8^+$ and $CD4^-$ $CD8^-$ cells, are found in thyroids of patients with autoimmune thyroid disease (Iwatani *et al.,* 1993). Martin and Davies (1992) have reviewed the characteristics of the intrathyroid T-cells and find that the epitopes recognized by T-cells can be quite specific. After injection of thyroglobulin into mice, an EAT results and cytotoxic T-cells attack the thyroid follicle cells (Weigle, 1980). Champion *et al.* (1987) isolated two murine T-cell clones that recognize iodinated thyroglobulin but not thyroxine-deficient thyroglobulin. In a synthetic nonomer peptide, these T-cell clones fail to recognize thyroglobulin when the residue at site 2553 is tyrosine (or any other of the standard amino acids) but recognize 2553 when the tyrosine has been derivatized to thyroxine. The MHC class II molecules from the thyroids of these mice bind the nonomer and activate such T-cell clones *in vitro.* When transferred to naive recipient mice, the

activated T-cells induced thyroiditis in their new host (Hutchings *et al.*, 1992). This is the first case of a precisely defined pathogenic epitope in thyroglobulin that activates T-cells; it is especially interesting that the residues in thyroglobulin must be iodinated.

The associations of particular TCR allelic subfamilies with multiple sclerosis in humans (Beall *et al.*, 1989; Seboun *et al.*, 1989) suggested a similar relationship for autoimmune thyroid disease. By PCR amplification of the TCR gene transcripts for the α-chain and the β-chain, Davies and his colleagues concluded that of 18 possible families of α-chains for T-cell receptors, an average of only 4 were present in thyroid glands suffering autoimmune attack. The β-chain usage did not show such bias (mean 14.1 out of 19 families) (Davies *et al.*, 1991, 1992). However, another careful study failed to find any restriction in the TCR α-chains of intrathyroidal T-cells for patients with Graves' disease (McIntosh *et al.*, 1993). The question remains unresolved. However, an intriguing recent report implicates γδ T-cells in thyroid autoimmunity (Iwatani *et al.*, 1992) .

E. Environmental Factors

Iodine deficiency is classically linked to thyroid dysfunction. This is even more true of dogs than people since dietary iodine requirements are greater for dogs than for people. Dogs are less effective at conserving iodine and excrete it at a higher rate in the feces as well as being less efficient in its utilization in the thyroid gland (Belshaw *et al.*, 1975). Dietary iodine is important not only as a constituent of thyroid hormones but also because it modulates the autoimmune response. In Europe, regional and seasonal increases in dietary iodine are associated with increased autoantibodies to thyroid antigens and increased lymphocytic infiltration of the human thyroid gland (McGregor, 1992). Animal models likewise demonstrate the important contribution of iodine to thyroid autoantibodies and disease [Bagchi *et al.*, 1985 (chickens); Allen *et al.*, 1987 (rats); Cohen and Weetman, 1988 (rats); Braverman, 1990].

Selenium deficiencies affect thyroid hormone concentration at several levels. The most obvious need for selenium is as a component of the liver deiodinase which converts T_4 to T_3 (Beckett *et al.*, 1993; Berry and Larsen, 1993). In addition, selenium is required for intracellular glutathione peroxidase activity; thus low levels of selenium mean increased peroxide supply and perhaps greater hormone synthesis (Corvilain *et al.*, 1993).

One source of thyroid damage due to high iodine could be the excess

production of free radicals (Mahmoud *et al.,* 1986; Hall and Lazarus, 1987). If free radicals play a role in the early stages of thyroid disease, their reduction might afford protection. When the antioxidants ethoxyquin and butylated hydroxyanisole were included in the diet of the OS chicken, infiltration of the thyroid by lymphocytes, increases in autoantibodies to thyroglobulin, and the onset of the spontaneous thyroiditis were delayed. Weaker antioxidants like β-carotene afforded no protection (Bagchi *et al.,* 1990).

Infectious agents affect the onset of autoimmune disease. Parasitic infections have been linked to the breaking of T-cell tolerance to self-antigens (Röcken *et al.,* 1992; Röcken and Shevach, 1993). In animal EAT, sterilization of the gut protects against the development of the disease while restoration of the gut microorganisms increased the incidence (Penhale and Young, 1988). Normal gut pathogens have surface proteins that can bind TSH (Ingbar *et al.,* 1987). There is potential for molecular mimicry in which the immune response to that pathogen might trigger an autoimmune disease (Sinha *et al.,* 1990). A more direct association between infection and thyroiditis has been suggested by Belfiore *et al.,* (1991) who report that a viral infection triggered the local elaboration of IFN-γ, which induced HLA-DR expression on the surface of thyroid epithelial cells, rendering them susceptible to immune attack. Endogenous retroviruses are potential etiologic agents in autoimmunity (Krieg *et al.,* 1992). The role of retroviruses, which can infect and transform rat thyroid cell lines, is intriguing (Weetman and Borysiewicz, 1990; Wick *et al.,* 1993). Retrovirus-like sequences have also been demonstrated in Southern blots of DNA from the thyroid glands of humans with Graves' disease (Ciampoillo *et al.,* 1989).

VI. Future Research Applications

A. Research Directions for Future Work

1. DLA Histotyping

It would be very advantageous to have reliable and convenient methods for DLA typing. The potential is not only for studies of immune function but also for characterizing the various breeds and populations and for deducing the relationships among breeds and lines (Bull and Gerlach, 1992). The recent advances in HLA typing with molecular techniques will need to be applied to DLA typing. The initial base sequence for a DLA-DRB allele provided by Sarmiento and Storb

(1990) has been followed by sequences for the variable regions of more alleles of this gene as well as the sequences of other DLAs (Sarmiento *et al.,* 1990, 1992, 1993); these base sequences allow one to precisely define the primers for PCR-based typing techniques of DLA genes. The PCR-RFLP approach offers great promise for distinguishing among the DLA alleles. Development of the techniques is now in progress in our laboratory.

2. DLA Types and Canine Autoimmunity

The evidence from humans and mice convincingly links specific MHC histotypes with predisposition to spontaneous autoimmune reactions and autoimmune disease. As Day and Penhale (1987) argued, it is almost certain that a significant portion of the genetic predisposition toward autoimmune diseases in dogs lies in the genes of the immune system. It is important to identify the genes that are putatively responsible. Spontaneous canine thyroiditis offers promising opportunities to look for correlations between disease incidence and the DLA types. We are attempting such correlations by using PCR-RFLP to compare the DLA in euthyroid and hypothyroid dogs within the same family.

3. Improving Diagnostic Criteria

Diagnosis of thyroiditis is problematical in dogs because (1) only about one-half of the affected animals demonstrate thyroglobulin antibodies, (2) clinical assays for these canine antibodies are not currently available, and (3) only a few affected dogs have circulating anti-T_4 and/or anti T_3 antibodies (Beale *et al.,* 1990; Thacker *et al.,* 1992; Gaschen *et al.,* 1993). Once the diagnosis is clear, the clinical management of thyroiditis is comparatively simple; in veterinary medicine as in human medicine, supplementation with levothyroxine usually ameliorates the symptoms. It could be argued that since there are likely to be several genes that contribute to predisposition, the specification of each and the unraveling of their respective roles will be largely of academic interest and irrelevant to clinical management of the disease. However, when one considers the present ambiguity in the clinic, additional genetic criteria should add an important dimension for more accurate diagnosis and realistic prognosis.

4. Genetic Improvement of Breeds

Once the connections between particular alleles and the predisposition to autoimmune diseases are discovered, one could screen for carriers of deleterious genes in dogs that are considered for breeding. Most autoimmune diseases do not become patent until middle age, and even

then their deleterious impact often is expressed only when particular sets of environmental factors, including infection and dietary factors, coincide. When the breeding dog is young or when the impact of environmental factors is absent or delayed, "silent" carriers of potential defects are bred. The unfortunate consequences appear in the descendants. An effective genetic screen would allow many such breedings to be avoided. As more information on inheritance patterns is collected and correlated with the molecular biological information (Smith, 1994), selection to minimize genetic disease should become increasingly efficient.

5. DLA Diversity and Endangered Species

In humans, comparison between races or well-defined ethnic groups often blurs the association between a particular HLA allele and thyroid disease. The population structure of the domestic dog is profoundly different from human populations, yet some evidence indicates that the genetic differences between dog breeds is rather like that between human races (Jordana *et al.*, 1992). Canine thyroiditis is apparently widespread (Dodds, 1988), found in many registered breeds and in outbred Alaskan huskies. Comparative studies using simultaneously both the well-defined breeds and lines and outbred mongrel dogs will permit the evaluation of the importance of a particular allele or haplotype in gene pools of large or small diversity. The impact of a particular allele may be more substantial in small structured populations, such as are characteristic of many endangered species. Research on autoimmune predisposition in structured and unstructured dog populations should provide useful guidelines for assessing the risks and planning management strategies for preservation of particular dog breeds as well as endangered wild mammals, birds, and other vertebrates.

6. Therapeutic Immunosuppression

In experimental models, autoimmune disease can be prevented by tolerization to the initiating target antigen. For example, a single injection of GAD into the veins (Kaufman *et al.*, 1993) or the thymus (Tisch *et al.*, 1993) of 3-week-old NOD mice, just before the onset of the spontaneous IDDM, prevented both insulitis and diabetes. Similarly, in EAT of high responder mice, preinjection of soluble thyroglobulin tolerizes against subsequent thyroglobulin administration (Lewis *et al.*, 1992; D. C. Rayner cited in Champion *et al.*, 1992). Such a procedure might protect young dogs known to be genetically predisposed from developing the symptoms in later life. There are many other very

promising strategies for selective immunosuppression that are beyond the scope of this chapter (Champion *et al.,* 1992; Adorini *et al.,* 1993; Zhang and Raus, 1993, Matsumoto *et al.,* 1994).

7. Correction of Genetic Defects

One great promise of molecular medicine is the potential for gene replacement therapy. Such treatments have been successful for hematopoietic cell function in dogs (Karlsson, 1991). Even more dramatic is the recent partial correction of canine hemophilia B following insertion of a cloned, functional copy of the gene for canine factor IX via a retrovirus vector (Kay *et al.,* 1993). It has recently been shown that the canine thyroid follicular cell is a particularly convenient target for retroviral gene delivery (O'Malley *et al.,* 1993). With retroviral constructs, the regulation of the thyroid and the succession of events during the development of autoimmune disease could be probed in many new ways. Regimens for rescue from congenital thyroid disease might be developed (O'Malley *et al.,* 1993). The complexity of this autoimmune disease makes complete rescue unlikely in the near future, but partial rescue might provide new basic information on thyroid physiology and might permit interesting new therapies.

8. General Mechanisms of Autoimmune Pathogenesis

A broader argument can be made for the study of thyroiditis as a model autoimmune disease, for both canines and humans (McGregor, 1992). The autoantigens and their important epitopes involved in thyroiditis are being defined with increasing precision; the target organ is discrete and accessible, the pathogenic processes in the gland are well described, and there are excellent groups working on the human autoimmune disease as well as animal models. In addition, thyroiditis in genetically predisposed individuals (human or canine) may be triggered as a normal response to a foreign antigen, perhaps one sharing antigenic determinants with the self-antigen. Part of the interest in studying thyroiditis in people stems from its convenience as a model to understand the factors that precipitate general autoimmune response and to see what tips the balance between benign response and disease (McGregor, 1992). Thyroiditis is an excellent model for canine research for the same reason.

9. The Importance of the Genetic Context in Disease Development

Canis familiaris is a species that offers unique advantages as an experimental model to study the role of immunogenetics in the onset of autoimmune disease. One problem that plagues attempts to evalu-

ate combinations of human MHC and TCR alleles as candidates for production of autommune disease is the very noisy genetic context (e.g., Hibberd *et al.*, 1992). The diverse genetic contexts available for dogs present opportunities to reduce the noise. The profound morphological and physiological differentiation among the hundreds of dog breeds reveals the significant genetic heterogeneity present in the species. Each breed comprises a subset of the total gene pool and is in effect a closed population. Within many dog breeds, the relationships of lines and the breeding histories are well documented. A candidate gene or a combination of candidate genes can be examined in unique genetic contexts—lines and breeds that are already established and readily available. The same candidate genes can be assessed in outbred mongrels. From such comparative studies, one could evaluate individual alleles, combinations of genes, or even inbreeding as factors that predispose toward autoimmune reactions or protect against them. The lessons learned from dogs might apply to other mammals, including humans.

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ADVANCES IN VETERINARY SCIENCE AND COMPARATIVE MEDICINE, VOL. 39

Veterinary Medical Specialization

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I. Historical Reference to Human Medicine

Veterinary medicine is the art and science concerned with the health and disease of animals. Veterinary medicine has evolved from the barnyard art of treating animals to a sophisticated practice of medical and surgical science by highly trained specialists. Early images of the veterinarian who provided medical and surgical treatment for all animals have changed to the modern day veterinarian who may limit practice to either large or small animals or specialize in the treatment of medical or surgical diseases. Veterinary medical specialization had its beginnings with observation of the human medical specialties that developed in the nineteenth and early twentieth centuries at most medical schools. Veterinarians desired to replicate the practice of medical specialization, which was leading to major scientific advances and medical breakthroughs in human medicine.

Modern biomedical research is based on the concept of "one medicine," which suggests that human and veterinary medicine are based

on similar scientific premises (Schwabe, 1984, 1991). This concept and practice has risen and fallen many times in the modern medical era. It is noteworthy that the art of medical practice in most folk medical systems is truly "one medicine," as the same doctors tend to both humans and animals. Only in modern "Western" medicine is there a professional and legal distinction between veterinary and medical doctors. However, the gap between human and veterinary medicine was evident as early as 200 B.C.

Although veterinary medicine has evolved separately from human medical practice there are several excellent examples that prove biomedical research is truly based on "one medicine." There are very few biomedical advances that have resulted in improved human health without prior observation or experimentation on animals other than humans. The biomedical research community is dependent on animal experimentation to improve the health of all animals, including humans. There are several specialties with frequent interactions between veterinarians and physicians. Epidemiology and the characterization of zoonotic disease is probably the best example. Comparative biomedical research, particularly animal models of human disease, is a second example of successful interaction between human and veterinary medicine that benefits both humans and animals. Laboratory animal veterinarians have traditionally provided medical care for research animals at most medical schools. In some instances, these veterinarians have limited their role to providing animal care without taking an active part in the actual research. As veterinary specialization has become more sophisticated, veterinarians now have the training and expertise to actively participate in all facets of biomedical research (AVMA Council on Research, 1994). No one is trained to observe and evaluate comparative animal research better than a veterinarian.

Veterinarians must continue to receive "cutting edge" training in biomedical research to be competitive with physician-scientists and to take advantage of unique opportunities to characterize animal diseases of biomedical importance. Although it is necessary and important to study animal diseases solely for the benefit of animals, funding for animal disease research is relatively minor when compared to the funds available for biomedical research related to human health. It is therefore prudent and often a necessity to develop comparative biomedical research projects. Veterinary specialization and biomedical research interact synergistically to enhance the quality of life for all animals. This chapter will briefly review the history of veterinary medical specialization, outline general training requirements and certification procedures for board certification, discuss research and

training alternatives of board-certified specialists for academic tenure-track positions, and present an example of a new specialty in molecular medicine based on clinical applications of new technology in molecular biology and gene therapy.

II. Early Development of Veterinary Specialization

A. Introduction

The origins of specialization are difficult to precisely identify, but it has been suggested that the beginnings of specialization occurred in the last decades of the nineteenth century (Vaughan, 1991). A number of local and private short-lived veterinary colleges gave way to the mostly state-supported veterinary colleges that exist today (Bierer, 1980). The American Veterinary Medical Association (AVMA) Committee on Education was formed in 1931 and specified curricula for all veterinary colleges were defined in 1936 by the Council on Education (Stalheim, 1994). Pressures for formal development of veterinary specialization came from a more knowledgeable public that had seen specialization improve the quality of human health care. Additional pressure came from the profession itself and the desire of practitioners to provide superior veterinary care and to have formal recognition as veterinary specialists. Informal veterinary specialty groups and allied organizations established objectives, training requirements, and minimal qualifications, and elected charter diplomates at the inception of most specialty colleges (Brightman *et al.*, 1987; Knecht, 1990; Pettit, 1990; Marshak, 1992). The success of most specialty colleges is in large part due to the combined wisdom of the charter diplomates.

The first specialty boards were formally recognized by the AVMA in 1951 when the American College of Veterinary Pathologists and the American Board of Public Health were recognized. The American College of Laboratory Animal Science was recognized in 1957. The Advisory Board of Veterinary Specialties (ABVS) was created by the AVMA in 1960 to oversee the development of new veterinary specialties. A chronology of veterinary specialization is illustrated in Table I. The ABVS considers applications for new boards as well as encourages specialty services within the profession and to the public. There are currently 20 specialty boards recognized by the AVMA. Each specialty board defines specific objectives for that discipline as relates to the veterinary profession and establishes prerequisites for board examination and minimal criteria for passing the specialty board examination.

TABLE I

CHRONOLOGY OF AMERICAN VETERINARY SPECIALIZATION

Organization of U.S. Veterinary Medical Association (USVMA)	1863
First state organization—New York	1880
Establishment of the Bureau of Animal Industries (BAI)	1884
First State Practice Act—New York	1886
USVMA changed name to AVMA	1898
AVMA Committee of Education created	1931
AVMA assumes sole authority from BAI for accrediting veterinary colleges	1945
First specialty boards recognized by AVMA—American College of Veterinary Preventive Medicine; American College of Veterinary Pathology	1951
Veterinary specialties and residency training programs established in most veterinary colleges	1950s, 1960s
Advisory Board of Veterinary Specialties created by AVMA	1960
Twentieth specialty recognized by AVMA, American College of Veterinary Behaviorists	1993

B. SCIENTIFIC ADVANCES AND CURRENT STATUS

Veterinary medical specialists are the bridge between basic scientists and the general veterinary practitioner. Veterinary medical specialists provide a superior level of patient care, diagnostic support, or surgical treatment because of additional post-D.V.M. degree training in AVMA-approved residency programs and a commitment to excellence in their disciplines. There are currently 20 AVMA-recognized colleges and specialty boards with more than 4400 certified diplomates. The specialty boards recognized by the AVMA as of 1994 are anesthesiology, dentistry, dermatology, emergency and critical care, internal medicine, laboratory animal medicine, microbiology, nutrition, ophthalmology, pathology, pharmacology, poultry, practitioner, preventive medicine, radiology, surgery, theriogenology, toxicology, zoological medicine, and behavior (American Veterinary Medical Association, 1994). The specialty colleges, membership numbers, and the year each college was recognized by the AVMA are summarized in Table II. The approval and governance of specialty boards is by the AVMA through the ABVS. Approval of a specialty board has three broad criteria: (1) the specialty must be a distinct and recognizable facet of veterinary medicine; (2) the specialty must establish critical standards for admission to membership and abide by them; and (3) the specialty must be incorporated (Vaughan, 1986). The objectives, pre-

TABLE II

VETERINARY MEDICAL SPECIALTIES

Organizational title	AVMA recognition	Membership
American College of Veterinary Preventive Medicine[a]	1951	399
American College of Veterinary Pathology	1951	1012
American College of Laboratory Animal Medicine	1957	430
American College of Radiology	1962	153
American College of Microbiologists	1966	170
American Board of Veterinary Toxicology	1967	71
American College of Veterinary Surgeons	1967	497
American College of Veterinary Ophthalmologists	1971	145
American College of Theriogenologists	1971	233
American College of Veterinary Internal Medicine	1972	592
American College of Veterinary Anesthesiologists	1975	95
American Board of Veterinary Practitioners	1978	320
American College of Veterinary Dermatology	1982	68
American College of Zoological Medicine	1983	31
American Veterinary Dental College	1988	22
American College of Veterinary Nutrition	1988	33
American College of Veterinary Emergency and Critical Care	1989	28
American College of Veterinary Clinical Pharmacology	1990	16
American College of Poultry Veterinarians	1991	112
American College of Veterinary Behaviorists	1993	8

Note. The membership totals are taken from the 1994 AVMA Membership Directory and Resource Manual.

[a] Originally recognized by AVMA as the American Board of Veterinary Public Health in 1951. The name was changed to the American College of Veterinary Preventive Medicine in 1978.

requisites for examination, examination procedures, and diplomate lists for each specialty are published in the annual AVMA Membership Directory and Resource Manual (1994). An abbreviated list is presented in Appendix I to this chapter. In general, the specialties strive to advance competency in the disciplines, provide didactic and practical training in that area, and administer an examination to establish minimal competency for specialty title. Additional goals of the specialty colleges are to encourage continuing education for professional ad-

vancement and to encourage research and dissemination of knowledge relating to the diagnosis, therapy, and pathogenesis of animal diseases.

Traditionally, veterinary specialists have been primarily employed by Colleges of Veterinary Medicine, governmental agencies, industry, and large specialty practices. However, as available positions at Colleges of Veterinary Medicine and other public institutions have dwindled, the number of specialists in private practice currently exceeds or will shortly exceed specialists in public practice for most specialties (Rawlings, 1994). The changing demographics have enhanced the quality of veterinary medicine available to the animal-owning public and is changing the practice of veterinary medicine from a "generalist era," in which a veterinarian treats and prescribes for all species and performs basic surgical procedures, to the "specialists era," in which treatment is limited to certain species (e.g., companion animal, food animal, equine, avian) or types of practice (e.g., dermatology, surgery, internal medicine). A three-tier system of veterinary practice will be firmly established as these demographic changes continue. The tiers will involve general practitioners, specialty referral practices, and university or large group practices that function as teaching hospitals, and tertiary referral clinics. This system has thrived and prospered in human medicine for many years and should result in a better quality of veterinary medicine as well. However, as specialty practice evolves into a largely non-university-based occupation, prerequisites and examination procedures for specialty boards will also change. For example, university-based residencies traditionally have required a research project as part of the residency training program to stimulate scholarly achievement.

Tenure in a university environment is correctly based not only on clinical service but also on scholarly achievement as evidenced by advancement of the discipline through publications, research grants, and continuing education lectures, and not just the application of one's knowledge to clinical service and teaching (Marshak, 1993; Tasker, 1993). University tenure requirements are not identical to specialty board requirements, and it can be argued in a legal sense that "time off from clinics" for research impedes rather than contributes to residency training. Residency training programs and certification requirements have done an excellent job of providing superbly trained specialists in the past and this should continue in the future. However, the nature of the training program and examination process must adapt to satisfy the changing demographics (Curtin, 1986; Council on Education of the American Veterinary Medical Association, 1992;

McGregor, 1992). This will result in little change for nonclinical specialties, but clinical practice specialties whose primary goal is to provide primary veterinary care must modify residency programs to train individuals for private specialty practice rather than a university-based practice, as was done in the past. These changes should not be viewed as undesirable as they should improve the overall quality of veterinary medicine available to the public. Clinical specialists desiring a university tenure-based academic position will need additional training other than a clinical residency, because the latter training does not necessarily prepare one to function critically as a university professor and scholar. Postresidency research fellowships and Ph.D. programs will be necessary to compete for university tenure-based positions in academic medicine (Curtin, 1986; Council on Education of the American Veterinary Medical Association, 1992; McGregor, 1992). Clearly, specialty organizations have been a major influence in raising the standards of veterinary medicine as practiced today. Training programs will continue to evolve as societal demands change, and new specialties will be recognized as new technology and outside pressures change the scope of veterinary practice.

C. Qualifications and Standards

Residency programs have established defined minimal criteria for admission that include a D.V.M. or equivalent degree. An internship or other postprofessional training may be required, and applicants should have satisfactory moral standards (American Veterinary Medical Association, 1994). A national standard "matching program" pairs intern and resident candidates with available positions. Most veterinary colleges, public institutions with veterinary training programs, and several large specialty practices participate in the matching program. The matching program is legally binding to the institution and the candidate, and prevents unfair competition and last-minute changes in the selection process. The total duration and extent of time proportioned between clinical training, research, and teaching is determined by each specialty board. The clinical specialties frequently require 24 months of actual clinic duty under the direct supervision of one or more board-certified diplomates following a 12-month internship or similar practice experience. In addition to the standard training programs, most specialty boards have established separate but comparable service requirements for nonconforming residency programs so as not to eliminate noninstitutional specialty practices from residency training, to make residency training available to nonstandard candi-

dates, and to fill training slots not participating in the matching program. Combined M.S. degree/residency programs exist at some institutions in an attempt to meet university academic as well as board-certification requirements. There is no evidence to indicate that M.S. degree residents are more or less qualified than non-M.S. degree residents. The M.S. degree is not a terminal degree for most academic disciplines and probably does not enhance academic credibility.

The goals of most clinical residency and Ph.D. programs are sufficiently different that neither is an adequate substitute for the other. Clinical specialists who desire research training for professional goals and academic competitiveness can obtain additional training in a Ph.D. program or postdoctoral research fellowship. Didactic training does not always equate with scientific competence. Developing characteristics and abilities to become a productive scientist requires research laboratory mentoring just as becoming an accomplished surgeon requires practical operating room experience. Most Ph.D. programs do not prepare one to be a productive scientist without a subsequent postdoctoral fellowship, and most board-certified specialists committed to academic medicine would be better trained as research fellows or clinician-scientists. The research fellowship program has been used very successfully by the medical profession for many years to help clinicians with the transition to becoming productive scientists. Academic institutions should initiate similar programs for clinical veterinary specialists. Nonclinical specialties such as pathology, laboratory animal medicine, and toxicology have effectively combined residency training and Ph.D. programs for many years to produce board-certified specialists who are nationally competitive in biomedical research. The ability to successfully combine residency and Ph.D. programs is in part due to the laboratory focus of the disciplines and the minimal to no primary clinical duty requirements.

Additional requirements such as case reports, first-author publications, and years of service beyond the formal residency vary by specialty board. A formal certification examination is the final requirement for specialty board certification. In a legal sense, the law states that any certification process must be rationally related to the stated purpose of the process (Stromberg and Schneider, 1994). With regard to veterinary specialty boards, the certification examination should measure competence in that specialty. Requirements that are neither demonstrably nor rationally related to practitioner competence may be illegal. Written and oral exams that test knowledge and practical experience in a specialty are appropriate. Prerequisites such as case re-

ports, first author publications, and research time away from clinical training may not be legally supportable since they do not directly measure practitioner clinical competence. There has been considerable discussion within the medicine specialties regarding the use of case reports in the minimum certifying credentials. The volley of Letters to the Editor describing the merits and biases of using case reports continues (Breitschwerdt, 1991; Center *et al.*, 1991; Green, 1991; Padrid, 1993; Brown, 1992). A consensus is unlikely due to the varied backgrounds, priorities, and expertise of the diplomates. Recently, an article describing a legally defensible due process for specialty board examination certification was published in the AVMA journal (Stromberg and Schneider, 1994). This article may serve as a blueprint for future changes in "veterinary specialties."

A considerable concern of candidates for specialty board certification is the actual examination and the process for determining a passing score. Validation procedures have been established by specialty boards in an honest attempt to make the certification examination as fair as possible (White, 1986). The examinations for most specialties have written and oral knowledge tests, practical clinical skills tests, and may or may not have subjective essay questions to evaluate in-depth knowledge and communication skills. Each specialty determines the passing score, which can vary from year to year. However, since each specialty determines its own passing score, a potential conflict of interest exists. Membership in a specialty board is a valuable economic and academic credential inversely related to the number of diplomates. Validation does not mean a test is without flaws, but indicates that a passing score is somehow related to the performance of board-certified diplomates that took the examination that year. The "pass point" is then determined based on the diplomates' performance, a statistical evaluation of the objective questions, and the subjective evaluation of the essay questions. Validation should be required of all specialty boards to make the examination legally defensible and as fair as possible. As if the issue of certification were not difficult enough, recertification for some specialties has been proposed.

D. Integrating Scientific Findings into Specialty Practice

Academic veterinary specialists are expected to provide superior clinical care, supervise professional and residency training programs, and improve the quality of veterinary medicine through clinical and laboratory research into animal diseases. Residency training programs

adequately prepare candidates to provide superior clinical care but are not designed to ensure competence in biomedical research. Academic veterinary specialists are in the ideal environment for biomedical research in animal diseases due to the large number of clinical specialists and basic scientists available for interaction, as well as top-quality research and clinical facilities. However, veterinary specialists in academic practice must have adequate research training to be nationally competitive for declining research funding. Although national competitiveness and extramural funding do not guarantee top-quality research, the peer-review process has time and again been shown to improve the quality of biomedical research through the process of criticism and revision. Academic veterinary specialties must take a proactive position for research in veterinary medicine so that the status of veterinary medical research in the biomedical research community is improved. Just as clinical specialty training is best achieved through an apprenticeship/mentoring program, becoming a competitive independent investigator in biomedical research is best achieved through a mentoring process with an experienced scientist. A research training program can be accomplished by several mechanisms: (1) Ph.D. degree program, (2) postdoctoral fellowship, and (3) faculty sabbatical. Regardless of the programmatic mechanism, the research training must include: (1) the development of an hypothesis for an important biomedical problem, (2) experimental design and laboratory research to test the central hypothesis, and (3) communication of the results for peer evaluation. Grant writing skills should be emphasized and practiced as the ability to convince funding agencies and peer-review panels to support original research is a requisite for success in modern biomedical research. A minimum of 80% effort should be devoted to full-time research in any postdoctoral research training program. Any less effort will impede the development of the research skills necessary to become an independent investigator.

The D.V.M. or equivalent degree is recognized as a terminal degree similar to the M.D., D.D.S., D.O., and Ph.D. degrees by most grant-funding agencies. It is not absolutely necessary to have a D.V.M. and a Ph.D. degree to be eligible for extramural grant awards. The most important criteria for a successful research grant application are a qualified investigator with appropriate training, a testable hypothesis to investigate an important but well-focused biomedical problem, and adequate preliminary data and experimental design to accomplish the proposed aims (National Heart, Lung, and Blood Institute, 1991). A key ingredient in a successful grant application is the background and research training of the investigator.

Research training can come before or after specialty board training, but regardless, it should be a practical research experience mentored by a successful scientist. There are several mechanisms to support postresidency research training besides institutional resources (Table III) (National Heart, Lung, and Blood Institute, 1991, 1993; AVMA Council on Research, 1994). Funding is available through U.S. Department of Agriculture (USDA) and National Institutes of Health (NIH) training programs. The USDA provides funding for postdoctoral fellowships through the National Research Initiative Competitive Grants Program. The NIH provides funding through postdoctoral fellowships (F32, T32, T32M), Clinical Investigator Development Awards (K08), the First Independent Research Support and Transition Award (R29), and the Intramural Research Training Award. The Comparative Medicine Program, National Center for Research Resources provides fellowship programs in laboratory animal science, but this can be broadly interpreted to include animal models of human disease (Bennett, 1994). The Special Emphasis Research Career Award, which is also administered through the Comparative Medicine Program, provides for extended research training support similar to the Clinical Investigator Development Award. Regardless of the type of support, the goals of these programs are to encourage research-oriented physicians and veterinarians to develop independent research skills and gain experience in advanced methods as well as experimental approaches in the basic and applied sciences, in order to address important basic biological and clinical questions.

Academic veterinary specialists should be encouraged and rewarded appropriately for the special efforts required to obtain extramural research awards. Postresidency fellowship programs should be established for clinical specialties to encourage scholarly endeavors and provide much needed research training programs. The veterinary profession has traditionally been poorly funded by the biomedical research community, in part due to the vocational attitude of the profession in the past. An increased number of well-trained specialty researchers are needed to serve as role models for future candidates and to provide a good environment for research training. Every clinician cannot also be a researcher, but a department with a balanced mixture provides a better environment than a department with either alone. Medical school clinical departments have demonstrated repeatedly that the most successful research emphasis programs, program projects, and clinical centers combine clinical and basic science expertise in the same group. Similarly, if the quality of veterinary biomedical research and therefore veterinary medical care is to improve,

TABLE III

RESEARCH TRAINING AND CAREER DEVELOPMENT MECHANISMS AVAILABLE TO VETERINARIANS

Career stage	Funding agency				
	Institutional	USDA[a]	NIH[b]	HHMI[c]	AHA[d]
Predoctoral	Individual investigator; departmental funds	—	Summer fellowships	Predoctoral fellowships	Summer fellowships
Postdoctoral	Individual investigator; departmental; special emphasis programs	NRICGP[e]	NRSA; clinician scientist	Postdoctoral fellowship; clinician scientist	Postdoctoral fellowship; clinician scientist
Faculty	Departmental; college; university	—	Clinician scientist; RCDA[f]	Medical investigator	Established investigator

[a] United States Department of Agriculture.
[b] National Institutes of Health.
[c] Howard Hughes Medical Institute.
[d] American Heart Association.
[e] National Research Initiative Competitive Grants Program.
[f] Research Career Development Award.

academic veterinary specialty groups must support discipline-based research enthusiastically. In this manner, veterinary academic leaders of the future will be productive scientists as well as competent clinicians.

III. Future Trends and Recommendations

A. Molecular Medicine

New veterinary medical specialties have developed due to internal pressures within the veterinary profession to provide expertise in defined disciplines, the public demand for superior clinical care for pets and other animals, and because of advances in technology. There have been revolutionary advances in molecular biology and biotechnology in the last few decades that have led to the development of new diagnostic methods, identification of new disease genes, pathogenic description of diseases in molecular terms, and finally, the treatment of diseases by gene therapy. The description of disease in molecular terms has been called "molecular medicine." Molecular medicine is the application of molecular biology to internal medicine (Bjorntorp *et al.*, 1991; Caskey and Rossiter, 1992; Pyeritz, 1992). Although we may not realize it, molecular medicine is already having major impacts on clinical practice. Application of new technological advances to clinical practice should be embraced by veterinary specialists.

The formal initiation of the human genome project will hasten the identification of disease genes in humans, which will likely transfer to improved understanding of disease genes in animals. Gene maps for domestic and companion animals are now being developed based on progress in the human and murine genome projects. Advances in cell biology and molecular biology techniques such as the polymerase chain reaction (PCR), production of monoclonal and anti-idiotype antibodies, development of viral and nonviral vectors for gene therapy, identification of intracellular signal transduction pathways, and defining cytokine networks that coordinate immune and inflammatory responses are but a few of the many advances in cell and molecular biology that now make understanding of diseases in molecular terms a reality. Although this improved molecular understanding may not directly correlate with new treatments for previously incurable disorders, it is certainly a major step in that direction.

B. Gene Therapy

Gene therapy will change the way most medical and surgical diseases are treated by the middle of the twenty-first century. Diagnostic plans and treatment strategies that seem advanced today will be antiquated by newer methods in molecular medicine and gene therapy. A veterinary medical specialty in molecular medicine and genetics will develop to provide clinical competence in molecular diagnosis of diseases and gene therapy. Gene therapy is the hallmark of molecular medicine.

Molecular medicine and gene therapy have been the goal of medical genetics ever since the genetic basis of inheritance was recognized. Most diseases, whether genetic or acquired, medical or surgical, metabolic or infectious, result from some change or mutation in DNA, expression of normal genes in inappropriate amounts or places, or elimination of normally protective genes. Since most disease processes originate with alterations in gene expression, it is only logical to strive to treat a disease at the site of origin rather than treat the symptoms that occur "downstream" of the primary abnormality. Defining disease in molecular terms, gene cloning, and the development of efficient vectors for gene delivery now make gene therapy possible.

Defining important veterinary diseases in molecular terms should quickly lead to the elimination of most inherited diseases by selective breeding. Bovine leukocyte adhesion deficiency is the first important genetic disease of domestic animals in which molecular genetic analysis has been used for diagnostic and selective breeding purposes (Shuster *et al.*, 1992). This problem has all but been eliminated from the Holstein breed using this strategy. Genetic eye and blood diseases of dogs are additional examples in which molecular medicine techniques have been used to identify breed-specific genetic defects and develop DNA diagnostic tests (Ray *et al.*, 1994; Whitney *et al.*, 1994; Whitney and Lothrop, Jr., 1995). Similar examples exist in all facets of veterinary medicine, and three examples are illustrated below.

1. Bovine Granulocytopathy

Bovine granulocytopathy, first described in 1983, was recognized as a disease syndrome causing chronic infections, lymphadenopathy, weight loss, hyperglobulinemia, and a characteristic leukemoid response (Gilbert *et al.*, 1993). Pedigree analysis of Holstein cattle of American descent in Japan suggested an autosomal recessive mode of inheritance (Takahashi *et al.*, 1987). In 1990, a deficiency in the Mac-1 (CD11b/CD18) glycoprotein was identified as the basis for defective

neutrophil function in affected Holstein cows (Kehrli *et al.*, 1990). A prominent bull was also identified as a carrier, which resulted in widespread transmission among Holstein cattle throughout the world.

Leukocytes express a family of cell surface molecules called β_2-integrins, which are vital in cell-to-cell and cell-to-substrate adhesion. The β-integrins are now termed CD11/CD18. Bovine leukocyte adhesion deficiency (LAD) is caused by an aspartic acid to glycine substitution at amino acid 128 in CD18, a β-integrin (Shuster *et al.*, 1992). A polymorphism was first identified in the CD18 locus in affected animals, which suggested CD18 was a likely candidate gene. Previously, several mutations that caused LAD in human beings had also been localized to the CD18 gene. A PCR-based diagnostic test was developed on the basis of the DNA polymorphism, which permitted identification of carrier animals in blood and frozen semen. The carrier frequency for LAD was determined to be 15% for bulls and 6% for cows, all related to the single founder bull. Bovine LAD had been estimated to cost the dairy industry at least 5 million dollars in annual losses in the United States alone. The dairy industry has used the molecular diagnostic tests to essentially eliminate this disease from dairy cows and bulls. All Holstein pedigrees now indicate whether a bull carries the LAD trait.

2. Progressive Retinal Atrophy

Progressive retinal atrophy (PRA) is an inherited disease seen in several different breeds of dogs (Ray *et al.*, 1994). Both autosomal recessive and sex-linked forms of retinal degeneration have been described. There are several nonallelic loci that cause PRA (Table IV).

TABLE IV

CANINE PROGRESSIVE RETINAL ATROPHY

Breed	Locus	Inheritance[a]	Gene
1. Irish setter	rcd 1	A	PDEB
2. Collie	rcd 2	A	?
3. Alaskan malamute	cd	A	?
4. Norwegian elkhound	erd	A	?
5. Poodle	prcd 1	A	
Labrador	prcd 2	A	?
Cocker spaniel	prcd 3	A	
6. Husky	XLPRA	X	?

[a] A, autosomal; X, X-linked.

Among the hereditary retinal disorders in human beings, mutations are frequently in one of three loci—rhodopsin, cGMP phosphodiesterase β(PDEB), and peripherin-RDS. A mutation in the PDEB gene has recently been identified as the cause of PRA in Irish setters (Ray *et al.*, 1994). The disease results from abnormal development and subsequent degeneration of rod photoreceptor cells. The pathologic changes are preceded by a tenfold increased accumulation of cGMP in the retina (Aguirre *et al.*, 1982). Dogs with rcd 1 have a nonsense mutation at nucleotide position 2420 of the gene. The G-to-A transition segregates perfectly with the rcd 1 phenotype. DNA diagnostic tests based on PCR amplification of exon 21 have now been developed to permit carrier detection (Ray *et al.*, 1994). The other genetic forms of canine PRA are apparently due to mutations in genes other than PDEB. Unique diagnostic tests must therefore be developed for each dog breed.

3. Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency is an autosomal recessive disease of the basenji, West Highland white terrier, Cairn terrier, beagle, and poodle breeds of dogs. The disease causes a moderate to severe, chronic, regenerative, nonspherocytic, hemolytic anemia due to impaired red blood cell glycolytic metabolism. Erythrocytic energy depletion causes markedly shortened red blood cell circulating lifespan. Recent studies have shown that a single base deletion of the pyruvate kinase L-gene (LPK) causes PK deficiency in basenji dogs (Whitney *et al.*, 1994). The deletion of cytosine at nucleotide position 433 of canine R-type PK cDNA predicts a translational frameshift and premature termination codon resulting in a truncated mutant protein lacking enzymatic activity. Measurement of erythrocytic PK activity has been used in evaluating dogs suspected to have PK deficiency. Considerable confusion in diagnosis of the disease has existed due to the paradoxical increase in erythrocytic PK activity in many affected dogs. This is attributed to pronounced reticulocytosis and to the abnormal expression of the M_2-type PK isozyme in place of the mutated R-type PK (Whitney *et al.*, 1994). Dogs having PK deficiency anemia may manifest increased, normal, or decreased erythrocytic PK activity. Absolute confirmation of PK deficiency anemia therefore requires demonstration of increased glycolytic intermediate concentrations or demonstration of altered erythrocytic PK activity. Aberrant expression of the M_2-type PK isozyme is responsible for the altered PK activity reported in PK-deficient beagles, West Highland white terriers, and Cairn terriers, as well as basenjis (Whitney *et al.*, 1994).

Carriers of PK deficiency present a particular diagnostic challenge because they show no clinical signs of disease and demonstrate no changes in standard hematologic parameters. Dogs heterozygous for the trait have been identified by demonstration of erythrocytic PK activity levels approximately half those of normal dogs. However, in some instances it is difficult to distinguish normal and carrier dogs by enzyme activity, and isozyme characterization and glycolytic intermediates are not helpful in detecting the carrier state. Test breedings with known heterozygotes are impractical and informative only when affected progeny result. Identification of the genetic basis for PK deficiency allowed the development of a DNA test for the trait based on the loss of an *AciI* restriction enzyme site in the mutant allele (Whitney and Lothrop, Jr., 1995). A molecular test is now available for basenji dogs (Fig. 1). The genetic test, now the gold-standard for the basenji breed, will not work with the other breeds because the mutations are different. Diagnostic techniques such as single strand conformational polymorphism (SSCP) and allele-specific hybridization should permit genetic diagnosis in other dog breeds by using the normal gene sequence and without having to identify breed specific mutations.

As more and more diseases are defined in molecular terms, it will be necessary to have qualified individuals who can request and interpret the appropriate molecular test, and subsequently develop the best treatment or breeding-plan based on the molecular diagnosis. Specific treatments for cancer will be designed to eliminate or inhibit specific oncogenes that have been activated in neoplastic cells, viruses will be treated with antiviral nucleic acids, surgical wounds will be healed with growth factor genes as well as surgical sutures, and immune diseases will be treated with specifically designed anti-idiotype antibodies (Pyeritz, 1992; Mulligan, 1993).

4. Gene Delivery

Genetic treatment of disease by gene delivery is in its infancy but nevertheless is now a reality. There are more than 60 human gene marking and gene therapy trials in progress. Limited gene therapy trials for cancer treatment of companion animals have begun at several veterinary institutions. A variety of viral and nonviral vectors can be used for gene delivery (Table V) (Mulligan, 1993). The retroviral and adenoviral vectors are currently the best developed vectors, but significant advances in other vector systems are rapidly occurring. Adenoviral vectors have been used to express the coagulation factor IX

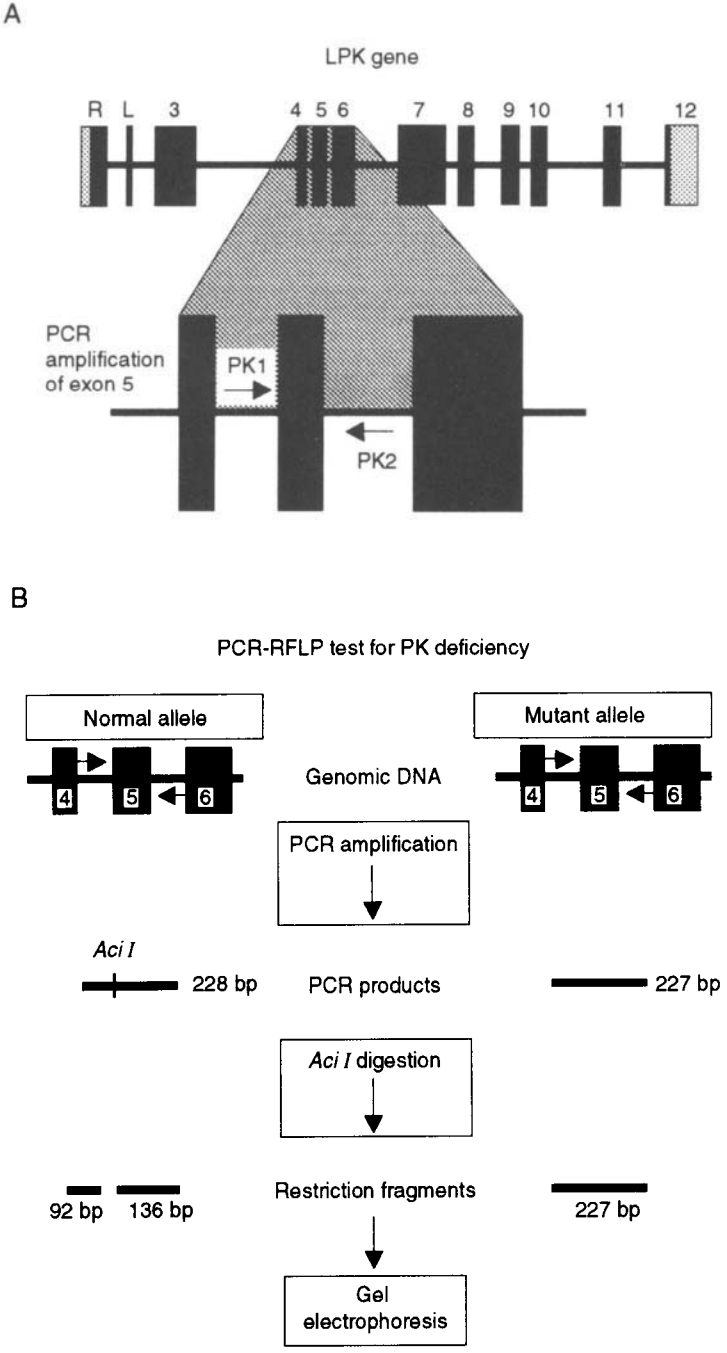
A
LPK gene
R L 3 4 5 6 7 8 9 10 11 12
PCR amplification of exon 5
PK1
PK2
B
PCR-RFLP test for PK deficiency
Normal allele
Mutant allele
4 5 6
Genomic DNA
PCR amplification
Aci I
228 bp
PCR products
227 bp
Aci I digestion
92 bp
136 bp
Restriction fragments
227 bp
Gel electrophoresis

TABLE V

CHARACTERISTICS OF GENE THERAPY VECTORS

Vector	Genome size (kb)	Insert size (kb)	Integration	Infections	Requires dividing cells	Stable
1. Retrovirus	11	7–9	+	+	+	+
2. Adenovirus	36	10–20	–	+	–	–
3. Adeno-associated virus	4	3	+	+	–	+
4. Herpes	150	>30	–	+	–	–
5. Liposomes	NA[a]	>20	–	–	–	–
6. Molecular conjugate	NA	>50	–	–	–	–
7. Naked DNA	NA	NA	–	–	–	–

[a] NA, not applicable.

(FIX) gene in dogs following intravenous administration of the vector construct (Fig. 2) (Mauser and Lothrop, Jr., 1995). Plasma FIX activity disappears over time due to immunologic elimination of hepatocytes that express adenoviral proteins as well as the FIX transgene. Second generation adenoviral vectors that express only the transgene must be developed to obtain longterm persistence of vector-transduced cells. Longterm and tissue-specific gene expression must be achieved for gene therapy to realize its full potential.

It is an exciting time for human and veterinary medical specialists alike. The potential to diagnose and treat incurable diseases is now within our grasp. Future specialists in molecular medicine will use

FIG. 1. PCR-RFLP test for PK genotype of basenji dogs. A. Oligonucleotide primers (arrows) used in PCR amplification of the canine pyruvate kinase L gene are shown in relation to a schematic representation of the exons (numbered boxes) and introns (intervening solid lines) of the region of the gene containing the basenji PK mutation. PK1, complementary to sequence contained in intron 4 and PK2, complementary to sequence in intron 5 define a segment of genomic DNA which includes the single nucleotide deletion of exon 5 in mutant alleles. B. Comparison of results of PCR-RFLP from normal and mutant genomic DNA. PCR amplification of the normal canine PK allele using primers PK1 and PK2 results in a 228 bp product containing the *AciI* restriction endonuclease site. The mutant allele yields a 227-bp amplified product lacking the *AciI* restriction site due to the deleted nucleotide. *AciI* digestion specifically cleaves the normal product into 92- and 136-bp fragments, but the mutant product is not cut. DNA fragments resulting from *AciI* treatment are visualized following agarose gel resolution.

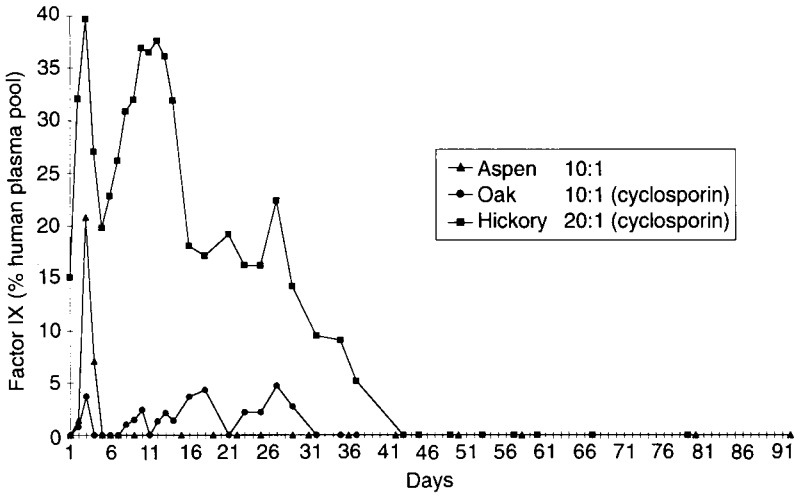

FIG. 2. Human factor IX production in normal dogs after *in vivo* adenoviral gene transfer. Levels as determined by enzyme-linked immunosorbant assay specific for human factor IX are represented as percent of normal human plasma levels for the indicated days following cephalic vein infusion of recombinant adenovirus. Dogs received replicative defective virus at multiplicities of infection (transfecting particles per hepatocyte) of 10:1 or 20:1 as indicated. Two dogs received immunosuppressive doses of cyclosporin, as indicated.

recombinant proteins and genes to treat diseases of man and animals that are considered fatal at the present time. Veterinary medical specialists will always provide superior animal care and contribute to an improved understanding of the basic biology of animals.

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Appendix I

AVMA Recognized Specialty Boards (Abstracted from 1994 AVMA Membership Directory and Resource Manual)

1. AMERICAN COLLEGE OF VETERINARY PREVENTIVE MEDICINE

Objectives

1. Advance the science and art of the specialty of veterinary preventive medicine.
2. Establish standards for experience and training for the qualification of veterinarians in the specialty of veterinary preventive medicine.
3. Provide continuing education for members in all disciplines and practice categories of preventive medicine at national, regional, and state levels.

Prerequisites for Certification

1. Have a total of 6 years experience beyond the D.V.M. (or equivalent) degree in one or more areas of preventive medicine of which up to 3 years may be a formal education program.
2. Be sponsored by a Diplomate (in good standing) of the College.

Examination Procedure

A candidate must apply to take the examinations by completing and submitting the appropriate application form with the application/examination fee. The forms and detailed instructions must be requested, in writing, from:

American College of Veterinary Preventive Medicine
Dr. Stanley O. Hewins
Executive Vice President
3126 Morning Creek
San Antonio, TX 78247
1-800-374-4944
210-524-3944 (Fax) (daytime only)

2. AMERICAN COLLEGE OF VETERINARY PATHOLOGISTS

Objectives

1. To further scientific progress in the specialty of veterinary pathology.
2. To establish standards of training and experience for qualification of specialists in veterinary pathology and veterinary clinical pathology.
3. To further the recognition of such qualified specialists by suitable certification and other means.

Prerequisites for Certification

1. Complete 4 calendar years of professional training in veterinary medicine subsequent to graduation from veterinary school; at least 3 of which shall be supervised training in pathology acceptable to the Council, and 2 of these shall be supervised training in the field in which certification is being sought.
2. Submit to the Council a detailed statement of qualifications, including references of any publications and other evidence of professional experience and competence.

Examination Procedure

Candidates for certification as "Veterinary Pathologist" will be examined in four areas of the discipline: (1) general pathology, (2) gross pathology, (3) microscopic pathology, and (4) veterinary pathology. The fourth portion is subdivided into: (a) large animal pathology, (b) dog and cat pathology, (c) laboratory animal, poultry, and wildlife pathology, and (d) clinical pathology.

Candidates for certification as "Veterinary Clinical Pathologist" will be examined in (1) general pathology, (2) hematology, (3) clinical biochemistry, and (4) cytology surgical pathology (CSP).

Ms. Coley Lyons, Executive Director
875 Kings Highway
Suite 200
West Deptford, NJ 08096
609-848-7784 (Office)
609-853-0411 (Fax)

3. AMERICAN COLLEGE OF LABORATORY ANIMAL MEDICINE

Objectives

1. To encourage education, training, and research in laboratory animal medicine.

2. To establish standards of training and experience for qualification of specialists in this field.
3. To recognize qualified specialists by certification.

Prerequisites for Certification

1. a. Have made application on a form provided and approved by the organization, and have paid the certification fee.
   b. Have completed the following training and/or experience requirements:
      i. Have completed a formal laboratory animal medicine training program and have a minimum of 4 years of combined training and experience in laboratory animal medicine following receipt of the veterinary medical degree, as approved by the Credentials Committee, or
      ii. Applicants may qualify to take the examination after 6 years of full-time experience in laboratory animal medicine, as approved by the Credentials Committee.
   c. Have had an article on some phase of laboratory animal medicine accepted for publication in a refereed journal.
   d. Have successfully completed comprehensive written and practical examinations and have satisfactory moral and ethical standing in the profession.
2. Candidates shall not be eligible to take the written or practical examinations until they have completed the application, training and/or experience, and publication requirements.
3. Certification of successful candidates shall require a majority affirmative vote by the Board of Directors.

Examination Procedure

Comprehensive written and practical examinations will be administered by the College.

Dr. Charles McPherson, Secretary-Treasurer
200 Summerwinds Drive
Cary, NC 27511
919-859-5985 (Office)
919-851-3126 (Fax)

4. AMERICAN COLLEGE OF VETERINARY RADIOLOGY

Objectives

To advance the art and science of radiology by:

1. Protecting the public against incompetence in the practice of veterinary radiology by conducting investigations and exam-

inations to determine the competence of voluntary candidates for certificates issued by the College.
2. Conferring certification upon candidates who have successfully demonstrated their proficiency in the field of veterinary radiology.
3. Encouraging the development of teaching personnel and training facilities in veterinary radiology.
4. Aiding in the evaluation of residencies and fellowships in the field of veterinary radiology under consideration by the Council on Education of the American Veterinary Medical Association.
5 Advising veterinarians who desire certification in the field of veterinary radiology as to the course of study and training to be pursued.

Prerequisites for Certification

1. Have satisfactorily completed an organized advanced program in Veterinary Radiology, as specified and approved by the Executive Council of the ACVR.
   a. Have supervised residency training in a program approved by the Executive Council for a minimum of 36 months.
   b. Submit evidence of qualifications that are judged by the Executive Council as being comparable to the requirements listed in paragraph (a) above.
2. Submit to the Executive Council a statement of his/her qualifications, including evidence of his/her professional experience and competence. Answer in detail a questionnaire presented by the Executive Council.
3. Submit names for letters of reference of his/her personal and professional competency, including a letter from his/her sponsor(s).
4. Submit to and pass a written and oral examination conducted by the College.

Examination Procedure

Examinations are given each year at a time and place fixed by the examining committee appointed by the Executive Council. The applicant must furnish a curriculum vitae, which includes the applicant's education prior to formal training in radiology, formal radiology courses, radiology experience, and supervisor. Radiologic experience should include the time spent in small and large animal diagnostic radiology, ultrasound, and elective training areas. Written and oral examinations will test knowledge in areas listed in guidelines for residency programs. The written examina-

tion consists of six sections: (1) anatomy, (2) physiology/pathophysiology, (3) radiation therapy, radiation biology, and radiation protection, (4) physics of diagnostic radiology, (5) radiographic contrast procedures, and (6) alternate imaging including basic physics and diagnosis with each modality. The oral examination encompasses radiographic interpretation, with some emphasis given to clinical management of the patient.

Dr. Myron Bernstein, Executive Director
P.O. Box 87
Glencoe, IL 60022
708-251-5517 (Office & Home)
708-446-8618 (Fax)

5. AMERICAN COLLEGE OF VETERINARY MICROBIOLOGISTS

Objectives

1. To further educational and scientific progress in the specialty of veterinary microbiology.
2. To strengthen and improve instruction at the pre- and postdoctoral level in veterinary microbiology.
3. To promote the highest professional standing of veterinary microbiologists.
4. To establish standards of postdoctoral training and experience for qualification of specialists in veterinary microbiology.
5. To certify qualified and competent veterinary microbiologists in subspecialty areas of bacteriology, mycology, virology, and immunology.

Prerequisites for Certification

1. The applicant shall have earned the Ph.D. with major emphasis in microbiology, or have earned the Master's degree with major emphasis in microbiology and have at least 2 years additional experience relevant to microbiology; or subsequent to earning the D.V.M., V.M.D., or equivalent professional degree, have at least 10 years professional experience relevant to microbiology in teaching, independent research, or diagnostics with increasing responsibility over this time period.
2. Have published at least two scientific articles in refereed journals. (The applicant must be the first author on at least one publication and have made a scientific contribution to the science and the writing of both.) The applicant is expected to have a knowledge of infectious disease (including the zoonoses) of

animals emphasizing etiology, pathogenesis, transmission, immunity, diagnosis, prevention, and control.

Examination Procedure

The examination is divided into two parts. The Qualifying Examination (Part 1) consists of multiple choice questions in the broad field of veterinary microbiology, including bacteriology, mycology, virology, immunology, and infectious diseases. Slides are projected in the Certifying Examination (Part II) for recognition, interpretation, and analysis of the material presented. Beginning in 1987, separate Certifying Examinations were administered in the subspecialty areas of bacteriology-mycology, virology, and immunology. The candidate must pass the Part I and at least one Part II examination within the five years following application.

Dr. H. Graham Purchase, Secretary-Treasurer
College of Veterinary Medicine
P.O. Box 9825
Mississippi State, MS 39762–9825
601-325-1205 (Office)
601-323-7139 (Home)
601-325-1066 (Fax)

6. AMERICAN BOARD OF VETERINARY TOXICOLOGY

Objectives

1. To further education, training, and research in veterinary toxicology.
2. To establish and maintain the highest possible standards of training and experience for qualification as specialists in veterinary toxicology.

Prerequisites for Certification

1. Has, subsequent to graduation from a school or college of veterinary medicine, made outstanding scientific contributions in the field of veterinary toxicology as evidenced by authorship of two accepted peer-reviewed publications or completing two funded research projects, or equivalent activity, and has provided the Board with sufficient evidence of these qualifications for admission to the examination.
2. An eligible candidate must: (a) have fully completed 4 calendar years of training in toxicology, including completion of an advanced degree. Experience in teaching of toxicology and/or toxicological research and/or the practice of veterinary clinical

and/or diagnostic toxicology must be obtained. A minimum of 2 of these 4 years will be subsequent to receiving the D.V.M. or equivalent degree, and under the direct supervision of an ABVT Diplomate; (b) Alternatively, a candidate may complete 2 calendar years in the clinical practice of veterinary medicine, subsequent to graduation from veterinary school or college, and at least 3 calendar years in a residency or other training program in toxicology and/or in the teaching of toxicology and/or toxicological research and/or the practice of veterinary clinical and/or diagnostic toxicology, acceptable to the Board. (c) Alternatively, for individuals who do not fulfill the requirements denoted under (a) or (b) above, the requirements for candidacy may be met through studies and experience obtained. The time necessary to obtain the required training and experience is dependent upon the amount of time available to be devoted to such activities. A candidate exercising this option should be first author on 5 research papers or clinical reports acceptable to the Board. The candidate is responsible for providing sufficient evidence of training and experience acceptable to the Board to gain admission to the examination.

Examination Procedure

Candidates will be examined for knowledge within the broad discipline of veterinary toxicology including but not necessarily limited to the following: (1) the concept of toxicology, its usefulness, definitions, and philosophies; (2) dosage-response relationships; (3) metabolism and detoxication; (4) toxicology of inorganic compounds; (5) toxicology of synthetic organic compounds; (6) toxicology of plant poisons and biotoxins; (7) toxicology of radiation and radiomimetic compounds; (8) residues and residual effects of chemicals and radiation in foods; (9) testing for safety, including experimental design and interpretation; (10) antidotal procedures; and (11) environmental toxicology—industrial, water, and air contamination.

Dr. Robert H. Poppenga, Secretary-Treasurer
University of Pennsylvania
New Bolton Center
382 West Street Road
Kennett Square, PA 19348
610-444-5800, ext. 2217 (Office)
610-444-0892 (Fax)

7. AMERICAN COLLEGE OF VETERINARY SURGEONS

Objectives

The objectives of the American College of Veterinary Surgeons shall be the advancement of the art and science of surgery and the protection of the public against incompetence by:

1. The development of methods of graduate teaching in veterinary surgery with particular reference to the resident system.
2. The establishment of a certifying agency to qualify members of the veterinary profession as specialists in surgery.
3. The encouragement of its members to pursue original investigations and to contribute to the veterinary literature.

Prerequisites for Certification

1. Have devoted at least 4 years, by the application deadline, to special education, training, and practice of veterinary surgery after the date of graduation from veterinary school. (See Veterinary Surgery Residency Program.) The following sequence of training is to be used.
   a. A rotating internship, or its equivalent, as defined by the ACVS.
   b. A 3-year Veterinary Surgery Residency.
2. Have made a significant contribution to veterinary surgery, as represented by publication and demonstrated by a high standard of proficiency in the specialty. In keeping with the constitutional objectives of the ACVS each applicant must demonstrate willingness to contribute to the literature. In addition to contributing to the literature, manuscripts originating from basic or clinical research enhance a Resident's education by the learning of scientific methodology, which may lead to the discovery of new concepts, or substantiate or refute established methods. Manuscripts should demonstrate the Resident's intellectual curiosity and should further the state of surgical knowledge or other closely related biological sciences.

Examination Procedure

The examination will test all phases of surgery in all species and types of animals as well as competence in areas of specialization. The examination is composed of three sections.

1. The written section consists of multiple choice questions with one correct answer. This part of the examination consists of questions that cover the areas of gastrointestinal, cardiovascular, respiratory, musculoskeletal, urogenital, neurologic (includ-

ing special senses), and integumentary surgery. In each organ system, questions will be asked on the basic sciences (anatomy, physiology, pathology), pharmacology/anesthesia, surgical techniques, diagnosis, and surgical treatment.
2. In the practical exam, the examinee can choose to be examined in either large or small animal surgery. The practical section of the exam is designed to test interpretive skills, and the questions are based on visual material or surgically related diseases or conditions.
3. During the oral examination, each candidate must also choose to be questioned on either large or small animal surgery. Three-case oriented questions will test surgical principles in and case management prior to, during, and after surgery.

Dr. Alan Lipowitz, DVM, Executive Secretary
4330 East West Highway, #1117
Bethesda, MD 20814
301-718-6504 (Office)
301-656-0989 (Fax)

8. AMERICAN COLLEGE OF VETERINARY OPHTHALMOLOGISTS

Objectives

1. To encourage education, training, and research in veterinary ophthalmology.
2. To establish standards of training and experience in this field.
3. To aid in the development of methods of graduate teaching in veterinary ophthalmology with particular reference to the residency system.
4. To recognize qualified individuals by certification.
5. To advise veterinarians desiring certification in the field of veterinary ophthalmology as to the course of study and training to be pursued.

Prerequisites for Certification

1. Have completed training in an ophthalmology residency program approved by the American College of Veterinary Ophthalmologists.
2. Have successfully passed the examination by the Board of Examiners of the American College of Veterinary Ophthalmologists.

Examination Procedure

Comprehensive written and oral, and practical examinations will be administered by the college.

Dr. Mary B. Glaze, Secretary-Treasurer
Veterinary Clinical Sciences
Louisiana State University
Baton Rouge, LA 70803
504-346-3333 (Office)
504-346-3295 (Fax)

9. AMERICAN COLLEGE OF THERIOGENOLOGISTS

Objectives

1. The advancement of knowledge, undergraduate, graduate and continuing education, research, and service in theriogenology.
2. The maintenance of a certifying agency to recognize veterinarians as specialists in theriogenology.
3. The development of methods and programs in continuing education for veterinarians, especially practitioners.
4. The development of graduate study and residency programs.
5. The establishment of high standards and guidelines for professional attainment and specialization.

Prerequisites for Certification

Be able to document evidence of advanced competence in theriogenology.

1. Through the standard route, a candidate shall have completed at least 1 year of clinical practice, or its equivalent, subsequent to attainment of a veterinary medical degree, and completed a minimum of 2 years in an established/supervised training program that includes experience in teaching, research, and/or practice of theriogenology.
2. In the alternative route, to be eligible to sit the examination an individual lacking formal advanced training shall have a minimum of 6 years of practice experience with a major emphasis in theriogenology, including successful completion of a 2-year preapproved study and mentorship program.

Dr. Dickson Varner
Texas Veterinary Medical Center
Texas A & M University

College Station, TX 77843
409-845-9150 (Office)
402-463-5683 (Fax)

10. AMERICAN COLLEGE OF VETERINARY INTERNAL MEDICINE

Objectives

To advance veterinary internal medicine and increase the competency of those who practice in this field by:

1. Establishing requirements for postdoctoral education and experience prerequisite to certification in the specialties of veterinary internal medicine.
2. Examining and certifying veterinarians as specialists in veterinary internal medicine.
3. Encouraging veterinarians to pursue a program of continuing education for professional advancement throughout their careers.
4. Encouraging research and other contributions to knowledge relating to diagnosis, therapy, prevention and control of animal diseases, and promoting communication and dissemination of this knowledge.

Prerequisites for Certification and Examination Procedure

The American College of Veterinary Internal Medicine is the body to which the Specialty Groups of Cardiology, Internal Medicine, Neurology, and Oncology are affiliated. Each Specialty Group has its own officers and by-laws and is responsible to the Board of Regents of the ACVIM. To qualify for membership: The candidate must successfully complete a general (qualifying) examination administered by the College and a certifying examination administered by the Specialty Group. The general (qualifying) exam consists of two-parts (1) a comprehensive exam dealing with general medical principles common to all species and (2) an examination oriented toward a field of practice. The minimum requirements for eligibility to take the general (qualifying) examination include: (a) 1-year internship or satisfactory practice experience; (b) 2-year residency or an intensive training program under the immediate supervision of a diplomate of the ACVIM or its equivalent as approved by the appropriate residency or training committee.

The requirements for the certification examination within the Specialty Groups differ slightly, but include (a) passing the gener-

al (qualifying) examination of the College; (b) submitting case reports; (c) submitting published material; (d) successful completion of a comprehensive written examination.

Ms. June Johnson
Executive Director
Suite 2125
7175 W. Jefferson Avenue
Lakewood, CO 80235-2320
303-980-7136 (Office)
303-980-7137 (Fax)

11. AMERICAN COLLEGE OF VETERINARY ANESTHESIOLOGISTS

Objectives

1. To establish, evaluate, and maintain the highest standards in the practice of veterinary anesthesiology by promoting establishment of educational facilities and clinical and research training in veterinary anesthesiology at the undergraduate and postdoctoral levels.
2. To arrange and conduct examinations to determine the competence of veterinarians who apply and to issue certificates to those who meet the required standards. The major criteria on which judgment of competence shall be based include:
   a. Facility in providing all technical services likely to be required in practice of the specialty and in training the specialist.
   b. Ready availability of mature medical judgment applicable to solution of medical problems associated with a patient's care as they arise in practice of the specialty.
   c. The talent, training and habits of study necessary for evaluating and applying new knowledge as well as seeking new knowledge.

Prerequisites for Certification and Examination Procedure

1. Submit proof to the college that he or she has satisfactorily completed special training in anesthesiology.
2. Successfully complete a certifying examination administered by the College. The qualifying examination will consist of two parts:
   a. A comprehensive written examination on all phases of veterinary anesthesiology including the basic science and clinical application.

b. The oral examination.

Application for admission to the examination may be approved only after a veterinarian has completed an approved training program. These requirements may be met by either of the following plans:

The residency program must be under the supervision of a Diplomate of the American College of Veterinary Anesthesiologists or by a Diplomate of the American Board of Anesthesiology.

Plan A - Residency Program

Residency training for not less than 2 years in Veterinary Anesthesiology in a clinical department of a School or College of Veterinary Medicine. The training period shall comprise application of methods for rendering patients insensible to pain during surgical and medical procedures, and the support of life functions, especially during the stress of anesthetic and surgical manipulation.

A publication in a refereed journal demonstrating results of experimental or clinical investigation in veterinary anesthesiology shall be included with the candidate's application for examination.

Plan B - Combined Residency and Postdoctoral Program

During the combined residency and postdoctoral training period, the candidate must accumulate a minimum of 2 years training in clinical anesthesiology which shall comprise application of methods for rendering patients insensible to pain during surgical and medical procedures, and the support of life functions, especially during the stress of anesthetic and surgical manipulation.

The remaining combined residency and postdoctoral training period shall be spent meeting the advanced degree requirements in a biomedical science directly applicable to anesthesiology and acceptable to the Credentials Committee. A publication in a refereed journal demonstrating results of experimental or clinical investigation in anesthesiology shall be included with the application for examination.

Dr. Ann E. Wagner, Executive Secretary
Department of Clinical Sciences
College of Veterinary Medicine
Colorado State University
Fort Collins, CO 80523-0002
303-491-0346 (Office)
303-491-1275 (Fax)

12. AMERICAN BOARD OF VETERINARY PRACTITIONERS

Objectives

The American Board of Veterinary Practitioners was established to certify veterinarians have a broad knowledge of many clinical subjects within a practice category. The practice categories currently recognized and certified by the ABVP are avian, beef cattle, canine and feline, dairy, equine, feline, food animal, and swine health management. Through certification the ABVP seeks to:

1. Promote advancement and recognition of high standards in the art and science of veterinary practice.
2. Establish a certifying agency for members who excel in species-oriented clinical practice.
3. Recognize veterinary practitioners who are qualified to fill a unique and specific functional role in the delivery of modern, comprehensive veterinary services.
4. Develop methods and locations of graduate teaching programs with particular emphasis on residency training for clinical practice.
5. Provide an incentive and reward for achieving education and experience by any means following graduation from veterinary school.

Prerequisites for Certification

To make certification accessible to more practitioners, the accreditation procedure does not require long, specific, formal training programs. Applications are obtained by written request from the Administrator of the ABVP. They are returned to the administrative office along with other required materials by March 1 of the year that the candidate wishes to be examined. To be eligible for examination, the following requirements must be satisfied:

1. Have 1 year of active practice experience or rotating internship approved by the Credentials Committee.
2. Have completed, before the examination, one of the following training programs:
   a. Residency. Residency programs must be at least 2 years in length. They must be approved in advance for each individual by the Residency Committee, unless otherwise approved by the Council of Regents. The program may be carried out at a veterinary college, practice, or institution. Residencies must include experience in all areas of importance to the practice category for which certification is being sought.

Daily supervision by an ABVP diplomate is required. However, in the absence of an ABVP Diplomate, a Diplomate of another AVMA-approved specialty board may be substituted upon approval by the council of Regents. Upon completion of the program, the director of the training institution must certify the satisfactory completion of all phases of training and recommend the candidate. In addition, the Residency Committee must certify that the candidate has satisfied all requirements of an ABVP Residency Program.

b. No Residency. Training must include 5 years of excellent experience in the practice category for which certification is sought. The candidate must demonstrate to the Credentials Committee their mastery of and contributions to the practice category and an ongoing commitment to continuing education within their practice category. Minimum time to qualify for examination will be 6 years.

3. Applicants must provide two case reports in the style of the *Journal of the American Veterinary Medical Association.*

Examination Procedure

Examinations for each practice category will be given annually in December. All examination requirements must be completed within 3 years from the date the candidate is first notified by the ABVP of acceptance of their credentials. There are separate examinations for each practice category, and candidates are required to select the category for which they wish to be examined. The examination for each practice category includes three parts. All candidates must take all parts of the examination upon the first application. Candidates may repeat any part(s) of the examination that they do not pass. The three portions of the examination are: Part 1. This part will test basic and preclinical science knowledge relating to the designated practice category; Part 2. This part will test information applicable to established disciplines within the designated practice category; Part 3. This part will test ability to recognize, analyze, and solve clinical problems by various methods.

Ms. Dee Ann Walker
Administrator
530 Church Street, Suite 300
Nashville, TN 37219-2394
615-254-3687 (Office)
615-254-7047 (Fax)

13. AMERICAN COLLEGE OF VETERINARY DERMATOLOGY
Formerly Specialty of Dermatology—American College of Veterinary Internal Medicine

Objectives

The primary objectives of the College shall be to advance veterinary dermatology and increase the competency of those who practice in this field by:

1. Establishing guidelines for postdoctoral education and experience prerequisite to certification in the specialty of veterinary dermatology.
2. Examining and certifying veterinarians as specialists in veterinary dermatology to serve the public by providing expert care for animals with dermatologic disease.
3. Encouraging research and other contributions to knowledge relating to pathogenesis, diagnosis, therapy, prevention and control of diseases directly or indirectly affecting the skin of all animals, and promoting communication and dissemination of this knowledge.

Prerequisites for Certification

1. The candidate shall have served a minimum of 1 year internship in a veterinary college, other institution, or have otherwise obtained appropriate clinical experience to be approved by the Credentials Committee.
2. The candidate shall have a minimum of 2 years of educational experience in the discipline of dermatology after completing the 1-year internship. An applicant must have a total of 3 years experience after graduation to be eligible for the examination.

Program Supervision

All residency programs are to be under the direct supervision of a diplomate of the ACVD.

1. The residency program must be documented completely before it is submitted for approval. Any individual, institution, or organization who will supervise a part of the residency must supply a written acceptance of their commitment, a description of their part of the program, and the date(s) of the training period. Approximate dates are acceptable. The Preceptor is to receive this information and include it in the program description.
2. After the program is accepted, the Preceptor must submit yearly progress reports. All individuals who are participating in the training of the Resident should document the Resident's perfor-

mance and forward this material to the Preceptor for inclusion in the yearly report.

3. If the training program does not develop as it was described in the initial application or if the Resident's performance becomes unsatisfactory, the Preceptor must notify the Education Committee immediately. The Committee has the authority to withdraw approval of the program permanently or temporarily until appropriate modifications can be made. The decision of the Education Committee can be appealed by notice to the President within 30 days.
4. A residency program is designed to train the Resident in all aspects of veterinary dermatology. All appropriate study areas such as basic dermatology, histopathology, comparative dermatology, and clinical dermatology should be included.
5. Residency programs must include clinical training periods under the direct supervision of a board-certified veterinary dermatologist. The Preceptor of the program may perform all or part of this clinical training. If the clinical training is to be done under multiple individuals, no more than four dermatologists or institutions should participate in the training.

Examination Procedure

The certifying examination is designed to test the candidate's broad and specific knowledge of skin and skin disorders (cellular and subcellular, microscopic and macroscopic, physiologic and pathologic, etiologic, and clinical).

The examination covers all phases of dermatology, as well as aspects of internal medicine related to the practice of dermatology, and is weighted approximately as follows: 80% small animal, general and comparative; and 20% large, laboratory, and exotic animals. In addition, the approximate weighing of subject content is as follows: (1) structure and function 20%, (2) parasitology 12%, (3) internal medicine (dermatology) 18%, (4) bacteriology 12%, (5) immunology 12% (6) endocrinology 8%, (7) mycology 3%, (8) neoplasia 3%, (9) miscellaneous (allergy) 12%.

Dr. Craig E. Griffin, Secretary
Animal Dermatology Clinic
13240 Evening Creek Drive
San Diego, CA 92128
619-486-4600 (Office)
619-486-4681 (Fax)

14. AMERICAN COLLEGE OF ZOOLOGICAL MEDICINE

The American College of Zoological Medicine was established in 1983 to recognize the specialty of zoological medicine. The objectives of the College are: 1) to advance competency and scientific progress in zoological medicine; 2) to establish standards for postdoctoral training and experience, and certify veterinarians as specialists in zoological medicine through a comprehensive examination; 3) to encourage research on the medical, surgical, and management problems of nondomestic species. Prospective diplomates are qualified to sit for the certification examination requirements after meeting requirements of training, experience, and publication as set in the bylaws of the College.

The certification examination is given in two parts. The first part is comprehensive, covering aspects of veterinary medicine relating to a broad range of animal groups including mammals, birds, reptiles, amphibians, and fish. The second part, taken after the successful completion of the first, covers in-depth knowledge in primary captive zoo animal, avian, wildlife, reptile/ amphibian, or aquatic medicine.

Dr. George Kollias, Jr., Secretary
Department of Clinical Services
Section of Wildlife Medicine
New York State Veterinary College
Cornell University
Ithaca, NY 14853
607-253-3049 (Office)
607-253-3708 (Fax)

15. AMERICAN VETERINARY DENTAL COLLEGE

Objectives

To promote advancement and recognition of the standards in the art and science of veterinary dentistry. Establish a certifying agency for members who excel in disciplines related to veterinary dental practice. Develop methods and locations of graduate teaching programs with particular emphasis on residency training for veterinary dental practice. By self-assessment and continuing education, promote continued improvement of practice standards and knowledge in veterinary dentistry. Encourage investigations and contributions to the literature on the field of veterinary dentistry.

Prerequisites of Certification

1. Submit to the Credentials Committee a dental log of veterinary oral/dental cases seen in a recent 12-month period.
2. A residency program of at least 2 years duration at an institution or facility approved by the Board of Directors of the American Veterinary Dental College, with at least 50% of professional activities relating to the oral cavity and significant time devoted to all major areas of dentistry (oral anatomy, pathology, periodontics, orthodontics, endodontics, restoratives, oral/ maxillo-facial surgery, and oral radiology), plus have at least 1 year of practice experience in which at least 50% of professional time is devoted to activities related to the oral cavity dentistry (oral anatomy, pathology, periodontics, orthodontics, endodontics, restoratives, oral/maxillo-facial surgery, and oral radiology), or:
3. Hold a degree in human dentistry (D.D.S., D.M.D., or equivalent) from an approved American Dental Association program, plus have 1 year or more clinical experience during which at least 50% of professional time is devoted to activities relating to all areas of animal dentistry.
4. Alternate route:
   a. A 6-year period of relevant practical experience during which at least 35% of professional time is devoted to activities relating to the oral cavity or 5 years at 45%. Substantial contact should be maintained with and performance evaluation accomplished by a member of the American Veterinary Dental College of AVDC-approved institution, facility, or individual capable of providing such instruction, guidance, and clinical support of the candidate.

   Plus:

   b. Show evidence of knowledge in all major areas of animal dentistry including oral anatomy, pathology, periodontics, orthodontics, endodontics, restoratives, oral/ maxillo-facial surgery, and oral radiology by submitting inclusive documentation.
5. Nonconforming residency program: a nonconforming residency program is a full- or part-time program that has been approved by the AVDC. The program will be substantially equivalent to full-time residency training as established by the AVDC. Curriculum must be submitted and approved by the AVDC prior to its commencement. Reporting requirements are identical to that of the Conforming Residency Program. The nonconform-

ing residency program is established to provide greater flexibility for candidates in their residency training programs.

Examination Procedure

The examination shall consist of at least two parts—a written section and a practical section—and shall cover oral anatomy, embryology, genetics, oncology, periodontics, endodontics, orthodontics, restoratives, oral/maxillo-facial surgery, and oral radiology.

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16. AMERICAN COLLEGE OF VETERINARY NUTRITION

Objectives

The primary objectives of the American College of Veterinary Nutrition (ACVN) shall be to advance the specialty area of veterinary nutrition and increase the competence of those who practice in this field by:

1. Establishing requirements for veterinary postdoctoral education and experience for certification in the specialty of veterinary nutrition.
2. Examining and certifying veterinarians as specialists in veterinary nutrition in further recognition of such qualified specialists.
3. Encouraging veterinary nutritionists to pursue a program of continuing education for professional advancement throughout their careers.
4. Promoting research and other contributions to knowledge relating to the nutritional needs and to the diagnosis, therapy, prophylaxis, and control of nutritionally related diseases in animals.
5. Enhancing the dissemination of new knowledge in veterinary nutrition through didactic teaching and post-graduate programs.

Prerequisites for Certification

1. Completion of 1 year of general clinical experience (internship, residency, or practice) or equivalent.
2. Completion of a 2-year residency program with emphasis in

veterinary nutrition as demonstrated by courses, research, and clinical experience. The program should include clinical, teaching, and research activities, but must include at least 6 months of full-time equivalent clinical experience in veterinary nutrition. Depending on the program track, i.e., comparative (small and large animal), small animal, or large animal, this must include experience in the large and/or small animal clinics and, where applicable, in field service, and should also include extension activities with referring veterinarians. Clinical experience must be under the supervision of a diplomate of the ACVN, or, until 1998 of a diplomate of another AVMA approved specialty.

3. Must have published or been accepted for publication in a refereed journal two scientific reports in the area of veterinary nutrition on which the candidate is a primary author. A report is considered to have been published in a refereed journal if the report itself was refereed.
4. Submitted reports of three clinical cases or herd problems having significant nutritional component(s) and in which the applicant has personally handled the nutritional management.
5. An alternate program for admission to the certifying examination is available.

Examination Procedure

The examination consists of three sections. Section one is a general examination. Sections two and three are either comparative (both large and small animal), small animal, or large animal examinations that candidates select according to their preference. The examination will be given over 2 consecutive days as follows:

1. A written 3-hour general examination covering principles of nutrition, and related aspects of biochemistry, physiology, pathophysiology, general medicine, and metabolic aspects of surgery.
2. A written 3-hour examination covering aspects of practical nutrition (feeding and nutritional management), clinical nutrition, and nutritional pathology.
3. A written 3-hour practical examination covering ration evaluation, ration formulation, and problem-solving clinical situations.

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17. AMERICAN COLLEGE OF VETERINARY EMERGENCY AND CRITICAL CARE

Objectives

The purpose of the ACVECC is to promote advancement and high standards of practice for those individuals involved in Veterinary Emergency and Critical Care Medicine. To accomplish this the ACVECC proposes:

1. To establish requirements and foster development of residency and alternative training programs for postdoctoral education and experience prerequisite to certification in the specialty of Veterinary Emergency and Critical Care Medicine.
2. To examine and certify veterinarians as specialists in Veterinary Emergency and Critical Care Medicine.
3. To encourage research and other contributions toward knowledge relating to diagnosis, therapy, prevention, and control of animal diseases requiring emergency or critical care management.
4. To promote communication and dissemination of knowledge relating to veterinary emergency and critical care medicine, and to recognize individuals for outstanding contributions to the specialty.

Prerequisites for Board Examination

1. Completion of 1 year of rotating internship or equivalent practice experience approved by the Residency Training Committee.
2. Completion of a Standard 3-year Residency program in veterinary emergency and critical care.
3. Completion of an Alternate Route Training Program in veterinary emergency and critical care. This program will allow prospective candidates to become eligible through training achieved in a noninstitutional setting as an alternative to the Standard Residency Program.
4. Provide the documentary evidence of advanced competence in veterinary emergency medicine and critical care through clinical experience, research, publications, and teaching.
   a. Candidates may choose one of the following options:
      i. Case Report Option—Four case reports of no more than five pages each, double spaced. Case reports must demonstrate expertise in management of a variety of veterinary patients requiring emergency and critical care. Case reports must be original work of the applicant, free of editing by peers, colleagues, or mentors.

ii. Publication option—one refereed publication (first author) relevant to the topic of veterinary emergency or critical care medicine that has been published or accepted for publication by the application deadline in a quality peer-reviewed refereed journal. Single case reports and review articles are acceptable.

iii. Applicants must demonstrate experience in teaching. Applicants must document 6 hours of lecture on emergency and/or critical care topics to veterinary students, AHTs, faculty, or veterinary audiences during their course of training. Experience must also include teaching in clinical or laboratory settings (minimum of 6 clinical days or 6 laboratories).

Examination Procedure

1. Candidates approved by the Credentials Committee and Council of Regents, upon receipt of the prescribed examination fee by the stated due date, will be advised of the exam format no less than 3 months prior to examination.
2. Examinations will be given once annually.
3. Examinations will be prepared and administered by the Examination Committee.
4. Passing scores on each section will be proposed by the Examination Committee and approved by the Council of Regents.
5. All examination requirements must be completed within exam cycles from the date the candidates's credentials are accepted by the College.
   a. Exception to this requirement may be made by the Council of Regents following written petition by the applicant through the Executive Secretary.
   b. A candidate who does not pass the examination within three exam cycles must resubmit credentials for approval. If credentials are reapproved, candidate has 3 years to pass the examination. Candidates who fail to pass the examination the second 3-year period cannot resubmit credentials.
6. A candidate will take all parts of the examination the first time.
7. The Examination Committee is responsible for preparing, administering, validating, and grading of all examinations. The format and content of the examination will be determined by the Examination Committee. Objectives of the examination will be consistent with those stated in the Constitution for promoting excellence in emergency and critical care medicine.

8. A minimum passing score as proposed by the Examination Committee and approved by the Regents must be achieved on each part.

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18. AMERICAN COLLEGE OF VETERINARY CLINICAL PHARMACOLOGY

Objectives

The primary objectives of the College are to advance the discipline of veterinary clinical pharmacology as a clinical specialty and assure the competence of those who practice in this field by:

1. Establishing requirements for veterinary postdoctoral education and experience for certification in the specialty of veterinary clinical pharmacology.
2. Examining and certifying veterinarians as having met the requirements to be a specialist in veterinary clinical pharmacology.
3. Encouraging veterinary clinical pharmacologists to pursue a program of continuing education for professional advancement throughout their careers.
4. Supporting and promoting education and research and other contributions to knowledge relating to veterinary clinical pharmacology.
5. Enhancing the exchange of new knowledge in veterinary clinical pharmacology.
6. Organizing committees of experts to research and make recommendations to the profession on current problems in veterinary therapeutics.

Prerequisites for Certification and Examination Procedure

1. Have completed 2 years or more of intensive residency training, primarily in veterinary clinical pharmacology, under the supervision of a diplomate of the American College of Veterinary

Clinical Pharmacology. Alternate methods for qualifying for examination will be considered, and may include graduate study, academic course work, and experience obtained through the practice of veterinary clinical pharmacology. The program must include at least 3 years of training, of which 2 years must be in the clinical practice of veterinary pharmacology.

2. Satisfactory completion of the initial phase of training will be evaluated by two criteria.
   a. Acceptable performance on an objective-type, comprehensive examination (Phase I) designed to test the candidate's knowledge of the areas specified above. This examination may be administered by a Diplomate of the College who resides in the geographical location closest to the applicant.
   b. Submission of a minimum of one manuscript based on work performed during the initial phase of training. The manuscript must be an original, refereed, drug-related article published in a peer-reviewed journal. A letter of acceptance from the editor will be sufficient for this requirement.
3. Satisfy the Phase II Program Requirements:
   a. Admission to the certification examination requires that the candidate has taken and passed the Phase I examination described above and subsequently has completed an approved program of study in clinical pharmacology designed to provide the applicant with the opportunity to apply the basic tools of clinical pharmacology within a clinical milieu. This may include, but is not restricted to: the implementation and evaluation of a therapeutic clinical trial, extensive management or consultative support of complex therapeutic problems in at least two important veterinary species, identification of adverse drug reactions, therapeutic monitoring, development of rational therapeutic protocols for extra label drug use in food animals, the determination of appropriate withdrawal times, and individualization of drug dosage.

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19. AMERICAN COLLEGE OF POULTRY VETERINARIANS

Objectives

1. To further educational and scientific progress in the specialty of poultry veterinary medicine.
2. To strengthen and improve instruction in poultry veterinary medicine at both professional and postgraduate levels.
3. To establish standards of postprofessional training and experience for specialists in poultry veterinary medicine.
4. To certify qualified and competent poultry veterinarians in aspects of veterinary medicine appropriate to breeders, broilers, commercial egg, and turkey production, and their ancillary disciplines.

Prerequisites for Certification

The Credentials Review Committee will examine the qualifications of applicants for examination or recertification and make recommendations to the Board of the College.

Candidates for Diplomate status must have capabilities extending over the broad area encompassed by poultry medicine. Candidates must be well informed in relevant aspects of microbiology, immunology, pathology, parasitology, physiology, management, toxicology, epidemiology, and preventive medicine. The candidate shall also have knowledge of infectious diseases, including the zoonoses, of poultry, with emphasis on etiology, pathogenesis, transmission, diagnosis, prevention, and control.

Academic training and experience should include an earned D.V.M. degree or equivalent, a Master's level degree or equivalent or higher postgraduate degree with a major emphasis in poultry health OR successful completion of an approved training program in poultry veterinary medicine, OR at least 5 years professional experience relevant to poultry veterinary medicine.

Training programs in poultry veterinary medicine will be approved and accredited by the Training Program Review Committee. Applicants must document their qualifications in an application package provided by the College. Applicants shall have published at least one scientific article or two research or technical case reports in refereed journals, OR prepared three case research reports of a standard suitable for publication, OR a combination of articles and reports.

Examination Procedure

1. The Basic Examination will include topics pertinent to poultry veterinary medicine, including nutrition, biochemistry, envi-

ronmental management, poultry housing, processing, food safety and quality, economics, and breeding. This examination will require a knowledge of modern commercial production and laboratory techniques in poultry veterinary medicine.

2. The Applied Examination will emphasize practical aspects of poultry veterinary medicine and will involve specimens, production data, laboratory findings, and other material representing a test of the ability to apply theoretical knowledge in solving problems. Candidates will be required to recognize, interpret, and analyze material presented.

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20. AMERICAN COLLEGE OF VETERINARY BEHAVIORISTS

Objectives

The primary objectives of the College shall be to advance veterinary behavioral science and increase the competency of those who practice in this field by: (1) establishing guidelines for postdoctoral education and experience prerequisite to certification in the specialty of behavior; (2) examining and certifying veterinarians as specialists in behavior to serve the public by providing expert care for animals with behavior problems; (3) providing leadership and expertise to the veterinary profession in behavioral therapy, psychological well-being and welfare of animals, and other appropriate areas of animal behavior; (4) encouraging research and other contributions to knowledge relating to etiology, diagnosis, therapy, prevention, and control of behavior problems and promoting communication and dissemination of this knowledge.

Prerequisites for Certification

1. Proof of completion of a behavioral residency program or nonconforming behavioral residency program approved by the credentials committee of ACVB.
2. Publication in a refereed journal.
3. Three case reports.
4. Three letters of recommendation from associates who have worked closely with the candidate in the area of animal behav-

ior. At least one of these associates must be a Diplomate of the ACVB.

Examination Procedure

The examination will be given over 2 consecutive days. It will be a written examination including both long and short answers that cover the basics of behavioral principles, basics of the behavior of various species, and the clinical application of behavior in various species.

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ADVANCES IN VETERINARY SCIENCE AND COMPARATIVE MEDICINE, VOL. 39

Standards for Veterinary Clinical Trials

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I. Introduction

A clinical trial is a prospective study that compares the effect(s) and value(s) of an intervention. Although the clinical trial is the most effective method for establishing the effects (efficacy or safety) of an intervention, proper planning and execution are paramount to its success and validity. The groundwork for high-quality clinical trials is

laid by observational studies that focus on new or existing interventions and provide the baseline data for these trials.

Unlike human clinical trials, the subject of a veterinary clinical trial may be owned by the research facility (experimental) or may be a client-owned patient. It is important to distinguish the source of experimental subjects for a veterinary clinical trial since certain types of bias can be more easily minimized when using experimental animals. Because subject variability (e.g., differences due to age, breed, sex, diet, environment, or disease) can be more easily standardized, some investigators do not consider studies that use experimental animals as clinical trials. Although it is important that results of an experimental clinical trial be extrapolated to the general population cautiously, the ability to minimize variability does not preclude the fact that a comparison has been made on the effects of an intervention. Recognizing these caveats, we have selected the following characteristics as our definition of a clinical trial (Friedman *et al.*, 1985; Meinert, 1986).

1. A clinical trial is a prospective study. The subjects of the study must be identified prior to implementation of the study and followed forward in time.
2. A clinical trial studies interventions applied under the control of the investigator. The intervention is intended to effect a change in the subject. The interventions may vary in nature, including prophylactic or diagnostic techniques; therapeutic agents, devices or regimens; etc. If there is no intervention applied by the investigator, the study is observational, and thus not a clinical trial.
3. A clinical trial studies both an intervention and a control group. If there is no control group, a comparison cannot be made and an effect cannot be proven. The study then becomes descriptive. Although the nature of the control group may vary among trials, there must be a basis upon which the intervention is compared. Control groups may include use of placebo, or a positive control (e.g., different levels or routes for a drug or a currently accepted alternative treatment). On rare occasions, a historical control may be appropriate.
4. The outcome measures upon which comparisons are based focus on either the efficacy or safety of an intervention. Clinical trials involving experimental animals do not necessarily include safety considerations. In contrast, clinical trials involving client-owned animals are ethically bound to consider all effects of the intervention.

In human patients, serious and costly consequences have followed the application of unproven interventions. As veterinarians, we tend to

be less ethically restrained in our use of unproven interventions due to the economic constraints of veterinary medicine. We frequently must use interventions that have not been proven to be effective. Yet the biological variation that characterizes disease among the species precludes our ability to determine the appropriateness of an intervention, and it is only through the well-designed clinical trial that the proper use of interventions can be firmly established. We must work against the financial and time constraints that limit clinical trials in veterinary medicine and focus on improving our scientific approach to this research method. This chapter will first summarize the history of human and veterinary clinical trials. Second, it will focus on the proper implementation of a veterinary clinical trial starting with the question to be answered and finishing with reporting of the results. A critique of the profession's current approach to the implementation and reporting of clinical trials will be followed by an assessment of the future of veterinary clinical trials.

II. History of Clinical Trials in Human Medicine

Lilienfeld places the first use of the term clinical trial in human medicine in the late 1920s (Lilienfeld, 1982). By 1948, the basic parameters were established for the modern clinical trial in human medicine. The double blind randomized clinical trial is currently considered the gold standard for therapeutic and preventive studies. Despite a relatively recent evolution into its present form, the development of this complex form of clinical trial has been a long process, drawing from statistical and other scientific disciplines.

There are several basic design components to the modern clinical trial: (1) having a prospective comparison group, which ideally requires a concurrent control group. A corollary of the control group includes the concept of a placebo treatment and the placebo effect, (2) random assignment to the groups to assure comparability between groups and decrease bias, and (3) blind assessment of a specific outcome whenever possible.

In setting the clinical trial into a larger context, several additional considerations have developed:

1. Ethical constraints, including issues that arise when doing experiments on human beings;
2. The need for collaboration and multicenter studies to carry out many types of research questions;

3. A shift to perform clinical trials that address questions about chronic diseases in addition to the traditional infectious and nutritional disease problems.

The Book of Daniel, verses 12–15, is commonly cited as the earliest conceptualization of the modern clinical trial (Medical Research Council, 1948; Lilienfeld, 1982; Meinert, 1986). These verses include the comparison between groups. One of the earliest specifications for using *concurrent controls* instead of historical or no control group probably occurred in 1747 (Lilienfeld, 1982). This was Lind's study on the use of lemons to prevent scurvy on long ship voyages. Concurrent controls were more generally accepted in the mid-1800s as is demonstrated by their inclusion in Claude Bernard's "Introduction to the Study of Experimental Medicine" (Lilienfeld, 1982). He clearly espoused the importance of the idea in this publication. Perhaps secondarily to the idea of concurrent control treatment is the concept of a *placebo effect.* This idea was first documented and described by Haygarth in 1799 (Meinert, 1986). It was reiterated in 1865 by Sir William Gull and Henry Sutton in their study of the effects of mint water on rheumatic fever (Meinert, 1986). Placebos were first used in clinical trials in 1931 during study by Amberson *et al.* (1931) on sanocrysin as a treatment for pulmonary tuberculosis (Hinshaw and Feldman, 1944). *Use of an inert substance* was first referred to *as a placebo* in 1938 (Feinstein, 1985). In 1944, Hinshaw and Feldman described the important components of a clinical trial, especially as needed for the study of tuberculosis (Hinshaw and Feldman, 1944). They included the use of a placebo to prevent the placebo effect as one of the important design considerations. The use of placebos in clinical trials also has ethical complications that will be discussed later.

Randomization of subjects to treatment groups occurred relatively late in the development of the clinical trial. The use of statistics and enumeration preceded the idea of random assignment of treatment by more than a century. Statistical comparisons of mortality were made between groups in smallpox studies in the 1720s by James Jurin (Lilienfeld, 1982). Lind's scurvy study also used comparative statistics (Lilienfeld, 1982). The field of statistics did not begin to address random samples until 1923 when Fisher introduced the idea in the analysis of agricultural studies (Box, 1980; Lilienfeld, 1982). The first mention of formal random assignment in clinical trials was in 1931 in the trial of sanocrysin in treating pulmonary tuberculosis (Lilienfeld, 1982). However, randomization did not become widely accepted until the 1940s as an important component of the clinical trial. Hinshaw

and Feldman and the Medical Research Council of Great Britain related the importance of randomization specifically in regard to the design of studies for the treatment of tuberculosis (Hinshaw and Feldman, 1944; Medical Research Council, 1948).

Blind assessment of outcome is the most recent refinement in the design of clinical trials. The primary purpose is to prevent individuals involved in the study from uncovering which patient received which treatment. This knowledge could bias the patient, clinician, or individual involved in the analysis of the results. In some situations, blinding can only be achieved by use of a placebo. The first double blind trial was the sanocrysin study (Amberson *et al.*, 1931). The use of blind assessment and the placebo effect was discussed by Hinshaw and Feldman in their review paper pertaining to tuberculosis (Hinshaw and Feldman, 1944). Blind assessment with the use of two "active" treatments was used in the streptomycin study of pulmonary tuberculosis (Medical Research Council, 1948). In that case, the radiologist who read the degree of improvement in the radiographs was blinded as to treatment.

As the level of sophistication of the public rose and the role of the medical profession evolved, larger societal issues were raised and had to be addressed. The *concern for ethical treatment of subjects* in medical research was a growing area of interest. From a historical perspective, the Nuremberg Code of Ethics arose as a result of the trial of war criminals from 1946 to 1949. The Code states a number of clear requirements for any research on human subjects (Beuchamp and Walter, 1978). The Declaration of Helsinki, which was adopted in 1964, further elaborates the responsibilities of individuals who conduct research on human subjects (Beuchamp and Walter, 1978). In 1977, a paper discussed the legal liabilities associated with epidemiologic research in general and clinical trials in particular (Berger and Stallones, 1977). Two papers from the 1980s are examples of literature that specifically addresses ethical concerns in clinical trials (Lebacqz, 1980; Freedman, 1987). These concerns have been well accepted and attention to these issues has continued. The specific concerns are discussed in detail in section IV.

About the time that the randomized clinical trial became a distinct entity, *multicenter studies* began to be conducted (Lilienfeld, 1982). This development also was supported by the Medical Research Council of Great Britain, which served as a resource for advice and assistance in the design and conduct of clinical trials (Meinert, 1986). An early multicenter study conducted in three different cities researched the use of serum treatment of lobar pneumonia (Medical Research Coun-

cil, 1934). The analysis for this study stratified by center, indicating an appreciation for the complexities that may occur in the analysis of these types of studies. Studies on tuberculosis in England in the late 1940s and in the United States in the 1950s are other early examples of multicenter clinical trials. In Great Britain, the first controlled clinical trial on streptomycin treatment of pulmonary tuberculosis was conducted in 1946 (Medical Research Council, 1948). Three centers were originally established, but it soon became clear that more centers would be needed to provide enough patients. Four additional hospitals were included. This was followed in 1952 by a multicenter randomized clinical trial in the United Stated which included 22 tuberculosis hospitals (Mount and Ferebee, 1952). This study evaluated isoniazid alone and with streptomycin, and streptomycin with para-amino-salicylic acid in more that 1500 cases of pulmonary tuberculosis. A coordinating center was cited as part of the design of the multicenter study.

As clinical trials increased in size and complexity, collaboration and the inclusion of specialists became more common. Personnel involved in the planning and conduct of clinical trials included physicians, biostatisticians, and epidemiologists (Meinert, 1986). Federal sponsorship of the National Cancer Institute in 1937 also promoted connections between researchers (Schneiderman, 1977). Additional impetus for conducting clinical trials came with the 1962 regulations of the FDA requiring proof of efficacy of new drugs (Feinstein, 1985; Meinert, 1986). Specialization and continued growth of clinical trial methodology led to the formation of the Society for Clinical Trials in 1979 and the establishment of the *Journal of Controlled Clinical Trials* in 1980. The combination of complexity and ethical constraints has also led to subdivisions in the organizational structure of large clinical trials. This division is especially consistent in the *separation of patient care from treatment evaluation.* As early as 1944, Hinshaw and Feldman suggested the beginning of this separation of the interpretation of results from the patient's care (Hinshaw and Feldman, 1944).

In the 1960s, the shift from infectious and nutritional diseases to *chronic disease studies* became strong. The emergence of chronic disease clinical trials also supported the need for multicenter studies to conduct the clinical trial in a timely fashion with the necessary number of subjects (Schneiderman, 1977). Studies on diabetes mellitus, hypertension, and cancer were implemented during this time. The University Group Diabetes Program was founded to evaluate several objectives concerning the treatment of diabetes mellitus. One was "to develop clinical and statistical methodology necessary for the conduct

of long-term clinical trials" (Goldner, 1970). Because of the long latency of some chronic diseases and the multicausal pathways, analyses had to account for many different confounders within heterogenous groups.

The growth of clinical trials as a specialized form of research and the development of affordable and powerful computers has also changed the *statistical analyses of clinical trials*. More sophisticated analyses became possible using the new software packages (Gordon, 1987). This led to increased specialization in the analysis as well as design of modern clinical trials. Clinical trials have become a very structured and complex form of clinical research in human medicine as a result of these factors (Meinert, 1986).

III. Early Veterinary Clinical Trials

Prior to 1966, there was no subject heading for clinical trials in *Index Veterinarius*. A few references were found under the pharmacology subject heading. This reflects the major focus area of early veterinary clinical trials, which was the testing of new drugs, usually in conjunction with industry. In this context, clinical trials were the link between veterinary practice and research, with the research agency usually being a pharmaceutical company. Of the 12 references in the 30 years prior to 1966, 9 were not in English, 2 were reviews in the human literature, and 1 was a clinical trial in humans. The earliest discussion of clinical trials specifically in veterinary medicine was an editorial on remuneration for clinical trials, published in 1966 (Editorial, 1966). The paper clearly refers to clinical trials only with regard to evaluation of new drugs. The author also espouses continuing education and additional training on clinical trials for veterinary practitioners, ideas that have only recently begun to be enacted. It was not until the past decade that clinical trials in veterinary medicine have been presented as studies that could be done using interventions other than new drugs.

A search of *Medline* was conducted from 1966 (when *Medline* began) until 1988. *Cab Abstracts* also were searched from 1972 to 1988. The headings used were "clinical trial" or "randomized controlled trials" in combination with "veterinary." The search was limited to the English language. Abstracts were obtained when available. When no abstract was available for an article, the entire published article was evaluated. For the first article in each year, the entire article also was read to look

for additional information on study design not available in the title or abstract.

The following information was collected for each article.

1. Presence and type of controls
2. Presence of random assignment of intervention
3. Use of experimental subjects versus clinical patients
4. Mention of blind assessment of outcome
5. Mention of placebos

All of these variables were reported separately when collected from the title and abstract or summary only. A study was considered a clinical trial if it was controlled, e.g., some comparison group was used (including historical controls). Random assignment was present if any mention of randomization was made by the authors. Animals that were bought for the study or owned by the company or university were considered experimental animals, and these studies were called experimental clinical trials. Studies using client-owned animals were not experimental clinical trials. Articles that were listed as clinical trials but which had no comparison group (uncontrolled and, therefore, case reports or series) or where the investigator did not control assignment of the intervention were classified as observational studies.

The numbers of clinical trials in veterinary medicine in the late 1960s were limited. The numbers of published clinical trials did not begin to increase until 1979 and appeared to peak in the mid-1980s. *Experimental clinical trials* continued throughout the period of 1966–1988 in low numbers. Descriptive studies called clinical trials appeared intermittently in the 1960s and 1970s and began to increase and then level off in the 1980s (Fig. 1). The labeling of descriptive studies as clinical trials highlights the confusion concerning the terminology and definition of clinical trials.

Review articles on specific diseases that also discussed clinical trial design or previous clinical trials in that area were published sporadically beginning in the 1980s. There was one each in 1980, 1982, 1983, 1986, and 1988 and three in 1984. None of these articles discussed design or conduct of clinical trials in veterinary medicine at length. One article in a set of review articles in 1985 (which did not appear in the data base searches) did discuss the critical review of clinical trials and the important components to consider when reading a journal article (Dohoo and Waltner-Toews, 1985). This represents the most in-depth discussion in the primary veterinary literature on the components of clinical trials until after 1988. Five review articles identified in the searches (one each in 1977, 1985, 1987, and two in 1988) state

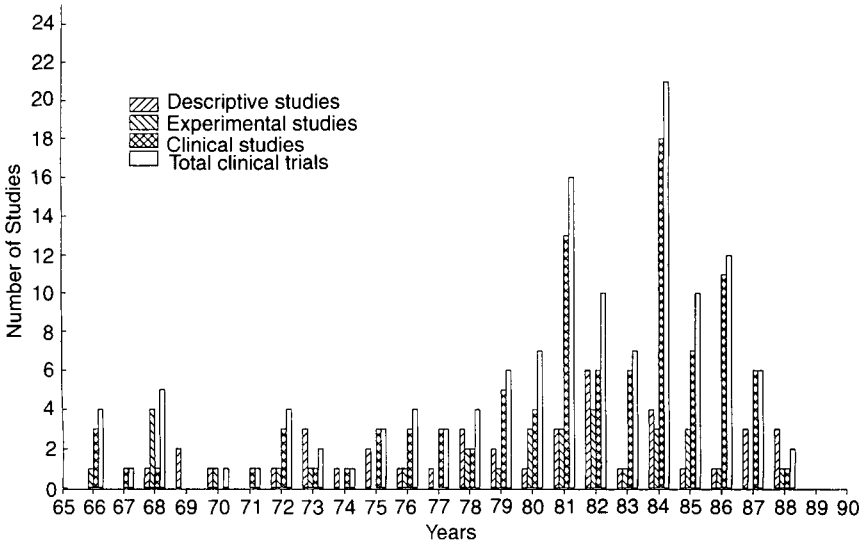

FIG. 1. The numbers of clinical trials found in database searches between 1966 and 1988.

that clinical trials needed to be conducted in the reviewed subject areas.

There were two sets of three articles (one in 1972 and one in 1980) that discussed drug trials and the role and cautions for veterinary practitioners involved (Kingma, 1972; McKinley, 1972; Williams, 1972; Allenstein, 1980; Muser, 1980; Powers and Powers, 1980). All were published in the *Journal of the American Veterinary Medical Association*. These publications focused on the implementation of new drug trials and the regulations affecting the trials, veterinarians, and sponsors. Although design was in the title, neither set of articles really addressed important design considerations except as they were affected by laws and regulations (Williams, 1972; Muser, 1980).

Random assignment of the intervention was first stated in the title or abstract in an article published in 1975. The article had one section consisting of a randomized clinical trial in client-owned animals (Averkin *et al.*, 1975). The next randomized clinical trial appeared in 1981 on foot bandaging as a treatment for foot abscesses in dairy cattle (White *et al.*, 1981). It was the first veterinary article that included the words randomized controlled clinical trial in the abstract. In any given year, not more than two clinical trials stated that they were randomized as determined by title and abstract. After reading the first

article of the year in its entirety, seven more studies were found to be randomized.

Design considerations of some importance in human clinical trials such as the use of *placebos* and *blind assessment* are mentioned infrequently in veterinary clinical trials. Both of these design elements may or may not be appropriate for any given study. In some situations where an established treatment exists, use of a placebo is inappropriate and unethical. For some types of interventions, blinding or double blinding is not possible. However, both of these techniques are important methods of reducing bias when their use is appropriate. Two of the 22 articles read in their entirety mentioned placebo use in the study. None mentioned the use of placebo in the title or abstract. No article addressed the use of placebos in veterinary clinical trials in general. One article discussed placebos but only as they pertained to therapeutics in clinical practice, not to clinical trials (Pesut and Kowalczyk, 1983). Fourteen authors stated that the studies were double blind in the title or abstract; two additional authors reported this in the materials and methods section. Three studies were single blind in design; one additional study was described this way in the materials and methods section.

An additional article published in Great Britain in 1986 again addressed the present and potential connections between general practice and industry or academe (Turner, 1986). The author proposed both mechanisms by which this collaboration could occur as well as benefits that would accrue. He specifically cited extending the existing arrangements with field trials conducted by industry in practice as one mechanism of collaboration between research and practice. Again, only this type of clinical trial, that of developing new drugs, appeared in the article.

Unlike the forced evaluation of the use of human subjects in research, there has been little written about the ethical considerations of the use of animal subjects in clinical trials. *Experimental clinical trials* involve animals owned by the research facility, university, or drug company and hence subject to the regulations regarding proper research animal care. The Animal Welfare Act of 1966 and its amendments provide national guidelines on the care and implementation of experimental protocols. Agencies such as the American Association for Accreditation of Laboratory Animal Care also oversee the welfare of animals owned by research facilities. Client-owned animals are not covered by the previously mentioned guidelines. Inclusion of these animals in clinical trials (which occurs in practice-based trials) was not

addressed specifically until after 1988. This topic will be covered in detail in a later section.

Unlike clinical trials in humans, researchers conducting clinical trials in veterinary medicine have been very slow to make the jump to multicenter, collaborative projects. Likewise, a change from drug therapies in infectious disease to nondrug interventions in chronic disease has been slow to occur. Little evidence of these trends is present in the veterinary literature prior to 1988.

One review article published in 1988 discussed the types of study designs published in the veterinary literature (Smith, 1988). The article evaluated publications appearing in the second half of 1986 in *Journal of the American Veterinary Association*. Uncontrolled clinical trials were included as a separate study design from case reports or case series. Uncontrolled clinical trials were defined as case series that evaluated therapies. Nine percent (14) of the articles evaluated fell into this category. There were three nonrandomized controlled clinical trials and two randomized controlled clinical trials. None of these controlled clinical trials was called a clinical trial or appeared in *Medline* or *Cab Abstracts* in searches for clinical trials. These data reinforce the rarity of clinical trials in veterinary medicine even into the mid-1980s compared with other types of study designs. The author also commented on the importance of journal articles in keeping current following graduation from veterinary school. Therefore, the application of research-related information such as clinical trials in the practice setting is another place where clinical trials can link research and practice.

Confusion about the terminology and lack of training in the importance of basic design components of clinical trials has led to the lack of informative and accurate publication titles and abstracts. *Distinction between controlled clinical trials and descriptive studies, regardless of the intervention, is vital.* While a controlled clinical trial can provide strong evidence for the efficacy of the intervention, descriptive studies can only report the findings without being able to provide conclusive evidence of efficacy. Descriptive studies may generate hypotheses, but cannot test them. Because of the importance of random assignment in decreasing bias and for the application of most statistical tests, authors of studies that are randomized should state this in the abstract and/or title. Blinding or placebo use if appropriate should be stated in the abstract or summary. Providing this information and consistently using the term clinical trial to mean controlled clinical trial would increase the ability of readers to discriminate among the many articles

published and determine which are most likely to provide the types of information needed for their particular setting. Early veterinary clinical trials were often not up to the standards currently expected in veterinary medicine and were far behind the progress made in human clinical trials. Adequate education of those involved in the design, conduct, and publication of trials would help address these problems.

IV. Designing Proper Clinical Trials

Recommendations regarding clinical trial design in human medicine are well established. Multiple textbooks address the topic in general (Armitage *et al.,* 1983; Shapiro and Thomas, 1983; Freidman *et al.,* 1985; Meinert, 1986; Spilker, 1991), and reviews can be found in journal literature. The Society of Controlled Clinical Trials publishes a bimonthly journal that reports results of human clinical trials and periodic reviews. Most of the recommendations found in these sources can be extrapolated to veterinary clinical trials. Modifications must be made because of some differences, e.g., use of experimental rather than client-owned animals, protection of the client and patient, differences in funding sources and amounts.

A. Posing the Question

1. Identifying Objectives

The primary question to be answered by the clinical trial should be determined prior to implementation of the study. The question should be simple and clearly stated. Although secondary questions might also be answered by the study, testing the secondary questions should not compromise the credibility of the findings in support of the primary question. Since the outcome of most clinical trials is a difference (or lack thereof) in outcome between one or more groups, the question can be posed in the form of a hypothesis.

If sufficient subjects are studied, secondary questions might be posed in the form of a subgroup hypothesis (e.g., different ages, types or stages of disease). As with the primary question, the subgroups should be identified prior to implementation of the study. The decision to include secondary objectives in a study should be based on the scientific importance of the questions, the suitability of the study protocol to pursue the objectives, the likelihood of successfully answering the ob-

jectives, and the increased study costs associated with additional data collection (Friedman *et al.*, 1985; Meinert, 1986).

2. *Initial Planning*

Factors that must be considered early in the planning process include cost, sources of funding, single vs multicenter studies, and specific rewards and responsibilities of all individuals involved. A common sequence of investigations focusing on a new treatment begins with observational studies, followed by small-scale clinical trials, which in turn often indicate the need for larger-scale clinical trials.

a. Study Location. In the context of a clinical trial, a center is an autonomous unit in a trial involved in collection, classification, assessment, or analysis of data or providing logistical support for the trial (Meinert, 1986). The center has a defined function that is performed during the trial and generally devotes two or more personnel to the functions of the center. A *multicenter* clinical trial involves two or more clinics: a common treatment and data collection protocol and a common center that receives and processes the study data. All other clinical trials are *single-center*. The primary difference between single- and multicenter trials is sample size, which is generally larger in multicenter studies. The selection of a single- versus multicenter trial may depend on the primary hypothesis of the trial. A single-center trial can suffice if the sample size is sufficiently large.

Single-center trials are easier to administer. Communication costs are limited and personnel efficiency is greater compared to a multicenter trial. In addition, the patient population may be more homogenous in a single-center trial, thus providing greater internal validity (a greater ability to draw an accurate conclusion about the subjects enrolled in the study) (Friedman *et al.*, 1985; Meinert, 1986). The primary disadvantages of single-center studies are sample size and resource limitations. Study design may be compromised in order to enroll a sufficient number of subjects if the number of eligible individuals is small.

Compared to the single-center trial, the *multicenter* trial may be a more powerful research tool. However, a major disadvantage is the need to organize and coordinate several distant groups of investigators. It is critical that the multicenter trial be exceptionally organized in the documentation of methods and procedures since the costs of straying from the protocol are more likely to be high (Meinert, 1986). Each center in the multicenter trial must have a clearly identified group of clinical investigators both willing and able to follow a com-

mon treatment and data collection protocol as well as support staff and facilities to implement the study. A disadvantage of the multicenter trial may be a decreased ability to detect a difference between treatments due to the increased heterogeneity. On the other hand, because of the heterogenous population, generalizations stemming from the study can be more broadly based. The heterogeneity of the multicenter study also is conducive to studies of subgroups and consistency of treatment response across clinics. It is important to determine publication rights, chain of organization, and individual responsibilities in a multicenter study with many people involved. However, establishing these elements prior to initiation of the trial is also important in a single-center study (Meinert, 1986).

b. Financing Clinical Trials. The design factors influencing the cost of a clinical trial are delineated in Table I (Meinert, 1986). The more carefully designed and executed a clinical trial, generally the greater its cost. In contrast, the cost can be prohibitive for poorly planned trials in which serious mistakes result in either false starts or total abandonment of a trial. Long-term trials generally cost more than originally budgeted, particularly trials that span several years. Shortfalls in patient recruitment and modifications in protocol contribute to additional costs. The addition of secondary study objectives will also increase study costs, particularly if the new objectives require additional data collection.

Costs are generally controlled at the level of the principal investigator(s). *Methods to control costs* include avoidance of:

TABLE I

DESIGN FACTORS IMPACTING COSTS OF A CLINICAL TRIAL

Patient eligibility criteria
Sample size
Outcome response measurement
Number of clinics
Need for specialty resource centers
Treatments
Blinding techniques
Complexity/frequency of data collection
Length of study (length of follow-up)
Frequency of follow-up data collections
Time for final analysis
Support for data analysis
Reporting methods

1. State-of-the-art technology when less sophisticated techniques are sufficient;
2. Overstaffing;
3. Collection of excessive data in anticipation of developing secondary objectives.

Although minimizing costs associated with the study is indicated, implementation of inappropriate cost-saving measures may, in fact, be more costly. A budget that underestimates the cost of a trial in order to increase the chances of funding should never be submitted. This includes not only reduction in patient number but also compromising on requests for technical support, one of the most critical factors for a well-organized, systematically implemented clinical trial. Similarly, support for data (including statistical) analysis should not be ignored. The budget should account for patient loss due to withdrawal, death, noncompliance, or losses to follow-up (Friedman *et al.,* 1985; Meinert, 1986).

Compared to human clinical trials, funding sources available to the veterinary profession are limited both in numbers and dollars. Funding sources include industry (i.e., pharmaceutical companies), government (e.g., regulatory or health agencies), and private foundations (e.g., kennel clubs, Morris Animal Foundation).

c. Miscellaneous Considerations. Initial planning of the clinical trial should include consideration of obligations of pet owners or collaborating investigators to the study and rewards to be realized from the study. Both of these considerations should be included in the informed consent. Rewards can include benefits to be realized from participation in the study or financial incentives. Collaborators or coinvestigators must be informed at the outset of their responsibilities and of authorship on publications and rights to present material at conferences. Recruitment methods should also be identified.

B. Selecting the Study Group

The group of animals to which the results will be generalized is the *target population.* This might be a population of healthy animals at risk for a disease if the intervention is prophylactic, or animals with a defined disease or syndrome if the intervention is therapeutic (Martin *et al.,* 1987). This must be established during the design of the trial. The animals that are actually included in the trial are the *sample population.* The *experimental unit* is the smallest number of individuals that is randomized to receive a different intervention. Each experi-

mental unit must be independent of the others. In many situations, the experimental unit is an individual animal, and therefore, each eligible animal would be randomized to a different treatment. In herd, house, farm, or tank situations, a given herd, house, farm, pen, or tank would be randomly assigned to a different intervention. The grouping depends upon the question to be answered; i.e., is the treatment effect on the individual or a population of animals important (Waltner-Toews, 1989). For example, if one treatment is assigned to all animals in one herd and the control or second treatment is assigned to a different herd, the experimental unit is the herd and there is only a sample size of one ($n = 1$) per group. The number of animals in the herd is irrelevant (Martin *et al.*, 1987). If the effect of intervention on an individual animal is important, it is inappropriate to allocate individuals to a treatment group and then house them together (separately from the control group) since the animals in the one pen are not independent. The choice of experimental units is dependent on the primary hypothesis to be tested and the practical constraints of the situation. The analysis must be done in a manner consistent with the choice of experimental units. This means that if a pen of eight pigs was an experimental unit, then one measurement of the outcome would represent that pen, not eight measurements (i.e., not one from each pig).

1. Methods of Recruitment

An estimate of the number of patients who meet the specific entrance criteria might be obtained from a review of hospital records. Each collaborating investigator may be able to provide an estimate of patients available through their participation. In contrast to human medicine, epidemiologic studies that describe the incidence of specific diseases are rare, and it is much more difficult to predict the number of patients afflicted with a disease, particularly within a geographical area. Ideally, if supportive data are lacking, the clinical trial might be proceeded by a well-designed pilot study that documents the incidence and prevalence of the disease in the available target population. Retrospective reviews of the medical records may also provide estimates of the numbers of subjects available.

The mechanics for recruiting should be established in advance. Confidence will be lost by animal owners if interviews or evaluations are delayed. Methods of recruitment may need to be modified after an initial pilot phase in order to resolve any difficulties with the process. Recruitment success should be monitored during the study to make sure it is progressing at a stable rate.

Various methods can be used to recruit patients. Some hospitals may

have a population sufficiently large to provide enough eligible patients. For larger studies, or studies involving diseases that occur infrequently, letters and announcements can be sent to veterinarians through state and local veterinary associations. Announcements or paid advertisements (depending on journal policy) can be published in veterinary journals. The journals may reach a wide population base or there may be a specialty journal that is more likely to target veterinarians who have access to qualified patients. Veterinary practitioner computer information networks might provide another means of accessing eligible patients. Animal owners targeted through breed journals or newsletters may prove to be the best source of potential candidates for some studies. Owners should be encouraged to make contact either through or in conjunction with their local veterinarian.

The investigators may need to modify their plan if the recruitment process fails to yield the expected or required number of subjects. Modification techniques include:

1. Decreasing the number of subjects to be studied, although the power of the study will also be decreased. This method is acceptable only if the original power calculation overestimated the number needed.
2. Relaxing the inclusion criteria for eligibility if the study design will not suffer. The incidence of adverse reactions to the intervention may increase if eligibility criteria are reduced. In addition, if the inclusion criteria are changed after the study has been in progress, the baseline characteristics data may become imbalanced.
3. Recycling of potential candidates—e.g., repeating measurements that rendered the patient ineligible in the hopes that the patient becomes eligible—is not recommended. Recycling does not include those patients who were initially denied eligibility because they were on a medication similar to the intervention and have since discontinued the medication.
4. The better alternative to recruitment problems is extending the time of recruitment or expanding recruitment efforts to larger geographical areas or to additional centers/hospitals. (Friedman *et al.*, 1985; Meinert, 1986).

Because of the availability of experimental animals for clinical trials in veterinary medical patients, recruitment may not be a major concern for many studies. Note that caution must be taken when extrapolating the results of an experimental clinical trial to the target population. Although study error cannot be eradicated by controlled conditions, it certainly can be reduced, particularly if the disease is experimentally induced. The investigators must consider the benefits

and detractions of using experimental animals. Discussions should include anticipated shortcomings when the results of the study are applied to animals with spontaneous disease. An appropriate sequence of studies might begin with an experimental study, followed by a similar study in client-owned animals. Current trends in the use of experimental animals are to minimize terminal studies and, when appropriate, adopt animals out upon completion of the study. Thus, animal subjects used in one study may be available for subsequent studies (assuming the facility's Animal Care Committee has approved this multiple use of the animals). If experimental animals are used in a clinical trial, investigators must be cautious to assure that the sequelae of previous studies to which an experimental animal may have been subjected will not introduce bias into the clinical trial.

2. Inclusion Criteria

The study population should be selected based on criteria that are well defined prior to implementation of the study. The criteria must be sufficiently broad to allow generalization of study results to a broader population but sufficiently narrow to allow standardization of the subjects. In addition, the criteria should be carefully considered for their impact on subject recruitment.

Criteria can be either inclusive or exclusive in nature. Reasons for the criteria should be stated in advance of the study. The more central a criterion is to the study, the more strictly defined it should be. Criteria should be based both on the question to be answered by the study and also on subject safety. Criteria development might include the following considerations (Friedman *et al.,* 1985; Meinert, 1986).

1. Subjects should potentially benefit from the intervention.
2. Selection might be based on homogeneity of the sample population (similarity in the mechanism of action of the intervention, level of disease, etc.). Alternatively, a heterogenous group might be selected in order to allow subgrouping or improve generalizability. The need for equal representation among sexes and ages should be considered as these factors are often important confounding variables. Similarly, equal representation among breeds should be considered.
3. The incidence of the outcome measurement (primary response) to be altered by the intervention must occur frequently enough during the course of the study for a change to be detected. For example, changes in heart rate can be continuously detected, while changes in seizure duration can be measured only if the patient seizures.

4. Subjects likely to react adversely to the intervention should be excluded.

5. Subjects predisposed to developing conditions unrelated to the study but which might preclude or confound measurements of the primary response should be excluded.

6. The exclusion of subjects whose owners are likely to be noncompliant may be indicated if the primary hypothesis is to determine the difference between interventions. However, inclusion of noncompliant participants maintains the realistic effect of the intervention and may be equally important to the hypothesis.

3. Sample Size and Power Estimates

The treatment effect observed in the trial (i.e., the difference in the outcome measurement in the control group compared to the treatment group) is used to predict or estimate the true effect in the target population. The treatment effect studied must be sufficiently large to be of clinical (biological or economical) importance to the target population. Generalizations of the results of the study to the target population can be incorrect in one of two ways. A type I error occurs when a difference between treatments is declared when, in fact, there is no difference. Generally, most investigators are willing to accept a type I error up to 5% of the time (i.e., a difference will be declared when there is none 5% or less of the time), or the confidence level of the study is 95%. A type II error occurs when a treatment effect occurs but is not detected (i.e., no difference is declared). Because a type I error is usually considered more serious than a type II error, investigators are willing to accept a type II error more frequently than a type I error. Generally, if information regarding the seriousness of a type I or type II error is not known, a type II error is accepted four times as often as a type I error (i.e., 20%).

The power of a study (the ability to detect a treatment difference if one is present) is one minus the type II error. Typically, the power of a study must be 80% or better to be considered valid. In general, one or two large-scale clinical trials are more appropriate than several small trials. If sample size is insufficient to allow a realistic power, a "no treatment effect" will be reported and the trial has not improved patient care. In addition, the greater the number of clinical trials, the greater the likelihood that a type I error will occur (i.e., the probability of a type I error occurring in two clinical trials with a confidence level of 95% each is 10%; the probability increases to 30% [6 × 5%] if six clinical trials are implemented).

Table II lists factors determining sample size and power calculations (Friedman *et al.,* 1985). The size of the study should be established prior to implementation of the study and should be sufficiently large to allow adequate levels of statistical significance and power. Such an approach is called a *fixed sample size design.* Most commonly, the number is based on practical considerations such as cost, patient availability, and time. Patients continue to be enrolled until the appropriate number or time has been reached. The calculated sample size is best based on estimates of sample variability, ideally determined from a population other than that to be studied (e.g., a pilot study). Overestimation of the sample size can result in an unreasonable study size and unneeded use of animals. Trivial treatment differences may be declared statistically significant in spite of being clinically unimportant. In contrast, underestimation may reduce the power of the study, i.e., decrease the ability to detect a true difference among interventions. The sample size calculation should be based on the specific statistical test that will be used to compare the response variables (interventions). The calculations can be based on formulas or tables available in standard epidemiology (Martin *et al.,* 1987) or statistical texts (Fleiss, 1981; Dawson-Saunders and Trapp, 1990).

A different approach is taken with *sequential designs.* With a classical open sequential design, patient enrollment continues until the observed difference in test–control treatment exceeds a predetermined boundary. Thus, the number of experimental units that eventually enter the trial is influenced by the results of the trial. For example, a study assigning treatments in pairs might continue to enroll patients

TABLE II

SPECIFICATIONS FOR SAMPLE SIZE CALCULATIONS

Standard information
Number of treatment groups
Type of outcome measurement(s)
Length of follow-up
Alternative objectives
Detectable treatment difference
Type I and II error levels
Group allocation ratio
Special consideration in clinical trials
Losses to follow-up
Losses to noncompliance
Treatment lag time

if the cumulative difference in outcomes measured in previously enrolled pairs is less than the defined boundary.

C. Designing the Intervention

Although the effect of the intervention is to be tested, the intervention should be selected based on careful consideration. The rationale for selection should include evidence of potential benefits and confidence that adverse effects will not jeopardize the subject or outweigh the potential benefits. The scheduling of the intervention must take into account the likelihood of patient (owner) compliance (including their ability to manipulate scheduling). The cost and availability of the intervention to the client after the study has been terminated (regardless of the reason) must be considered. Methods of blinding should also be taken into account during selection of the intervention. What constitutes successful intervention must also be defined (e.g., what percentage change in the outcome measurement must occur before the intervention is considered successful?)(Friedman *et al.*, 1985; Meinert, 1986).

1. Controlling Bias

Bias is a systematic difference from the true situation. Bias can be introduced any time during the design, implementation, and reporting of a study. Selection and measurement bias are managed primarily in the design and implementation stages of the trial, while confounding may be controlled in all stages. *Selection bias* refers to bias introduced if the groups under study differ in regard to some factor that influences outcome. Animals may be selected for inclusion in the study with different susceptibility to the outcome, different extent, duration, or treatment of disease, or with some unforeseen lack of similarity (Budsberg, 1991). Losses to follow-up, especially if they are unequally distributed between treatment groups, is a common cause of bias. Randomization, restriction (limiting the study group to a more homogenous population), matching, and stratification are all mechanisms to decrease selection bias. *Measurement bias* results from dissimilar measurement methods among groups or from inaccuracy. A common problem occurs if one treatment group is more likely to have the outcome determined than another group (Fletcher *et al.*, 1988). This can be managed by strict criteria for outcome and follow-up protocols and blinding of group assignment. Bias due to different levels of expertise is likely to occur if the levels of skills used in administration of the intervention (e.g., surgery) varies (Budsburg, 1991). *Confounding bias*

occurs when two factors or processes are associated and one distorts the effect of the other. For example, age of a dog could be a confounding factor if you are evaluating the effect of caloric intake on disease outcome (e.g., osteochondritis dissecans) since age is associated with caloric requirements and with the time period when this disease occurs. Age, breed, and sex are common confounders in veterinary medicine and should be considered as possible sources of bias in clinical trials. Confounding by unforeseen confounding variables is controlled by randomization. Methods used to control selection bias may also help. Confounding can be accounted for by use of multivariate or stratified statistical analyses if the level of the confounding factor can be measured. Overall, control of bias is accomplished in clinical trial by four primary considerations: comparability of study groups, the type of control, the methods of randomization, and the use of blinding.

a. Comparable Study Groups. A valid test of comparison assumes that the baseline characteristics of the comparison groups (i.e., control and treatment) are similar. Ideally, the characteristics are identical in all aspects, although this is almost never achieved in practice. In such cases, homogenous patients can be randomly assigned to the test and control treatments. Alternatively, patients can be paired, with one going to the control group and the other to the treatment group, prior to enrolling in the study. The pairs should be matched on important baseline characteristics. Unfortunately, the number of patients necessary for suitable matching is large and tends to render such studies unreasonable. Stratification procedures prior to implementation of the study can successfully control the distribution of a few variables. Comparability can also be partially met by selecting appropriate criteria for patient selection (Meinert, 1986).

b. Types of Control. Rarely, results of an intervention are so dramatic that a historical control will suffice for comparison. However, the wide spectrum of clinical signs associated with a disease and the variability in clinical response to interventions generally necessitates a defined control or comparison group. Several types of control groups can serve as comparison to the group receiving the test intervention. Occasionally, a clinical trial attempts to study a disease that occurs so rarely that a sufficiently large population cannot be identified and studied within a reasonable time frame. In such instances, an observational study design such as the case-control study (which is useful for rare diseases) may be possible, but this is no longer a clinical trial.

A *randomized clinical trial* involves at least two treatment groups, one test and one control; concurrent enrollment; and follow-up of both treatment groups. The method of treatment assignment in a ran-

domized clinical trial allows neither the patient, pet owner, nor persons responsible for selection or treatment to influence the assignments (Meinert, 1986). The control can be no treatment, a negative control, a placebo, or a positive control (generally the best treatment currently available). The selection should be based on ethical considerations and availability of other therapeutic alternatives. In situations where there is a currently accepted "best treatment" available, this best treatment is almost always the most appropriate control choice because of ethical reasons.

The crossover design is a type of randomized clinical trial that allows each subject to serve as its own control (Meinert, 1986). The simplest design is a two-period crossover, in which each subject receives either the intervention (A) or the control (B) in the first period, and the alternative in the succeeding period. The order in which A and B are given to each subject is randomized, with approximately half of the subjects receiving each intervention each treatment period. The major advantage of the crossover design is that smaller sample sizes can be studied since subject variability is minimized by using each subject twice. The crossover design is limited by the assumption that the effects of the treatment in the first treatment period will not carry over into the second period and that treatment order is not important (Meinert, 1986). These assumptions are inappropriate if the intervention cures the disease or if the disease progresses or becomes irreversible or fatal. Statistical tests of the assumption of no period–intervention interaction are not strong enough to detect violations of the assumption unless a large number of subjects are studied or more complicated patterns with more than one period of treatment with each intervention are used. Since the mean response of the AB group is compared to the mean response of the BA group, subject variability must still be considered in tests for carryover. Thus, the crossover design is useful in very specific instances but should not be undertaken without careful consideration of carryover effects, changes in severity of disease with time, and number of treatment periods.

A factorial design compares two simultaneous interventions with a control in a single experiment (Meinert, 1986). In simple terms, two studies are accomplished simultaneously: each intervention is studied alone and both are studied following concurrent administration. An advantage to this study is that two treatments can be studied with the same number of units needed to study one treatment. In addition, the interactions between treatments (i.e., additive, synergistic, or antagonistic effects) can be studied with this design. The design is handicapped by the impact of interaction on sample size. The effect of each

intervention may differ depending on the presence (or absence) of the second intervention. As with crossover designs, the power for testing for interaction is less than the power for testing the main effects of intervention. Thus, the total sample size must be increased. The larger the interaction, the smaller the sample size needed to detect the interaction.

Withdrawal studies begin with baseline measurements collected while the patient is receiving the intervention. The outcome is measured after the intervention is changed (e.g., withdrawn or decreased) (Meinert, 1986). The design is used most appropriately to assess response to a decrease in dose or discontinuation of an intervention following chronic administration. The benefits of the intervention are understood prior to a withdrawal study; the duration of benefit of the intervention becomes the focus. For example, a withdrawal study might establish the need of lifelong therapy, or assess the efficacy of an intervention that has never conclusively been shown to be beneficial. Withdrawal studies should be implemented according to the same standards (randomization, blinding, unbiased assessments, and appropriate data analysis) that are applied to other clinical trial designs.

A nonrandomized concurrent trial includes a control group selected and evaluated at the same approximate time that the treatment group is evaluated. However, subject allocation to the two groups is not random, as might occur in patients at one institution receiving one intervention and patients at another institution receiving an alternative (or no) intervention (Meinert, 1986). Chance does not dictate the intervention to be assigned, which may be desirable to pet owners. The possibility of groups not being comparable is a major risk associated with this design. For example, the level of supportive care routinely provided by one institution may result in a more favorable outcome. Also, unknown confounding variables will not be evenly distributed in this type of study. There is rarely justification for this type of clinical trial.

Historically controlled studies compare outcomes in a group of subjects receiving the intervention with those outcomes measured in a previous series of comparable subjects (Friedman *et al.*, 1985; Meinert, 1986). Thus, the studies are nonrandomized and nonconcurrent. Historical data are obtained from one of two sources: the literature or medical records. Collection of data from comparable groups in the medical literature may be difficult if not impossible. Collection of data from medical records is most likely to be successful if the data were recorded at a large clinical center. Data collected as part of a different clinical investigation might serve as a control group for a future study.

The major advantage of the historically controlled study design is

that all subjects can receive the intervention. Historically controlled studies might be preferred when therapeutic benefits of an intervention are considered likely, a positive control does not exist, and denial of the intervention to a placebo control group is considered unethical. In general, historical controls are only appropriate if there has been a standard, well-designed treatment protocol used, good data are available, and there are predictable levels of disease or response for all animals enrolled currently or historically in the study. A historically controlled study may be the only feasible design for studies in which the diagnosis of a highly fatal disease has been clearly established. Study subjects may be more willing to participate if all subjects receive the intervention. Because all subjects can be assigned to receive the intervention, the study can be completed in half the time necessary to complete a randomized study.

Historically controlled studies are extremely vulnerable to bias. Changes in population or management over time may result in outcome improvement that may be inappropriately attributed to the intervention. Improvements in diagnostic techniques or preventative health care can cause changes in the frequency and stage of disease diagnosis as well.

c. Allocation Process: Methods of Randomization. Randomization implies assignment to (or selection of) a treatment by a random process. The randomized studies are comparative studies with an intervention group and a control group. Such studies are considered the gold standard for clinical trials. The process of randomization assures that all subjects are equally likely to be assigned to either the intervention or the control group. Randomization offers several advantages over other methods of group assignment. First, and most importantly, randomization decreases the potential for bias. Second, groups become comparable when randomly assigned as characteristics of the groups tend to become balanced. Note that covariates may be imbalanced even in randomly assigned groups; stratified randomization or analysis may compensate for such imbalances. Third, randomization satisfies one of the assumptions needed for the validity of most statistical tests (Friedman *et al.*, 1985; Meinert, 1986). An appropriate randomization (allocation) method should be characterized by the following:

1. The patient, pet owner, clinician, or clinic personnel are unaware of the treatment group until the information is needed;
2. Future assignments cannot be predicted based on past assignments;
3. The methods of generation and administration are reproducible;

4. The process is based on known mathematical properties;
5. A clear trail of assignment is available;
6. Departures from the assigned method of allotment can be detected (Meinert, 1986).

Any method of group assignment that can be accessed prior to patient enrollment is susceptible to bias. This includes:

1. Odd–even assignments (such as patients assigned to one group if enrolled on odd dates, and another if enrolled on even dates);
2. Every other assignments, in which every other patient is enrolled in the second treatment group;
3. Coin flips (often not a 50:50 chance of heads or tails and easily manipulated). Sealed envelopes containing group assignments may be acceptable, but remain vulnerable to manipulation if contents are known to clinic personnel (Meinert, 1986).

The randomization scheme to be used should be defined and written down prior to implementation of the study. The report should be sufficiently detailed to allow an outside reader to repeat the procedure. Publication references (citing the source of numbers used) and the order in which numbers were read should be included. Once written, several safeguards might be implemented to assure adherence to the procedure. These include avoiding the use of schemes that are highly predictable (such as using blocking units that are small), maintaining blinding procedures, designating the responsibility for treatment assignment to personnel not included in the treatment loop, and maintaining a clear audit trail of assignment. Several methods can be used for random allocation of subjects.

Fixed allocation procedures assign the intervention to subjects based on a prespecified probability that should be equal among the subjects and should not alter as the study progresses (Friedman *et al.*, 1985; Meinert, 1986). Occasionally, an unequal allocation ratio may be used (e.g., two animals are assigned to the treatment group for each animal assigned to the control group) when information regarding the toxicity or side effects of an intervention are of interest. If less information is needed from the control group, an unequal allocation may be appropriate. Even if subjects are unequally allocated to a control and intervention group, blinding procedures should be based on the assumption of equality so that there is no indication to pet owners or investigators that one intervention is preferred over another.

a. *Simple randomization* is best exemplified by the toss of an unbiased coin each time a subject is to be randomized. The disadvantage of

this system has already been discussed. Alternatively, the coin can be replaced by a random digit table consisting of digits 0 through 9 which are arranged by rows or tables. Each digit is equally likely to occur if a row or column is randomly selected. For example, intervention A might be assigned to each even digit assigned to a subject; intervention B would be assigned to each odd digit assigned to a subject. Computer programs producing algorithms are available for most digital computer systems. Randomization procedures can be easily adapted to more than two groups. Although simple randomization procedures are easy to implement, a major disadvantage is the possibility that at any point in the randomization, allocations may be disproportionately balanced even though each group will be in proportion by the end of the study. Alternating assignment of subjects to intervention A and intervention B (or control) is not a method of randomization since only the first subject is randomly assigned. The major criticism of alternate assignment is that in unblinded studies (and even some blinded studies), the investigators know the next assignment, which can lead to bias.

b. *Blocked randomization* is used to avoid the imbalance that might be encountered using the simple randomization procedure. Using blocked randomization, at no time during the randomization will the imbalance be large. At certain points, using this procedure, the number of subjects in each group will be equal. With blocked randomization, subjects are randomly assigned with equal probability to each of two treatment groups. However, for each block of a predetermined, even size, one-half of the subjects are assigned to A and the other half to intervention B. If three interventions are studied, each block should be divisible by 3, with each intervention receiving one-third of the subjects. In order to avoid bias in assigning the last subject of a block, the blocking factor should not be known to the study investigators. Alternatively, the blocking factor can be altered during the course of the study as long as the previously described techniques are maintained. Blocked randomization can complicate data analysis since the statistical analysis should reflect the randomization process. Blocking provides balance between groups, thus increasing the power of the study.

c. *Stratified randomization* is based on prognostic factors that are measured either before or at the time of randomization. If a single factor is used, two or more subgroups are identified. Several factors (e.g., age, species, sex, center site) can be used to stratify subgroups; the total number of strata is the product of the number of subgroups in each factor. Thus, the stratified randomization process involves mea-

suring the level of the factors for each subject, determining the stratum to which the subject belongs, and randomizing allocation within the stratum using simple or blocked randomization procedures. Smaller studies with a high probability of heterogeneous subjects may benefit from stratification provided that there is not too much heterogeneity that will result in very small numbers of subjects in each strata. Only important variables should be used to identify strata. The power of a study can be increased if stratification procedures are included in the analysis. Variability in group comparisons is reduced by stratification since smaller group differences in response variables can be detected. Stratification of data can occur after data have been collected, particularly if the study is large.

Nonrandom allocation methods were developed as alternatives to fixed allocation methods due to concerns regarding the ethical nature of randomization procedures. *Adaptive allocation* allows group assignment based on prior knowledge of treatment efficacy as well as current results of the ongoing trial. Assignments of each new unit to a group are based on response of the previous unit to its assigned intervention. "Play-the-winner" is a method of adaptive allocation in which units continue to be assigned to an intervention as long as the last unit assigned to the intervention responds favorably (Budsberg, 1991). If a failure is observed, the alternative treatment is used and continued until treatment failure occurs again. Thus, the most efficacious treatment is ensured to each unit. Disadvantages of this method become obvious as the treatment difference between the groups decreases (i.e., no assurance that one treatment is more beneficial than the other). The definition of "failure" must be strictly defined in order to avoid subjective bias in assessing results (Martin *et al.*, 1987). Overall, nonrandom allocation methods are controversial and are not recommended in the majority of veterinary clinical trials.

d. Blinding Procedures. Collection of data that are free of bias is difficult. Treatment-related bias is particularly important to reduce since it can inappropriately cause or obscure a treatment difference. *Blinding procedures* are intended to protect against treatment-related bias. Blinding is used to withhold treatment assignment or other information from one or more individuals as a means of improving the objectivity of the study. It is particularly important in trials for which outcome measurements are subject to measurement or assessment errors. Bias affecting study treatment, data collection, reporting, or analysis procedures can be minimized using appropriate blinding procedures. In an *unblinded* study, both the animal owner and the clinician have knowledge of the treatment assignment. In contrast,

either the animal owner (most common) or the clinician is aware of the treatment assignment in a study that is *single-blinded.* With a *double-blinded* study, neither the animal owner nor the clinician have knowledge of the treatment assignment (Meinert, 1986). Data can be collected in a blinded fashion in unblinded or single-blinded studies if the person who collects the data needed for assessing study treatments is blind to group assignments. The person performing the data analysis can also be blinded to treatment.

Blinding is possible only if all treatments can be given in the same manner and if knowledge regarding treatment identity is not necessary in order to provide appropriate patient care. Blinding procedures are most commonly used in drug trials where bottling, labeling, and dispensing of test drug and control can be done in an identical fashion. If more than one drug is to be studied, two different placebos may be necessary: one to mimic each drug. Gelatin capsules and enteric coating are two methods by which treatments can be reformulated to mimic one another. Bottles should be similar in appearance; relabeling should be by the same facility. Treatments are usually dispensed by bottle number. Although the simplest method of dispensing is to label all bottles bearing the same treatment similarly, once a single bottle is unblinded, all bottles bearing that number then become unblinded. A new number for each bottle may be indicated for clinics that have more than two patients participating in the study (Meinert, 1986).

The efficacy of blinding procedures might be evaluated at the end of the study (or as follow-up reaches completion for each experimental unit) through a questionnaire. The questionnaire should, in a confidential manner, establish the pet owner's or attending clinician's knowledge regarding the intervention assigned to that unit. These results should be reported in the publication to allow the reader to evaluate the results in light of the efficacy of the blinding procedure.

2. Designing the Regimen

The dosing regimen, including route of administration, frequency, and dose should be clearly stated prior to implementation of the study. Dose–response relationships should be identified prior to establishing the dosing regimen; alternatively, more than one dosing level can be included as a group or as a subgroup. Care must be taken that each group is independent of one another, and other than the difference in dose, the groups are treated similarly. Sufficient sample size must be maintained with the addition of new groups or subgroups. Additional treatment regimens or therapeutic manipulations that will or will not be allowed must be specified. Differences in disposition kinetics be-

tween interventions may necessitate different dosing regimens between treatments. Attempts should be made to minimize these differences (i.e., use of therapeutic drug monitoring) and to include blinding procedures that minimize bias based on dosing differences. The regimen must be considered with client compliance in mind. Multiple doses and unwieldy methods of administration should be avoided. Methods to assure compliance (such as therapeutic drug monitoring or drug accountability) should be identified.

D. Follow-up

Follow-up refers to the process of periodic client contact after the client's animal has been enrolled into the trial. Follow-up should provide a method of administering the assigned treatment, observing its effects, collecting the data, and if indicated, modifying the intervention. *Closeout* is the process of termination of regular follow-up, including cessation of treatment. *Post-trial follow-up* can be used to collect information of the outcome measurements (primary or secondary) after closeout is completed (Meinert, 1986).

1. Outcome Measurements

Outcome measurements are measured during the course of the study and will define and answer the study question. The measurement can be categorical or continuous, but should be well-defined, discreet, and established prior to implementation of the study. Examples include a clinical event (rate of death or disease event, such as pancreatitis or seizure) or a surrogate outcome measurement (e.g., change in blood pressure, hypoglycemia) (Meinert, 1986). The rate of occurrence of the outcome event will affect the power and duration of the study. Studies dependent upon surrogate measures generally require less time for data collection than those dependent upon a clinical event (Meinert, 1986).

Preferably, a single response variable answers the primary question in order to increase the likelihood that a statistical difference will be detected. The variable should be capable of being studied in all subjects and must be measured in the same manner for all subjects. Measurements should be precise and accurate. The variable must be measured as completely as possible. Studies based on long-term follow-up of patients are at greater risk for failure because data may not be collected.

If a single response variable is not sufficient, several primary variables can be listed. Alternatively, events or variables can be combined

to make a single response variable, particularly if the occurrence of one of the variables is infrequent (Meinert, 1986). However, only one of the combination events should be counted in each subject. Response variables that reflect multiple events (such as multiple seizures in the epileptic patient) can be difficult to analyze correctly. Rather than simply establishing the mean numbers of events for all subjects studied, alternative methods of assessment should be used since the events any one patient experiences are not independent of one another. Subgrouping subjects according to the type or number of events may be an acceptable alternative. In the case of food-producing species, a relevant outcome measure may include an indicator of productivity.

Assessment of outcome measurement must be unbiased. Blinding methods should be used for assigning interventions. If blinding is not used, bias can be reduced, although not consistently avoided, if outcomes are measured by individuals not aware of the study group. For example, administration of the intervention by one investigator and measurement of outcome by another can be biased if the pet owner is aware of the intervention. The use of objective response variables that are not subject to interpretation will also help reduce bias.

2. Adverse Effects

The potential risks of an intervention must be monitored during a clinical trial. In contrast to the primary outcome, multiple adverse reactions may need to be monitored and reported during the course of the trial. The adverse reaction is most appropriately defined prior to implementation of the study. Clearly, it is not possible to define all possible adverse reactions. However, adverse effects most likely to result from the intervention and likely to be clinically important can usually be anticipated. Their description and manifestations (e.g., clinical signs or laboratory effects) can be well delineated prior to the study.

Assessing the true frequency of an adverse reaction is difficult. Adverse effects can be detected because their occurrence was volunteered or was reported as a result of direct questioning. Animal owners are less likely to report an adverse response if they are not in compliance with the study protocol. Adverse reactions that occur with a low frequency or that have vague or subtle signs are easily overlooked. The duration of a trial will impact the assessment of adverse effects. Drug reactions are often time dependent. An adverse response may vary with patient characteristics, such as age, sex, or breed, further complicating assessment.

Adverse effects can be reported four ways:

1. The incidence on a per-animal basis can be reported;
2. The severity can be estimated (e.g., generation of a severity score);
3. The frequency that study subjects are affected (e.g., the number of episodes of vomiting) can be established for each animal;
4. Time patterns of the reactions can be identified (Friedman *et al.*, 1985; Meinert, 1986).

The clinical trial can be an excellent means of establishing the safety of a specific intervention. The expected frequency of an adverse reaction can be reported, although which patients will react adversely cannot be predicted. *Note that it is unlikely that uncommon adverse reactions will be detected by a clinical trial.* In addition, subjects participating in a clinical trial are not truly representative of the population since they were likely to be excluded from the study if they were predisposed to adverse reactions. Long-term follow-up after completion of the trial should be considered to better assess the frequency of certain types of adverse reactions.

3. Quality of Life

In some cases, studies focus on quality of life instead of morbidity or mortality as an outcome. Thus, assessment of the animal's quality of life may become an important response variable. Quality of life is particularly important for trials in which the intervention has a potential effect on symptoms rather than mortality, or if the intervention is likely to cause a high incidence of unpleasant adverse reactions. Quality of life assessment is also important in clinical trials that assess preventative interventions, or when the benefit of an intervention is fewer adverse reactions compared to a standard, currently available intervention. Defining quality of life in human medicine has been studied extensively and is based on social, physical, emotional, and intellectual function of the subject. In veterinary clinical trials, assessment must be based on animal owners' perceptions or on indicators of health as assessed by the veterinarian. Perceptions are subjective. What constitutes a sense of general well-being to one animal owner may be entirely different to another animal owner. Socioeconomic, demographic, and age differences will affect owner perception. Distinguishing the effect of the intervention from the effect of the disease on the quality of life may be particularly difficult with long studies. In some instances, the distinction may not be important since an animal owner may withdraw from the study regardless of the cause of poor life quality.

4. Subject Compliance

Noncompliance results in underreporting of both therapeutic and toxic effects of an intervention. Several methods can be established before the study to compensate for noncompliance. The study might be designed so that each subject is studied in a clinic since noncompliance is much more likely in animals not hospitalized. Calculations of sample size should be based on a realistic estimate of noncompliers (Meinert, 1986). The study design should be as short as feasible and the intervention should be simple. Multiple drugs or doses and differing doses or schedules should be minimized. Selection of study subjects might be based in part on clients who are likely to follow the study protocol. The informed consent should be as informative as possible to facilitate the client's understanding of the importance of compliance. The informed consent might be accompanied by an information brochure. Maintaining close contact through office visits or telephone calls (e.g., to remind clients of visits) will encourage compliance. This is particularly true if continuity of health care occurs through contacts. Monitoring compliance is critical in a clinical trial since noncompliance to either the intervention and/or the study protocol can profoundly affect the results. Monitoring is more difficult when the intervention is administered by the client. Measures of compliance might include pill or capsule counting at each visit or refill, laboratory measurements (i.e., drugs, physiologic response variables), interviews, or client records. The effectiveness of each method varies.

5. Data Collection

Data can be one of two types. *Categorical data* describe characteristics of an individual that are discreetly grouped such as breed and type of housing. Ordinal categorical variables have categories that are related to one another in a progression of size or severity (e.g., mild, moderate, or severe disease and lameness or body condition scores). Dichotomous categorical variables have only two categories—presence or absence of disease.

Continuous data can be measured on a continuous scale (e.g., weight, height, age, volume, etc). Duration, survival time, and serological data (often logarithmically transformed) are other examples. Data represented by dates are usually transformed into time intervals and analyzed as continuous data (Bigras-Poulin, 1989).

Data collection is often the weak link in a clinical trial. The quality of data is a major determinant of the quality of the trial. Sources of

data in a clinical trial include interviews, questionnaires, physical examinations, and laboratory measurements. Several problems can arise with data collection. Causes include inability of the animal owner to provide the data (including poor instructions by the investigators), inadequate physical examinations, laboratory mishaps, or improper data entry. Incomplete data are often irretrievable, and erroneous data may not be recognized. Measurement error may be due to variability between observers and decreases the power of the study. Errors in data collection can reflect vague definitions, inadequate laboratory methodology (e.g., old technology), poor sample handling, unreliable or invalid questionnaires, poor training of personnel, and carelessness (Meinert, 1986).

Data collection can be improved by clearly explaining definitions. This is helpful for both entry and diagnostic criteria and in determining outcome measures that are subjective (such as the description of a seizure). A well-designed form for data collection can minimize misunderstanding. Forms should be as short as possible, yet contain all information necessary to collect the data, or refer to a page number in a manual that contains the information (Spilker and Schoenfelder, 1991). Forms should be numbered for identification. Color coding can be used to distinguish among the different types of forms. Forms should be pretested if possible. If animal owners are responsible for data collection, they should be trained or tested for their ability to respond appropriately. Method of reporting results should be identified, consistent, and stated in advance. If necessary, staff and investigators should have training sessions. Measurements should always be collected in the same manner; these methods should be tested to assure results are consistent. If possible, data should be collected without knowledge of group assignment in order to reduce bias.

Extreme results should always be flagged and checked. Laboratory error should be considered when appropriate. Samples should be saved in case reevaluation is indicated. Recording equipment, scales, measures, etc. should be evaluated periodically; these performance checks should be recorded. The quality of drug preparations should be measured throughout a drug study. Labels should be periodically monitored for mislabeling. Medication should also be examined for damage, disintegration, etc. Storage life of medications should be adhered to. Dispensing of medications should be monitored with emphasis on proper drugs in the correct dose, sent to correct recipient, and with the correct code placed on the outside label. If weaknesses are identified at any stage of data collection, modifications should be made and recorded.

6. Data Monitoring

Ethical responsibility dictates that collected data be monitored for both benefits and adverse reactions intermittently during the trial. If adverse reactions are evident, or if one treatment is clearly better than another, early termination of the study should be considered. Monitoring can indicate that data collection is insufficient to clarify questions that have arisen during the trial (i.e., toxicity or benefits need to be better documented). Various methods of repeated or multiple testing (sequential analysis) of accumulated data are available (Meinert, 1986).

7. Length of Follow-up

Investigator and client enthusiasm for the trial is likely to be maintained if the trial is short. Interest can be maintained among investigators by periodic meetings. Understanding of the trial goals and protocols is critical to maintenance of client interest. The perceived importance of the follow-up visits and the potential health benefits or side effects will influence client participation. Client interest is further encouraged if staff are courteous and if the data are collected in a clinic that is pleasant and convenient. Written and telephone contact will also encourage interest.

8. Losses to Follow-up

Follow-up loss occurs whenever a datum is not collected at the allotted time as defined by the study protocol. The loss may reflect failure of the staff to collect or record the information or failure of the client to provide the datum either by recording the information or returning for appropriate measurements. Decisions must be made in the design phase about whether to include variables in the analysis that have missing data because of losses to follow-up. Reasons for losses should be established whenever possible to determine if the losses were due to the treatment protocol. In some statistical analyses (e.g., survival analysis), data collected up until loss to follow-up can be used.

9. Closeout

Depending on the length of the trial, closeout can be emotional for both the trial investigators and client. The details of closeout should be established prior to the first patient termination. The information collected at closeout should include that collected at follow-up. In addition, the date that the client was informed of the intervention (if blinded) and the date that treatment was discontinued (if appropriate) should be included. The method of discontinuation should be consid-

ered. Abrupt withdrawal of drug therapy may be inappropriate. Other considerations include: the recommendations to be given to clients or participating veterinarians regarding subsequent or alternative treatments (preferably based on the results of the trial); assurance that clients have alternative and reasonable sources of the treatment; methods by which the care of the animal can be transferred to the client's veterinarian; and assurance that clients have had ample opportunity to ask questions regarding the trial, its outcome, or subsequent care. Baseline and follow-up data might be summarized during closeout. If blinding procedures were used, methods of validating the blinding procedure should be considered. Finally, the need for post-trial follow-up should be considered. The decision should be based on the aims of the study, and clients should be informed of continued communications.

10. Post-trial Follow-up

Post-trial follow-up takes place after the trial has been terminated. Justifications for post-trial follow-up might include a need to extend the period of observation for death or serious, nonfatal events or to identify disease or conditions aggravated or caused by the intervention. Assuring a smooth transition for the animal to a new maintenance treatment for chronic diseases may also require post-trial follow-up. Additional treatments instituted after post-trial follow-up may complicate interpretation. If the intervention is continued after trial termination, compliance may be an important focus for post-trial follow-up.

E. Data Analysis

Several ground rules must be adhered to during data analysis of a clinical trial.

1. Subjects used in the comparison should be counted in their original treatment group assignment. The reason for randomization is lost if subjects are not analyzed in their original assigned group.
2. The denominator for that treatment should be all patients assigned to the treatment. Subjects cannot be excluded from analysis because their treatment was not in accordance with the study protocol.
3. All events—not just those believed to be related to the disease process—should be counted in the comparison of primary interest.

Justification for exclusion of events perceived to be unrelated to the intervention or disease studied is usually subjective and easily criticized (Friedman *et al.*, 1985; Meinert, 1986).

1. Exclusions and Withdrawals

Candidates that are screened as potential subjects of a clinical trial and subsequently are not enrolled because they fail to meet the eligibility criteria are considered to be exclusions (Friedman *et al.,* 1985). Reasons for exclusion may be lack of disease or appropriate severity of disease; presence of complicating diseases; medications which might predispose to interactions with the intervention; increased susceptibility to adverse reactions; unacceptable size, age, etc.; or refusal to participate. Reasons for exclusion should be established with eligibility criteria. Withdrawals consist of subjects that are enrolled in the study but are not counted in the analysis. In contrast to exclusions, withdrawals can alter the comparability of the study groups, and thus bias the study results. Withdrawals can occur for several reasons. Unqualified subjects may be inadvertently approved and ineligibility may not be realized until after they have been randomized and received their intervention. This might happen if the intervention must be started before the baseline data can be analyzed. Policies that might prevent or reduce the effects of withdrawal of ineligible candidates on study results include:

1. Not enrolling subjects until all diagnostics have confirmed eligibility and allow no withdrawals of any candidate considered ineligible after the study starts;
2. Enroll unconfirmed cases and withdraw them in a blind fashion (with respect to group assignment and results) when ineligibility has been established based on data collected before the study started;
3. Enroll such patients prior to confirmation and allow no withdrawals.

The latter policy assures patency of the random process, but reduces the power to detect a difference if the ineligible candidates do not benefit from the intervention. Subgroup analysis on the basis of eligibility might be used after completion of the study to ascertain if the results agree regardless of eligibility status (Freidman *et al.,* 1985).

Noncompliance is another reason that patients might be withdrawn from the study. Noncompliance may reflect the choice of the animal owner or the investigator due to adverse effects, loss of interest, changes in the medical condition of the subject, or other reasons (Friedman *et al.,* 1985). Including noncompliers in data analysis reduces the ability of the test to detect differences in the intervention. On the other hand, including the noncompliers in the data may be more representative of the actual target population since noncompliance reflects a response to the

intervention. Excluding noncompliers may bias the data if the rate of noncompliance differs among groups. This may occur, for example, if the incidence of adverse reactions (which may lead to noncompliance) differs between groups. Establishing rules for noncompliers prior to implementation of the study does not necessarily avoid the possible effects of excluding their data from analysis. Thus, inclusion of data from noncompliers is probably more appropriate than exclusion.

Investigators are frequently tempted to withdraw patients because the data are poor. Poor data may reflect data collected improperly, data that are erroneous and inconsistent with other results, an outlier (the value is extremely significant from other patients), or data that are missing either by failure to collect the data or loss to follow-up (Friedman *et al.*, 1985). Although it is tempting to remove data that appear erroneous and inconsistent with other results, exclusion may bias the results and is not recommended. Extremely inconsistent results (i.e., outliers) may reflect correct results and are important indicators of the possible variability in response. Outliers should not be excluded from analysis unless the data are clearly erroneous. Analysis with and without the outlier might be compared. Loss to follow-up is more likely with long-term trials. Results may be biased if the rate of follow-up loss is different among treatment groups, particularly if the loss is due to the intervention.

2. Basic Analytical Methods

It is beyond the scope of this paper to describe methods of comparison analysis. Statistical support is integral to the successful clinical trial, and funding should be established for this portion of data analysis. Simple analysis can be used if the following conditions are met by the trial: subjects were enrolled over the same time period and subjected to the same follow-up; loss to follow-up is low and is the same for all treatment groups; and treatment groups have comparable baseline characteristics. Comparisons of proportions can be used for binary data (e.g., alive/dead or recovered/relapsed) as well as continuous data that can be converted to binary form; and for comparison of baseline or outcome measurements. Other methods can be used to compensate for more complicated analysis. Changes that vary over time and data collected from subjects whose participation in the trial varies in duration require more sophisticated analysis techniques. Changes over time can be tracked with rate calculations using lifetable methods (Meinert, 1986).

Descriptive statistics can facilitate interpretation of trial results. If

baseline characteristics of subjects are not comparable among groups, stratification procedures can be used to assure comparability for a few selected variables, although distribution among other variables cannot be controlled. Subgrouping is the simplest stratification procedure, but may result in too many groups with too few subjects. Multiple regression is an alternative, albeit more complicated method that might be used to control for several simultaneous sources of variation.

Although the *P* value resulting from conventional tests of significance is an important component of the analysis, it should not be overinterpreted. The statistical test only provides an estimate of the effects of chance on the difference between the treatment groups. Other considerations should dictate acceptance or rejection of a treatment. The decision to keep a treatment may be best based on the persistence of beneficial effects, which requires long-term follow-up after a statistically significant difference in treatment has been established (Meinert, 1986).

For many statistical tests, an assumption of normally distributed data is necessary for statistical validity. Lack of normality occurs when the outcome variable being tested has more individuals at one extreme than at the other (e.g., data do not form a bell-shaped curve if plotted). Data that are not normal might be normalized by mathematical transformations (e.g., logarithmic transformation) (Bigras-Poulin, 1989). Another option is the use of nonparametric statistical tests. Again, close consultation with a statistician early in the planning process will direct the optimal method of statistical analysis for a trial.

F. Data Reporting

1. Publication Questions (Meinert, 1986; Spilker and Schoenfelder, 1991)

a. When. A clinical trial is not complete until the information is disseminated. Publications of results should be prepared and made available as soon as possible. Although most manuscripts are prepared after closeout, in certain instances interim publications are prepared.

Interim publications provide access to study results as they occur. Additional benefits might include easier preparation of the final manuscript and greater exposure of the study to the public. However, there are several disadvantages to interim reporting (Meinert, 1986). Results that are inconclusive may be confusing. If the results are discouraging, investigator enthusiasm may wane. Most critical is the possibility of

bias in subsequent treatment assignment and data collection. Finally, data analysis and presentation may differ from and thus diminish the impact of the final report.

b. Where. Results of the study can be disseminated through publications in peer reviewed journals and presentations at national meetings. Some journals (those that focus predominantly on human medicine) will not publish papers that have been nationally presented. The time gap between presentation and publication should be minimized.

The choice of journal should be limited to refereed journals that are covered in *Index Medicus* and *Index Veterinarius*. Unrefereed journals should be avoided if they lack a critical review process. In addition, they may reach a smaller public and be more difficult to identify or retrieve. A specialty journal (i.e., *Internal Medicine, Neurology,* or *Surgery*) might be considered if the results are of primary interest to the specialty group.

c. What. The potential importance of many veterinary clinical trials is not realized because of failure to publish the appropriate information. The goal of the publication should be to provide a clear, concise description of the study (Meinert, 1986). Studies that report nonstatistically significant findings should also be published.

The clinical trial may only result in a single publication that is published upon completion of the trials. The organization of the publication will vary with the targeted journal. Typical components of the publication include the following.

1. The *title* is probably the most important component of the publication. It should be concise and as short as possible while indicating the main thrust of the paper. The term *clinical trial* should be included in the title. The title section should also include the authors, source of financial support, acknowledgments, and address for reprints. Finally, a list of key words selected by the author should be included to allow for retrieval.

2. The *abstract* is often the only part of a paper that is read, and as such should provide a summary of the paper. The abstract will be included in *Medline,* the computerized version of *Index Medicus* or other computerized data bases. The abstract should include the study purpose or objective, primary outcome measure, intervention, type of control, method of allocation, blinding procedures, number of animals enrolled and studied, and conclusions.

3. The *introduction* should be short and succinct. Its purpose is to provide a historical background for the study. Included is a literature review, what led to the initiation of the study, the study objectives, and

the rationale for the study. This might include a rationale for the study design, intervention, or outcome measurements.

4. The *materials and methods section* should be sufficiently detailed to allow readers to make informed judgements regarding the quality of the methods. Recommended contents are listed in Table III. Citation of a previously published paper describing the methods can reduce the content if the paper was devoted primarily to the design and methods of the trial.

5. The *results section* is generally the longest. Recommended contents are listed in Table IV. The crux of the paper should be represented by tables, charts, and figures that should be understood without reference to the text.

6. The *discussion* should highlight the important findings of the study. Positive as well as negative findings should be reported. The clinical implications of these results and consistency with previous findings should be discussed.

7. The *conclusion* may either stand alone or complete the discussion. The conclusions must be drawn from the results of the trial. If appropriate, the statistical power of the study should be noted if the conclusion favors the null hypothesis. Finally, a statement regarding the extent of generalization should be included.

8. *References* should be limited to those that support the rationale of the objectives and document methods of data collection and analysis. The journal of publication will dictate the organization of the references. Original articles should be referenced whenever possible. Secondary sources are acceptable if the primary cannot be found, if the primary is published in a foreign language, or if the secondary expands upon information provided by the primary paper. Checks of accuracy of title, authors' names, etc., should be based on the article itself, and not citations listed in other bibliographies (i.e., *Medline*).

9. The *appendix section* is optional and may not be allowed by some journals. Contents should be limited to information regarding methods that are too technical or detailed to include in the body of the text. Examples of information to be contained in the appendices are: details of sample size calculations; sample data forms; data collection schedules; special charts, figures, equations; consent statements; and data listings.

d. Internal Review. The submission process can be facilitated if the manuscript is reviewed prior to submission. Authors should review the paper for inconsistencies in format and style (including tables and figures), for redundancy, and for reporting deficiencies. Figure and

TABLE III

CONTENTS OF THE MATERIALS AND METHODS SECTION OF A PUBLISHED CLINICAL TRIAL REPORT (MEINERT, 1986)

Study population
Numbers
Eligibility criteria
Recruitment methods
Intervention
Description of the intervention
Method and times of administration
Description of the blinding procedures
Methods of confirming compliance
Methods of packaging and dispensing medicaments
Outcome measures
Primary and secondary
Rationale for choice of primary
Diagnostic criteria for measures
Methods for coding and classifying outcomes
Design specifications
Blocking specifications
Method of randomization
Methods to assure validity of randomization process
Length of follow-up (planned and actual)
Type I and II error protection level
Patient safeguards
Specifics regarding informed consent
Methods of monitoring data for treatment effects
Data collection and processing
Sequence of baseline and follow-ups
List of data collected
Descriptions of forms / coding procedures
Definition of missed visits and patient dropouts
Cutoff date for data reported in manuscript
Description of how missing data was handled
Description of departure for protocols
Literature citations of analytical methods
Description of analytical methods not described in literature
Data purges resulting from problems with reliability or accuracy
Quality control procedures
Data editing
Clinical laboratory and other tests
Performance monitoring
Measures used to assess study participants
Treatment monitoring
Frequency of interim analyses
Methods
Procedures for implementing protocol changes based on results
Organization
Number and location of study participants/centers
Location of data center
Funding

TABLE IV

CONTENTS OF THE RESULTS SECTION OF A PUBLISHED CLINICAL TRIAL REPORT (MEINERT, 1986)

Numbers
Patients enrolled in each treatment group
Missed examinations
Drop-outs
Patients lost to follow-up
Deaths by group
Comparison of treatment groups
Primary and secondary outcome measurements
All analytical techniques
Indicators of treatment adherence
Count of patients that received none of the assigned treatment
Count of patients that received an alternative treatment
Assessment of comparability of group baseline measurements
Treatment group comparison differences
Serious side effects
Rate of hospitalization
Other health indicators
Treatment comparison by selected baseline characteristics
Multiple regression analyses of baseline characteristics for adjusted treatment comparisons
Treatment comparisons by adherence level
Treatment comparisons by clinic
Other special analysis relating follow-up data for one variable

table numbers should match text citations. Citations should be reviewed to assure that information cited is correct. Colleagues should provide the second review. Their primary function is to identify confusing aspects of the manuscript. Total rewrites may result from this internal review. The final review by the authors should focus on the format of the journal to which the manuscript is to be submitted. The number of copies and prints submitted, title page, style, and format, etc. should match the specifications set forth in the journal's "Instructions to Authors." After publication, the primary authors should establish an archive consisting of all documents related to the paper, beginning with raw data and finishing with a copy of the printed manuscript. This information should be kept at least 3 years (Meinert, 1986).

2. Reviewing a Clinical Trial

When reviewing a manuscript that reports the results of a clinical trial, the reviewer should be unbiased regarding the results of the

trial. An opinion regarding the report should be based on the merits of the study rather than the opinions and critiques of others. The purpose of the report should be strongly considered, particularly if sponsor support was critical to the study and the sponsor stands to gain financially from the report. The information provided in a report should allow adequate review of methodology so that critical aspects of the report can be evaluated. Reproducibility of results and generalization of results to a large population (rather than a small subset) should be assessed. Exclusion of patients should be well justified. The study design should be well safeguarded against biases during the assignment and administration of the intervention and during data collection and analysis. Methods used to edit the data for errors or inadequacies should be cited. Statistical methods should be appropriately sophisticated. Major differences in baseline group comparability, dropout rates, or compliance should be evaluated for a possible role in treatment differences.

V. Ethical Considerations in Clinical Trials

The use of animals in prospective research studies is becoming increasingly important. Considerations for client-owned animals will be contrasted with experimental animals that are owned by the research facility. Included in this latter group are animals that have been donated by clients. This section will include a discussion of ethical consideration for humans, experimental animals, and client-owned animals in clinical trials. General ethical considerations for all clinical trials will also be presented.

A. Rights of Human Subjects

It is not unreasonable to expect the same concern and consideration for veterinary patients that is given human patients used as clinical research subjects. Human patients involved in clinical research are very well protected against inhumane use. The Nuremberg Code of Ethics in Medical Research (1948) emphasizes the rights of the experimental human subject (Table V). The Declaration of Helsinki (1964) as adopted by the World Medical Association went further and mandated that:

1. Such research must conform to scientific principles;
2. Design and performance of the research must be clearly formulated

TABLE V

GUIDELINES FOR THE IMPLEMENTATION OF MEDICAL RESEARCH IN HUMANS AS RECOMMENDED BY THE NUREMBERG CODE OF ETHICS

1. Voluntary consent of the human is absolutely essential.
2. The experiment should yield fruitful results for the good of society unproveable by other means of study.
3. The study should be based on a knowledge of the disease or other problem under study such that the anticipated results justify the performance.
4. All unnecessary physical and mental suffering and injury should be avoided.
5. The study should not be conducted if there is reason to believe that death or disabling injury will occur.
6. The degree of risk should not exceed the importance of the problem to be solved.
7. Proper preparations should be made and adequate facilities should be available.
8. The study should be conducted only by scientifically qualified persons.
9. The subject should be at liberty to bring the experiment to an end.
10. The scientist must be prepared to terminate the experiment if the experiment is likely to result in injury, disability, or death to the subject.

in a protocol, and transmitted to a specially appointed, independent committee;
3. Publication of results should accurately reflect the results of the study.

The Declaration emphasized the right of informed consent and specifically addressed human medical research that is combined with professional patient care. Research facilities such as academic institutions that direct clinical research in humans are guided by Institutional Review Boards whose primary charge is to assure compliance with the Nuremburg Code and Declaration of Helsinki. With minor modifications, most of the guidelines delineated in these two documents also are applicable to the veterinary patient (client-owned animal) used in clinical research. Like human medical counterparts, veterinary clinical research facilities should institute a mechanism by which adherence to these guidelines is assured.

B. WELFARE OF EXPERIMENTAL ANIMALS

Experimental animals are protected by guidelines offered by the Animal Welfare Act of 1966 and its subsequent amendments. The Public Health Service Policy (PHS) on Humane Care and Use of Laboratory Animals (i.e., the National Institutes of Health Policy) requires compliance with this act of all institutions receiving PHS funds. Most

research facilities (academic institutions) have laboratory animal care committees that assure compliance by individual investigators with the PHS policy. However, these committees are not necessarily charged with the care of client-owned animals, and often (as at Texas A&M University) will agree that research involving client-owned animals does not fall under their purview.

C. Welfare of Client-Owned Animals

In order to protect the welfare of client-owned animals, some type of review board should be in place. In a university setting, a Hospital Review Committee (HRC) evaluates clinical trials and other types of clinical research (Boothe *et al.*, 1992). The term HRC will be used in this manuscript to distinguish from Institutional Review Boards (IRB) which address human subjects in clinical research. The goals of a HRC should be:

1. To protect the patient, client, institution, and attending veterinarian(s) from the intended or inadvertent application of investigations which are inhumane or unethical;
2. To promote the advancement of science through clinical research.

The HRC functions to safeguard the welfare of the patient through the approval of proposals involving clinical research. While its mission is not to provide rigorous review of the scientific merits of a proposed study, decisions regarding the ethical nature of a proposed clinical trial may require the HRC to question the scientific basis and the scientific and statistical design of any proposal (Elliot, 1989).

D. General Ethical Considerations

General considerations on the ethical use of animals in clinical trials arise from the human research guidelines. Some of the following considerations are most applicable for clinical trials using client-owned animals. An overarching truth is that it is more ethical to perform a randomized clinical trial than to use treatments of unproven efficacy. The patient's best interest cannot be forsaken for the sole purpose of "therapeutic progress"; rather, treatment of the patient is the priority. Completion of the trial must not be rushed if it creates risks to the patient. In the study design phase, protocols should be developed for use in the situation where the risk/benefit ratio of the therapeutic intervention becomes too high during the course of therapy (e.g., the trial will be terminated, the currently recommended ther-

apy will be used). Trials should include methods to evaluate the incidence, frequency, type, and severity of side effects of test treatments. This is especially important if client-owned animals are used.

In determining the treatment protocol, if periodic therapeutic withdrawal or use of a placebo is planned, assurance must be provided that the subject's life or comfort is not threatened. The use of an inactive placebo is not appropriate (except in very unusual, experimental situations) if there is a standard treatment protocol for the disease to be investigated. The double blind technique (defined here as blinding of the investigator and client or caretaker) should be abandoned if one treatment can be clearly recognized by the investigators to be preferred because of its beneficial effects, or if a treatment entails any risks that prove to be unreasonable. The code indicating a subject's treatment must always be available to appropriate participants in case of an emergency. For trials using client-owned animals, if a client withdraws an animal from the study, assurance should be given that patient care will remain available. There should be a system of verification of results that avoids the possibility of manipulating results after the study.

Additional questions regarding the scientific merits of the study that should be answered by the investigator include the following:

1. Is there a need for the study? The answer to this question includes evaluation of the importance and clarity of the primary objective without unnecessary duplication of previous studies (in target or other species). Considerations of the applicability of the results is also important.
2. What is the justification for the study? Considerations include the inclusion of appropriate numbers of patients and controls, explicit inclusion/exclusion criteria, and ethical risk/benefit ratios.
3. Are the risks to the subject reasonable in relation to the possible benefits to the subject and/or the importance of the knowledge that may be reasonably realized from the study? A sound research design that does not unnecessarily expose subjects to risk is critical to address this question. Currently accepted or proven procedures that are already available for diagnostic or therapeutic purposes should be applied when available.
4. Has informed consent been obtained? The legal client representatives of the animal patients must be informed regarding the study and allowed voluntary choices. Informed consent should be sought from each prospective animal patient's legally authorized representative and should be properly documented.

E. Guidelines for Completing an Informed Consent

The informed consent should be perceived as a document that provides information to the owner, in layman's terms. The information should be pertinent to the study and should include anything that is likely to be important to the animal owner. This recommendation might seem nebulous and needlessly all-inclusive. However, the intent of the consent form might best be appreciated by answering the following questions: If you (or your child) were the subject of this study, what would you want to know about it? Is there anything left out of the consent form that the client is likely to get mad about when he/she finds out? The informed consent should be succinct, clear, and above all else, informative.

Bolding can be used to emphasize points that the investigator feels are particularly important to the client. The following consent form is organized for purposes of discussion. The organization should be tailored to the study in a manner that is not confusing to the animal owner.

Figure 2 is an example of an informed consent that might be used for a study comparing the efficacy of two drugs. The italicized information and underlines would be replaced with the appropriate information prior to owner's presentation. The *first paragraph* identifies the animal and animal owner. The reason for this animal's inclusion should be stated. Additional information might be included in a brochure. This should be submitted along with the protocol. Note that information in the brochure is not part of the informed consent and cannot replace the information required in the consent document.

The *second paragraph* provides information about the experimental protocol. The following information should be included:

1. The study name and study location (this might include both the central location and the site where the animal is to be studied);
2. Statement that the study constitutes research, an explanation of its purposes, and the expected duration of involvement;
3. Description of the procedures to which the animal will be subjected.

Those procedures that are experimental should be noted as such. Additional information might include the funding agency and the number of animals to be studied.

The *third paragraph* focuses on the risks and benefits associated with the study. The following must be included:

1. A description of the risks and discomforts that are reasonably fore-

seeable (this should include the clinical signs that the animal owner will recognize);
2. A description of the possible benefits to the animal and animal owner as well as other animals;
3. A description of appropriate alternative treatments.

If a placebo or negative control is included in the study, it must be clear to the animal owner that there is a possibility that the animal may receive no therapeutic benefit from the study.

Additional information might include:

1. A statement regarding the approval status of the drugs / therapies / tests to be studied;
2. A statement that unforeseen risks may occur;
3. A statement about the safety of the test intervention;
4. A description of the costs of the study assumed by the investigators (e.g., 50% of all clinical laboratory tests);
5. A description of the obligations of the animal owner.

This latter statement might include costs to be incurred by the owner for participating in the study, the number of follow-up visits to a veterinarian, record keeping, telephone calls, etc.

The *fourth paragraph* focuses on client options should an adverse reaction occur or client withdrawal be desired. Relevant information that should be included in this section is as follows:

1. An explanation of whether compensation or treatment will be available if injuries occur;
2. A statement regarding the client's right to withdraw at any time with no change in patient care;
3. Who is to be contacted in the event of an adverse reaction/injury or if questions regarding the study arise;
4. Conditions that might lead the investigators to withdraw a patient from the study (e.g., noncompliance, poor record keeping, or "escape criteria," such as a worsening of the disease being tested);
5. A statement regarding financial obligations of the client should their animal be withdrawn from the study;
6. A statement allowing the client to seek a second opinion regarding the cause of death should their animal die while participating in the study.

The *fifth paragraph* assures the confidentiality of the study. Points to be included:

INFORMED CONSENT FORM (EXAMPLE) Page 1_____

I/we, the undersigned, acknowledge that I am/we are the owners or agent of __(*animal name or number*), a ___(*age*), __(*sex*), ____(*breed*) (*species*) who has been diagnosed to have_(*diagnosis being studied*) . I/we have read and understand the attached information and questions regarding the study were answered to my/our satisfaction.

I/we acknowledge my/our willingness to enter my/our animal in the research study entitled _____ _____(*Proposal Study Title*) _____ which is funded by the ___(*Funding Agency*) __and which is guided by _____(*Investigator's name*) ____ at the Texas Veterinary Medical Center (Texas A&M University). I/we understand that the intent of the study is to ___(*goal of study*) _____. This study will include __(*number of experimental subjects*) . The duration of study is anticipated to be _____; my pet/animal's will be involved in the study for _____. The study will be conducted at _(*location where research is to take place*) _. I/We have been informed of the procedures which will be performed on our animal to validate eligibility for inclusion into this study and that these procedures may include ___(*examinations, collection of blood, other procedures*) ___.

I/we understand that the __(*methods, techniques, drugs, etc*) ___ used in this study have not been approved for use in (__ *species* __). We also understand that the_(*methods, techniques, drugs, etc*) generally are considered to be safe (or are anticipated to be safe) in our animal, but may induce some side effects (complications, etc.). The most likely potential side (undesirable consequences) effects of these (methods/drugs/etc) include but are not limited to_(*list potential adverse effects etc associated with methods/drugs/techniques being studied*) __. I/we have been informed that the currently accepted and thus alternate therapy for the animal's illness is _____. I/we recognize that benefits anticipated from our participation include _____. Our financial obligations during participation in the study consists of _____. The investigators will provide for the cost of ___(*procedures, medications, etc.*) ____. I/we understand that my/our obligations to the study will consist of _____(*delineate obligations such as record keeping, visits to veterinarians, administration of medication, financial obligations, etc*).

This research has been reviewed and approved by the Hospital Review Committee of the Texas Veterinary Medical Center. If questions arise regarding your rights, the Hospital Review Committee may be contacted through ___(*Designated representative of the Hospital Review Committee*) _____.

FIG. 2. An example of the Informed Consent Form.

In the event that complications resulting from the ____(*methods, techniques, drugs*)____ arise in our animal, the persons to contact include __(*referring veterinarian, or study investigators, names and phone numbers*) _____.I/we agree that Texas A&M University and __(*funding agency*)_____ will not be held financially liable and financial compensation is not expected in the event that the prescribed therapy fails, unexpected reactions occur to the drug or complications result from the implementation of this protocol. I/we agree that the study can be discontinued by the investigator in the event of unforeseen undesirable circumstances, but that the care of my/our animal will not be jeopardized if such an event should occur. Reasons for which the investigator may discontinue the study include __(noncompliance, escape criteria etc)___ I/we understand that I/we can withdraw my/our animal from this study at any time with no change in patient care provided by my/our referring veterinarian or the Texas Veterinary Medical Center. However, I/we also have been counseled regarding the risk that such a decision may have on my/our animal's health. In the event that the study is discontinued by the investigators or because I/we choose to withdraw my/our animal, I am/we are financially obligated to pay for any further expenses incurred if patient care is continued. In the event that my/our animal should die or be euthanized while in the study, I/we agree that a full necropsy examination will be performed by pathologists at Texas A&M University in order to identify the cause for my/our animal's illness. We understand that we are entitled to a second opinion regarding the cause of death, but we are financially obligated to pay for any costs associated with the second opinion.

I/we understand that our animal's participation in this study will remain confidential. Although publications will include data collected by this study, no individual animal will be identifiable. As the owner of the of a participant in the study, we will be kept informed of any significant findings that may benefit or harm my/our animal's health. I/we will be informed of the findings of the study in a timely period following its completion.

I agree that my/our participation in this study is on a voluntary basis.

_____	_____
Signature of Owner or Agent	Date
_____	_____
Signature of Investigator	Date
_____	_____
Witness	Date

1. A statement that assures that the data collected from the animal will be kept confidential;
2. A statement regarding notification of findings that might affect the willingness of the client to continue participation;
3. A statement regarding notification of the results of the study upon its completion;
4. A statement verifying that the client has participated in this study willingly.

Each page of the informed consent should be numbered and accompanied by a place for the client's initials next to the number.

Informed consent may be *waived* under extenuating circumstances if the investigator and an unbiased clinician (one not participating in the study) certify in writing all of the following:

1. The patient is confronted with a life-threatening situation that necessitates the use of the intervention being tested;
2. Informed consent cannot be obtained from a legal representative of the patient (i.e., stray animals);
3. There is not sufficient time to obtain informed consent from the patient's legal representative;
4. There is no alternative method of approved or generally recognized therapy that provides an equivalent or greater likelihood of saving the patient's life.

VI. Current Status of Clinical Trials in Veterinary Medicine

The current status of clinical trials in veterinary medicine was based on a review of the literature. The review took place in two phases. The first review focused on a search in computer data bases, and the second review focused on a single, recently published volume of the *American Journal of Veterinary Research.*

A. Review of January 1989 through December 1993

This review was intended to provide information regarding both the quantity and quality of clinical trials in veterinary medicine during the past 5 years. Reports were identified and reviewed for appropriateness as a clinical trial. Reports cited in *Medline* using "clinical trial" or "randomized controlled trial" as the key words were studied. The study was limited to the last 5 years (January 1989 to 1994), to the English language, and to domestic or food animals. Each citation was evalu-

ated for appropriateness of inclusion as a clinical trial. Abstracts, and when necessary, the original article were reviewed. The designation of a report as a clinical trial was considered appropriate if the report was a description of an original comparison of an intervention, a summary of clinical trials focusing on an intervention, or a review of methodology in clinical trials. Case studies and review of therapeutic agents that did not focus on reports of original clinical trials were not considered as clinical trials. The definition of clinical trial given in the introduction is used throughout this discussion. Any report that met these guidelines was considered a clinical trial, regardless of the type of animal subject (i.e., client-owned versus research facility-owned) or the source of illness (i.e., spontaneous disease or experimentally induced disease). Inclusion of studies based on experimentally induced disease or using experimental animals may be controversial, particularly to epidemiologists who focus on human studies. Since diseases are not induced in human clinical trials, rarely will the issue of inclusion of such trials be considered. In contrast, clinical trials for which the target disease is induced are not only common in veterinary medicine, but provide the basis of most comparison studies. Because the principles of implementation do not differ with the source of animals or disease, it is appropriate to include "experimental" (as opposed to "spontaneous") trials in this review.

1. Summary

Using the above guidelines, 65 reports on clinical trials were cited in 36 journals between the years 1989 and 1994. Journals in which reports were published are listed in Table VI. Among the 36 journals represented, 16 are published in the United States. *Acta Veterinaria Scandinavica, American Journal of Veterinary Research, Journal of the American Veterinary Medical Association,* and *Veterinary Clinics of North America Small Animal Practice* were the journals most frequently cited.

Of the 65 citations, 42 reported the results of a clinical trial. Of the remaining 23 citations, 13 represented a review of an intervention (most commonly drugs or vaccines) or a review of disease and various interventions. Three represented reviews of clinical trial methodology that included, in some instances, a focus on a special method (e.g., constrained randomization) or problem (e.g., single patient versus field trials; swine). Five citations were considered nonclinical trials; one was an announcement seeking prospective candidates for a clinical trial; a second was a case report.

Among the 42 clinical trials, 6 reported on interventions designed to

TABLE VI

JOURNALS REPORTING CLINICAL TRIALS AS CITED BY *MEDLINE* DURING THE YEARS 1989 THROUGH 1993

Number	Journal
2	*Acta Veterinaria Hungarica*
6	*Acta Veterinaria Scandinavica*
5	*American Journal of Veterinary Research*
2	*Annales de Recharches Veterinaires*
1	*Annals of the New York Academy of Science*
1	*Antiviral Research*
3	*Australian Veterinary Journal*
1	*Biometrics*
1	*British Veterinary Journal*
1	*Canadian Journal of Veterinary Research*
1	*Cancer Chemotherapy and Pharmacology*
1	*Cancer Research*
2	*Cornell Veterinary Journal*
1	*Current Opinions in Immunology*
2	*Equine Veterinary Journal*
1	*Electromyographic Clinical Neurophysiology*
5	*Journal of the American Veterinary Medical Association*
1	*Journal of International Cancer Research*
1	*Journal of Neurotrauma*
2	*Journal of Veterinary Internal Medicine*
1	*Journal of Veterinary Medical Sciences*
4	*Journal of Veterinary Pharmacology and Therapeutics*
1	*International Journal of Health Services*
1	*International Journal of Hyperthermia*
1	*Oondersport Journal of Veterinary Research*
1	*Seminars in Veterinary Medicine and Surgery*
1	*Tijdschrift voor Diergeneeskunde*
1	*Tropical Animal Health Products*
3	*Vaccine*
1	*Veterinary Clinics of North America, Equine Practice*
5	*Veterinary Clinics of North America, Small Animal Practice*
1	*Veterinary Parasitology*
1	*Veterinary Quarterly*
3	*Veterinary Record*
3	*Veterinary Surgery*
1	*Year in Immunology*

control parasites; 6 focused on antibacterial therapy (including establishing dose–response relationships); 2 reports focused on vaccine efficacy; 7 reported on the use of preanesthetics, anethesthetics, or their

reversal; 3 reported the results of anti-inflammatory agents; 3 reports focused on treatment of cancer; 3 compared dermatological agents; and 4 reported cardiovascular interventions. The remaining reports included comparison of surgical interventions, and a survival analysis. One of the 42 citations was an interim report.

Assessing the quality of reports of clinical trials relating to veterinary medicine is difficult. Among the reports considered of lesser quality, deficiencies reflect either inappropriate methodology or insufficient information in the report to allow adequate assessment.

2. Deficiencies in Methodology

Several deficiencies in methodology can be noted in the review of veterinary clinical trials. It was often unclear what group was being used as a control or comparison group. Although by default such studies may have had historical controls, rarely were the groups comparable. Incomparability among groups also occurred in studies using controls. Failure to identify the appropriate experimental unit was a common mistake in veterinary clinical trials. Failure to treat a herd as a single unit or housing animals in the same treatment group together are two examples. Many studies failed to randomize treatment assignments and others failed to use blind assignment and administration when practical. Studies compared baseline measurements with those following withdrawal of an intervention without randomizing to a withdrawal or control group.

The different appropriate study designs used in veterinary clinical trials were limited. Crossover studies were rare and placebo controls were not frequently reported. Groups whose baseline characteristics were not comparable were often compared; treatments (other than the test intervention) often varied within and among groups. Subgrouping or stratification were rarely used. Statistical methods were not used for comparisons in some reports.

3. Reporting Deficiencies

For some reports, the title was not representative of the study. Words such as comparison or trial were not included; occasionally, they were included even though a comparison was not made. The abstract often failed to describe the study design, control type, or blinding techniques. The methods section of veterinary clinical trial reports was the most deficient one. Often, the description of eligibility or exclusion criteria was poor to absent. Blinding methods for masking and administration (including reformulation or packaging techniques) and methods to assure compliance were seldom included. Descriptions of prima-

ry and secondary outcome measurements often were vague and their selection often was not rationalized. The diagnostic criteria for the measurements often were not provided. Methods of randomization often were poorly described, and in no report reviewed was the method to safeguard the integrity of the randomization procedure given. Descriptions of how withdrawals, drop-outs, or missing data were defined or handled, or how deviations from the protocol were handled were rare. Forms used for data collection were not mentioned. Reports rarely mentioned informed consent. Quality control methods were largely ignored, including those that assured accurate collection and transcription of data and those that validated assays used for analytical procedures. Descriptions of statistical methods were often inadequate. Within results, indicators of the completeness of follow-ups (e.g., missed examinations, drop-outs), were seldom reported. Treatment comparisons were usually reported only by the primary (or secondary) outcome measurements, and only occasionally by selected baseline characteristics, or levels of adherence. In the conclusion section, veterinary reports seldom discussed limits on generalization of conclusions or statistical power if no treatment difference was detected. These problems may be due to author's lack of knowledge or to limitations imposed by the journal.

4. Positive Assessment

Despite the deficiencies noted above, reports of veterinary clinical trials have improved in the past decade. Comparisons are the rule rather than the exception and we are becoming more sophisticated in our randomization methods, blinding techniques, and statistical analysis. As the technology becomes more sophisticated, outcome measurements are becoming less subjective. As a discipline, field trials focusing on the incidence, prevention, or treatment of infectious diseases or parasites are particularly well reported. Perhaps the most favorable indicator of improvements in the implementation of clinical trials in veterinary medicine is the number of articles dedicated to clinical trial methodology. Several textbooks focus on veterinary clinical trials, and in Canada, a symposium dedicated to clinical trials in veterinary medicine was published in 1989; several additional articles have been published since then. Several reviews have been published in sources directed toward the veterinary practitioner, an indication that clinical trials have expanded in importance. Within these publications, the term clinical trial is defined implicitly in different terms (Lulich *et al.*, 1989).

B. Single Issue Review: December 1993

Variability in reporting styles and inefficiency in recalling veterinary clinical trials from *Index Medicus* and *Index Veterinarius* complicate evaluation of recent trends in veterinary clinical trials. The intent of this review was to establish the most recent trends in improvement and to identify continued areas of deficiency in the reporting and implementation of veterinary clinical trials. This review focused on very recent publications that were likely to represent our current approach to implementation and reporting. The focus of this review was the December 1993 issue of the *American Journal of Veterinary Research*. Abstracts of each manuscript were reviewed and identified as clinical trial or nonclinical trial. If designated clinical trial, the abstract was further reviewed for content and scientific design. For selected components found to be missing in the abstract (e.g., number of animals studied; blinding procedures) or unlikely to be in the abstract (e.g., statistical methods), text of the manuscript was reviewed. Note that the absence of information does not necessarily indicate that the method was not implemented by the investigators, but may simply reflect a failure to report the information.

Of the 37 articles published in the December 1993 issue of the *American Journal of Veterinary Research*, 22 reported the results of comparisons between two or more interventions. An additional article focused on an intervention but did not use a control group for comparison. This latter article was not considered a clinical trial, but represents a common mistake made in veterinary clinical research, (i.e., the absence of controls). Of the 21 articles, one used the term "comparison" in the title. Another report used the term comparison in the title, but it represented a case–control study, and thus was not a clinical trial. Only one of the articles studied spontaneous diseases (i.e., used client-owned animals). None of the articles was cited in *Medline*. It is likely that studies using experimental animals are not considered to be clinical trials. The species represented included horse (eight); cow (five; three of these were calves); dog (five); pig (one); and sheep (two). Three compared a diagnostic intervention [infectious diseases (three) or metabolic disease (two)]. The remaining comparisons (17) were made of treatments (15 drugs, two surgeries, one bandage). One of these "drugs" was nontherapeutic (the effects of endotoxin). Among the drugs, six focused on anesthetic regimens; two focused on anthelmintics; three compared the effects of anti-inflammatory agents, and the remaining represented several other therapeutic drugs.

Within the abstract, 16 noted the study was controlled, although it was not clear that controls were studied in two articles. The remaining five noted the use of controls within the text. Five used the term "random" in the abstract when describing the control. Another five noted in the text that control was randomized. Ten failed to mention randomization. One article used the term "chance" when describing the method of randomization. No article noted the method of randomization used. Among the controls, five were positive (alternative intervention, dose, or time). Most of the remaining controls were placebo or sham surgery controlled. Six studies failed to report the number of animals studied within the abstract. In one of these six reports, it was difficult to determine how many animals were studied. It was difficult to summarize the appropriateness of grouping of animals among the studies. Often, the number of animals studied was not sufficient for the number of groups studied. Only one study reported that intervention assignments were blinded. Blinding methods were not reported for any other aspect of data collection. No study reported the methods by which group size was determined. Ten of the studies used the same subjects for comparisons, but only two reported a crossover design. Although the intent of this review is not to focus on statistical methods (this is best accomplished by a statistician), obvious inappropriate use of statistical analysis are evident in some reports. Evidence of statistical analysis (including P values) was rarely evident in the abstract. Statistical methods were not reported in two papers, although significant differences (with P values) were reported in the text.

Two studies published back-to-back exemplify some of the difficulties encountered when implementing and reporting a veterinary clinical trial using the same animals for multiple comparisons. The two studies apparently used the same experimental subjects, even though this was not stated in the report. The studies focused on an intervention designed to prevent an adverse effect induced by a challenge drug in animals exposed to two different types of anesthesia. Between the two studies, subjects (n = 5 to 7 depending on the treatment) were treated with 12 anesthetic protocols. Although the report indicates that animals were assigned to treatment groups randomly, no indication was given that the sequence of treatments was random for each animal. Statistical analysis reported for the study does not include techniques used to detect interactions among the independent variables. It is not surprising that the study found no significant differences among treatment groups. The use of statistical methods that take into account repeated measures increases the power of studies that use the same subject and might have made detection of treatment

differences easier in the studies. The studies also used *t* tests for multiple comparisons (i.e., multiple *t* tests were used to compare data within groups, but at three times: baseline, challenge, and posttreatment) which increases the likelihood of a type I error. Standard error of the mean (mean ± SEM) was reported rather than standard deviation. Use of SEM, while not common, is not unusual in veterinary clinical trials (three reports in this one volume). Because SEM is SD divided by the square root of the sample size, the number is smaller and thus more tempting to report. However, SEM is a measure of the variability of a sample of populations (i.e., the standard deviation of a set of means, each mean reflecting a different population sample). In contrast, SD is the variability of a single sample population around the mean of that sample population. As a general rule, unless multiple populations are studied, SD should be used to reflect sample variability.

Only three studies reported or described animals excluded from data analysis. Two of the studies noted that exclusion was due to death. The third study explained, but did not justify why animals were excluded. Further examination of this study reveals that 50% of the animals were excluded from data analysis because the outcome being measured in response to the intervention was too large. Without (and probably even with) a more informed explanation, the validity of this study should be questioned. Quality control continues to be a subject that is not addressed in many reports. An exception is made for reports involving quantitative assays, particularly those establishing a diagnostic intervention or studies whose outcome assessments include an analytical assay. In such instances, reports of quality assurance are becoming the rule rather than the exception.

VII. Recommendations

Despite the deficiencies in methodology and design that are evident in our most recent research journal, the veterinary profession has dramatically improved its research style. We have the desire and intent to focus on the quality and thus validity of our clinical trials. A major obstacle to improving the quality of our studies is the lack of funding. Mechanisms must be identified through which we can make available to our clinical investigators funds that will allow proper implementation of a clinical trial. Two critical points that will add substantially to funding requests are number of animals and technicians. While current review boards recognize the need, and often negatively critique a proposal for the lack of adequate numbers, these same agencies are

reluctant to provide adequate funds for appropriate technical support. Yet, as is evident in the discussion of proper implementation, no study is better than its technical support. One feasible avenue of funding for veterinary clinical trials is human medicine. As a profession, we have not emphasized the relative importance that animals with spontaneous disease might have in the evaluation of interventions intended to treat human patients with similar diseases. With current trends reducing the number of experimental animals used to study human diseases, it is timely to focus our funding energies toward support of human clinical research. This is perhaps best accomplished by proving the soundness of our research (i.e., resolving the continued deficiencies in our scientific design and reporting methods).

Many improvements can be made without a substantial increase in funds. We must expand our definition of clinical trials and improvement in methodology to include experimental animals. Our methodology will have to improve as our ability to control subject variability decreases with the use of client-owned animals and spontaneous disease as the primary subject source. Simply improving our reporting methods will enhance the soundness of our studies. We need to focus on increased sophistication of study design, specifically subgrouping and stratification procedures and statistical analysis. Despite added costs to our funding requests, we must increase subject numbers and use proper controls. Techniques as simple as random assignment must first be used, then improved. Better appreciation of what constitutes an experimental unit is indicated, particularly in multiple pet households or herd animal situations.

Development of information networks can facilitate not only recruitment of subjects for clinical trials involving client-owned animals, but also help us become more efficient in the collection and reporting of data. Because of our funding constraints, investigators focusing on clinical trials must be willing to share ideas, coordinate studies, and avoid unnecessary duplicity of studies or implementation of studies whose results are invalidated by poor scientific design. Our professional journals must set standards of acceptance, assuring that studies are valid while avoiding exclusion of reports that "don't fit the mold." Finally, as practitioners, we need to improve our ability to critique reports on clinical trials and thus become more sound in our judgment regarding the applicability of the results to our practice.

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ADVANCES IN VETERINARY SCIENCE AND COMPARATIVE MEDICINE, VOL. 39

Benefits and Burdens: Legal and Ethical Issues Raised by Veterinary Specialization[1]

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The increasing influence of specialization in veterinary medicine will bring more than improvements in medical care for animals. As the number of specialties and specialists grows, so will legal and ethical

[1]This chapter presents general information about legal principles and is not intended to offer legal advice to anyone. Laws and regulations vary from jurisdiction to jurisdiction, and legal requirements within jurisdictions are subject to change. Additionally, minor variations in factual circumstances that might seem insignificant to a practitioner can have important legal consequences. Those with specific legal questions or concerns should consult an attorney.

responsibilities faced by all veterinarians—both specialists and generalists. This chapter provides an overview of distinctive legal and moral issues presented by veterinary specialization. These issues are of enormous practical importance. Veterinarians who ignore them can face litigation and its potential aggravation, economic cost, or loss of reputation. For the profession as a whole, the legal and ethical implications of specialization pose fundamental questions about how veterinarians want to relate to patients, clients, and colleagues.

I. Veterinary Specialization and the Law

A. Overview of General Principles of Veterinary Law

1. Intentional Wrongs and Negligence

There are two major kinds of wrongdoing for which veterinarians can be sued in civil lawsuits by clients and others: intentional wrongs and negligence.

Intentional wrongs (often referred to by lawyers as "intentional torts") are actions the law recognizes as justifying a civil suit and are done with knowledge and intent. An example of an intentional wrong is disposing of the body of a deceased animal without the client's authorization. The law regards even a dead animal as the property of its owner. A veterinarian who disposes of it without permission has deprived the owner of his property rights in the animal. The law classifies this as an intentional wrong because such disposing of an animal is not accidental, but intended by the veterinarian; the veterinarian knows what he is doing even if he does not have a malicious intent (such as to make the client unhappy). Other intentional wrongs veterinarians can commit are performing a necropsy on an animal without the client's permission, defrauding a client (e.g., charging for a procedure that was not done), and defamation of character (e.g., calling a client a "worthless deadbeat nonpayer" to the client's friends). Intentional wrongs represent a small proportion of civil lawsuits against veterinarians. However, intentional wrongs are still dangerous and should be avoided vigorously by all veterinarians. In many jurisdictions practitioners held liable for committing such a wrong can be ordered to pay a plaintiff punitive damages if the practitioner's behavior has been especially egregious. Punitive damages are intended not to compensate the plaintiff but to punish the defendant. Therefore, punitive damages can be quite large and need bear no relationship to

the amount of injury or damage actually suffered by the plaintiff. Worse, most states prohibit malpractice insurers from covering veterinarians for punitive damages, which means that a veterinarian who must pay such damages must do so out of his or her own personal assets.

Unlike intentional wrongs, acts of negligence do not require the intention to perform an act that the law considers wrongful. Negligence is the failure to behave as an ordinarily reasonable, prudent, and competent person would behave under the same or similar circumstances. If, for example, I deliberately drive my car onto your lawn and through your front window, I am committing an intentional wrong. However, if I decide to read my newspaper while driving my car on the road in front of your house, do not pay attention to where I am going, and as a result wind up inside your house, this is not an intentional wrong but negligence. I have no intention of driving into your house; I would not do so if I were paying attention. The law considers my actions negligent because an ordinarily reasonable and prudent person does not read a newspaper while driving a car. This example illustrates why the great majority of lawsuits against veterinarians allege negligence rather than an intentional wrong. A veterinarian who does something that harms a patient will rarely be alleged to have intended to harm the animal. Rather, the client will usually claim that the doctor did not exhibit the degree of skill or care that an ordinarily reasonable, prudent, and competent veterinarian should have exhibited under the circumstances.

2. "Ordinary" Competence

From its beginnings in early English law the concept of negligence has required people to behave in their interactions with others in ways their peers would regard as *ordinarily* reasonable, prudent, and competent. This, it has been thought, is all the law can *require*, even if one might hope people would try to conform to a higher standard. When the concept of negligence was applied to professionals like physicians, lawyers, and veterinarians, the concept retained this minimal requirement of *ordinary* reasonableness, prudence, and competence. The concept of negligence also retained the principle that in determining whether someone is negligent, a jury usually must look to what one's peers regard as ordinarily reasonable, prudent, and competent behavior.

Thus, to avoid negligence—to perform competently as the law defines competence—veterinarians are not expected to exhibit the highest possible level of knowledge and skill. It is sufficient to perform with

a level of competence that would be regarded by one's fellow practitioners as average, that is, ordinary.

The law regards the relationship between a veterinarian and a client as contractual: typically, the practitioner promises to provide veterinary services in return for the client's promise to pay the doctor's fee. The law therefore places its requirement of ordinary competence within this contractual context. The contract between practitioner and client is considered to contain an implied representation by the doctor, and an implied understanding by the client, that the veterinarian will exhibit ordinary competence, and only ordinary competence. This representation of ordinary competence is implied into the veterinarian-client relationship even if neither veterinarian nor client are aware of it. The representation of ordinary competence is part of the contractual agreement unless there is an understanding between veterinarian and client that the doctor shall be held to a higher standard. The law does not permit a veterinarian to hold himself to a lower standard than that of ordinary competence. Moreover, practitioners can be sued by clients for negligence even when services are provided free of charge.

3. Forms of Veterinary Negligence

It is not possible to provide an exhaustive list of all the possible kinds of veterinary negligence. Any action, however unusual or unprecedented, can be negligent. In each lawsuit a jury in a veterinary malpractice trial will ask whether (1) the veterinarian-defendant conformed to the level of competence that would be expected of the ordinarily competent practitioner, and (2) any such negligence caused the plaintiff compensable injury or damage. Table I relates several common kinds or categories of negligence. These categories illustrate the broad range of actions to which the legal concept of negligence can apply. The categories have no independent legal significance; the only concept on which a jury will focus is that of negligence. Clients do not sue, for example, for "failure to take a proper medical history," but for negligence.

4. Informed Consent

As Table I indicates, one form of negligence is the failure to obtain from a client an informed consent to a procedure or service. Both the words "informed" and "consent" are crucial. Except in rare or emergency situations where it may be impossible to obtain a client's authorization, a veterinarian may not do anything to a patient without first obtaining the consent of the client or of someone authorized by the client to give consent. Moreover, this consent must consist of more

TABLE I

SOME GENERAL CATEGORIES OF VETERINARY NEGLIGENCE

Failure to take an adequate medical history
Failure to properly examine the patient
Failure to discover the cause of the patient's symptoms
Failure to obtain from the client an informed consent to the treatment or procedure to be performed
Failure to provide adequate treatment
Failure to properly monitor the patient or to schedule follow-up care
Failure to adequately instruct the client regarding the requirements of the patient's care
Dispensing the wrong drug or medication, or incorrect calculation of the proper dosage
Failure to utilize and properly interpret laboratory or diagnostic testing
Failure to make, maintain, and secure adequate medical records
Abandonment (withdrawal from the case prematurely)
Failure to keep one's knowledge current and to obtain and use equipment expected of the ordinarily competent practitioner
Failure to adequately protect the health and safety of the client or the client's family (e.g., improperly permitting the client to hold the animal during examination; failing to dispense medications in a child-proof container when there are children in the client's family; failure to advise the client to consult a physician if the animal has a zoonotic condition)
Failure to refer a case to a specialist

than assent, more than merely saying "yes." The consent must be *informed.* It must be based on sufficient information upon which to base a knowing and voluntary consent.

Although failure to obtain an informed consent is but another form of negligence, it requires some special discussion. Informed consent differs from other forms of negligence in that in some jurisdictions the veterinary profession does not set the standard for what constitutes acceptable practice.

In about half the states the standard for informed consent is the same as that for any form of negligence. In these jurisdictions a veterinarian must disclose and explain what an ordinarily reasonable, prudent, and competent veterinarian would disclose and explain under the circumstances. This is called the "professional" rule of informed consent because it bases what must be explained to a client on what members of the *profession* would judge appropriate under the circumstances.

However, approximately half the states use what is termed the "lay" rule of informed consent. In these states, a veterinarian must disclose

and explain what an ordinarily reasonable and prudent *layman*—that is, an ordinarily reasonable nonveterinarian client—would want to know under the circumstances before agreeing to a procedure or service.

What a veterinarian must tell a client might not differ significantly in states adopting one or the other of these standards. Court decisions that have elucidated the legal concept of informed consent indicate that in ordinary, that is nonemergency, situations, a practitioner should reveal to a client the diagnosis, the nature and potential consequences of the patient's condition, approaches that would be considered efficacious or reasonable by the profession generally, and the reasons for one's recommended approach. A clear difference between the "professional" and "lay" standard concerns the kind of testimony a jury must hear to determine whether a defendant veterinarian obtained an informed consent. In states adopting the "professional" standard, a jury must base its determination on the testimony of expert witnesses, that is, veterinarians who testify regarding what the ordinarily competent practitioner would explain. In states using the "lay" standard, no expert veterinary testimony is required for a jury to determine whether a practitioner obtained an informed consent. Jurors are, by definition, ordinary lay people. In these states jurors are therefore allowed to decide for themselves whether a veterinarian conveyed information an ordinarily reasonable client would want under the circumstances.

B. How Specialists Are Held to Higher Standards

With regard to intentional wrongs, the law treats specialists and general practitioners identically. Specialists have no stronger obligation than generalists not to commit intentional wrongs, and the very same acts constitute the commission of an intentional wrong whether done by a specialist or generalist. Thus, if a veterinarian performs a necropsy without authorization from the animal's owner, the wrong of "conversion" (the civil law analog of theft) is committed, whether the doctor is a specialist or a generalist.

1. Higher Standards of Competence for Specialists

However, with regard to negligence, the law imposes much higher standards on specialists than generalists. This happens in two ways.

First, the law of negligence applies its requirement of "ordinary" competence differently to specialists and generalists. A generalist is

required (with variations discussed below) to perform as any ordinarily reasonable, prudent, and competent generalist would perform under the same or similar circumstances. In contrast, to avoid negligence a veterinary specialist must perform as would any ordinarily reasonable, prudent, and competent *member of his or her specialty* under the same or similar circumstances. What the veterinary profession considers ordinary competence for members of a specialty includes the possession of knowledge, skill, experience, and sometimes equipment or facilities that are not expected of the average general practitioner. In this sense the law views the standards imposed on specialists as "higher" than those imposed on generalists. This does not mean that the law considers specialists to be "better" in some sense than generalists. Nor does the law prefer that patients be seen by specialists rather than generalists. In fact, the law—which reflects the behavior of the veterinary profession itself—considers that routine, primary care can be provided by generalists, and that specialists function to provide advanced services that are not within the province of ordinarily competent generalists.

Each specialty must make its own determinations of what constitutes ordinary competence for its members at any given time. Such decisions can be made formally, by edict of officers or designated members of a specialty board. Typically, however, a specialty's standards of ordinary competence are expressed in the day-to-day behavior of its diplomates. One can attempt to infer these standards from such things as textbooks, specialty board examinations, and opinions of members of the specialty. For example, it can be said with a reasonable degree of confidence that an ordinarily competent veterinary ophthalmologist must have diagnostic and treatment equipment, knowledge of certain conditions, the ability to do certain kinds of surgery, and facility in the use of certain kinds of drugs, including many utilized in human medicine (Gelatt, 1993), that would not be expected of the average general practitioner. However, the definitive job of determining what is ordinary competence for a veterinary ophthalmologist or a member of any other specialty must be done afresh in each particular lawsuit involving an ophthalmologist or other kind of specialist, just as discovering what constitutes ordinary competence for a generalist is determined by individual juries in negligence trials involving individual generalists.

2. Imposition of Uniform Standards on Specialists

A second way the law demands more of specialists than generalists results from the fact that virtually all jurisdictions impose the same

standard on specialists, while what constitutes ordinary competence for generalists can vary substantially from state to state. The various states have developed different rules regarding where to find the standards of ordinary competence for generalists. In some states a general practitioner must perform as would any ordinarily competent generalist in his or her locality (city or town). Many states look to what would constitute ordinarily competent practice in the practitioner's locality or in a community of *similar* size, geography, and demographics. A growing number of states require that a generalist perform as would any ordinarily competent generalist *in the United States*. These differences can affect significantly what is expected of a generalist. In a state that restricts the relevant location to a generalist's city or town, or even a similar city or town, it may be possible for a general practitioner in a sparsely populated, rural, or isolated area to convince a jury that what might constitute negligence in a community with larger, more technically advanced practices is perfectly ordinary and average where he practices.

In contrast, the "location" to which the law looks in assessing the competence of specialists is the entire country. This is so because for each specialty there is one specialty board, and each of these boards expects all diplomates to meet its minimum standards. In maintaining specialty status, a veterinarian is considered not only to want to be held to the higher standards of the specialty, but also to understand that the specialty board is a national entity whose standards do not vary from state to state or community to community.

C. The Specialist's Standard of Care

Not every aspect of practice requires specialists to conform to a higher standard of care than generalists. For example, it is doubtful that specialists have a different standard from generalists regarding abandonment (withdrawing from a case when doing so would cause immediate risk to the life or health of the patient). Although it is possible that in a particular situation a specialist would know more than a generalist about when it would be safe to attempt to withdraw from a case, in most circumstances all veterinarians would probably know equally well when a patient requires continuing care lest it die or suffer injury.

However, in many situations specialists will be held to a higher standard than generalists. Certainly in diagnosis and treatment the average specialist dealing with a condition or procedure covered by his specialty will often be expected to have knowledge and skills not pos-

sessed by the average general practitioner. A jury sometimes might expect a specialist to tell a client more in obtaining an informed consent than it would expect of a generalist by virtue of the specialist's presumed greater knowledge. Specialists might even be expected to keep more extensive or different kinds of medical records than generalists where this is part of a specialty's ordinary approach.

In short, whenever a specialist diagnoses or treats an animal for a condition covered by his specialty he is likely to be held to an exceedingly high standard of care—and a much higher standard than would be applied to a generalist practicing in the same community, in a similar community, or anywhere in the country. Moreover, while a specialist need not perform as would the most highly skilled member of his specialty, the standards of "ordinary" competence within the specialties are very high. Specialty boards expect their members to keep current with the latest developments, including techniques and equipment that may never find their way into the practice of the average generalist. The standards toward which specialists must aim are a rising target, and a steadily rising one at that.

D. Who Is Subject to the Higher Specialty Standards?

The law regards as a "specialist" (and therefore subject to higher specialty standards) any practitioner who is certified by a specialty board recognized by the American Veterinary Medical Association.[2] One is subject to these standards as long as one maintains board certification. A specialist will be held automatically only to the higher standards applicable to his specialty. Thus, a board-certified dermatologist who is doing abdominal surgery on a particular patient will not be required to perform this procedure as would the ordinarily competent board-certified veterinary surgeon.

[2]The AVMA has two categories of specialty recognition: provisional and full. Upon being granted provisional recognition, a specialty has up to 10 years to gain full recognition. The official annual AVMA *Directory* lists provisionally approved bodies as "specialties," and members of the profession speak of and recognize them as such (Ames, 1993b). Therefore, a court would regard as a specialist and not a generalist a member of one of these provisionally recognized specialties. As of 1993, the specialties of dentistry, clinical pharmacology, poultry medicine, emergency and critical care, and nutrition had provisional recognition; all were regarded by the AVMA as making satisfactory progress toward full recognition (Ames, 1993b). As in human medicine, "subspecialties" administered partly by a larger specialty group (e.g., cardiology, neurology, and oncology within veterinary internal medicine) are regarded by the profession (American Veterinary Medical Association, 1993) and therefore by the law as full-fledged specialties.

Specialists are generalists in fields in which they are not board-certified. Whenever a specialist attends to a case or condition not comprehended by his specialty he has the same duties (e.g., the duty to refer to a specialist, discussed below) that a generalist would have under the same or similar circumstances. This is an important principle. It means that any specialist who sometimes works outside his field of specialization must also understand the legal obligations of generalists, because he too will sometimes be a generalist.

It is not entirely clear what level of performance is required of residents in training for specialty certification, or of so-called "board-eligible" doctors who need only to pass their examinations to be board-certified. A strong argument can be made that such veterinarians should not be held to the standards of competence of their future specialty. That they have not been certified indicates that the specialty board does not yet consider them sufficiently qualified to claim specialty status. Nevertheless, in one well-known case, a resident in human medicine was held to specialty standards because his experience and training greatly exceeded that of a generalist (Valentine v. Kaiser Foundation Hospital, 1961). It is possible that given the proper set of facts a plaintiff suing a resident or board-eligible veterinarian for negligence could convince a court to apply either the specialty standard or a standard higher than that which would be applied to a generalist.

There are circumstances in which practitioners who are not, or who are not yet, board-certified will certainly be held to the standards normally applicable to specialists. Although generalists must perform as would any average generalist (in the relevant location used by the practitioner's state to determine ordinary competence), the law will hold to a higher standard any veterinarian who *wants* to be held to a standard higher than that which would ordinarily apply. *Therefore, any veterinarian who represents, suggests, or indicates to a client that he is a specialist relating to the kind of procedure to be done will be held to the higher standards required of a board-certified specialist—even if he is not board-certified or is certified in a specialty not related to this procedure.*

The test of whether a generalist made a representation sufficient to invoke higher specialty standards is whether the generalist said or did something that was reasonably understood by the client to contain a representation of specialization, and the client relied on this representation in deciding to employ the doctor's services or to authorize a particular procedure. Juries decide on a case-by-case basis whether

these conditions have been met. There are no magic words or formulas necessary to constitute a claim of specialization. Certainly, a generalist who tells the client that he is a "specialist" can be expected to be held to higher specialty standards.

In 1993 the American Veterinary Medical Association (AVMA) adopted its "Guidelines for Use of Specialty Titles," which is intended to guide specialists and generalists regarding appropriate representations of their level of expertise. These Guidelines state that generalists should not use the terms "specialist in . . . ," "specialty of . . . ," "specializing in . . . ," "special training in . . . ," "expert in . . . ", "advanced knowledge or training in . . . ", " . . . ologist, e.g., dermatologist, echocardiologist," and " . . . ist, e.g., internist" (American Veterinary Medical Association, 1993).

The AVMA Guidelines are useful. However, they are not gospel and may or may not coincide with a jury's interpretation of what a generalist represented under all the facts of the case before it. A jury is always free within the context of a particular lawsuit to determine that language approved by the AVMA for generalists in fact amounts to a representation of specialty status. For example, until 1995 (Ames, 1995), the AVMA Guidelines considered it appropriate for a generalist to represent a "special interest" in a field. I would urge extreme caution regarding the use of such language. A client might hear "special" as "special*ist*." Moreover, there can be a danger that in explaining the nature of a "special" interest one will lapse into language that is reasonably interpreted by a client as a representation of specialty status. The AVMA Guidelines also state that if a facility offers "specialty services," "any and all listings of that staff must identify which are diplomates," and the "majority of the staff veterinarians should be certified specialists." However, a jury could find that a client reasonably interpreted a claim of "specialty services" as a representation that *all* veterinarians in the facility are specialists, unless this client was actually told that *his* veterinarian was *not* board-certified.

Table II contains statements that in my view would and would not constitute claims of specialization. It cannot be emphasized strongly enough, however, that a jury can decide that a representation which ordinarily would not constitute a claim of specialization was, under all the circumstances, a statement that the practitioner is a specialist. For example, a generalist who advertises in the Yellow Pages that his practice is limited to cats, but then regales clients with statements about his "expertise" in feline medicine may be viewed as having represented himself as a specialist. Many different kinds of behavior

TABLE II

REPRESENTATIONS RELATING TO SPECIALIZATION

Statements that claim specialty status
"I am a specialist in dermatology [cardiology, ophthalmology, etc.]."
"I am an expert in dermatology."
"I have special expertise in dermatology."
"I am a dermatologist."
"I'm as skilled at this as any board-certified veterinary dermatologist."
"This is a specialty practice."
"This practice specializes in cats."
Statements that do not claim specialty status
"I have much experience treating skin problems."
"I have a long-standing interest in dermatology."
"I have a particular interest in animal skin problems."
"Practice limited to cats."

(including, perhaps, failing to contradict a client who states he believes the doctor is a specialist) can under certain circumstances be interpreted as a representation of specialty status.

Both generalists and specialists should be particularly careful about statements in advertisements, informational brochures, and newsletters distributed to present or potential clients. Overly enthusiastic or careless promotional language can result in the practice being held to higher specialty standards even when care is provided by generalists who do not themselves make inaccurate representations. Thus, a brochure stating that one's hospital provides "specialty care in all areas" may result in the practice being held to higher specialty standards when care is actually provided by a generalist, a resident training to become board-certified, a board-eligible doctor, or a doctor who is certified in another specialty. If a client reasonably interprets a statement made on behalf of the *entire* practice to imply that a particular veterinarian is a specialist, the higher specialty standards might be applied by a jury to this veterinarian and the practice.

Specialists should also be careful not to make representations that can be interpreted as asserting a level of competence higher than that of the ordinarily competent member of their specialty. Here too the law will allow practitioners to hold themselves to a level of performance higher than that ordinarily implied into the doctor–client relationship. For example, some specialists (and generalists) state in advertise-

ments or brochures that they provide the "highest" level of care or the "best" veterinary care. However, even an ordinarily competent member of a given specialty is not required, simply by virtue of board certification, to provide the *highest* level of services or the *best* services. Specialists are only required to be *ordinarily* competent specialists. Thus, a specialist who promised the highest level of care and is sued by a client for negligence may still be held liable, even if he performed as would the ordinarily competent member of that specialty—if the patient was injured by his failure to provide the highest possible level of care.

1. Consequences of False Representations

Making a false claim of specialty status does not result in automatic liability in a negligence lawsuit. There will not be liability if in fact the veterinarian performed at the level that would be expected of the ordinarily competent member of the claimed specialty. The plaintiff must also prove that any injury or damage was caused by the doctor's failure to perform as would the ordinarily competent specialist and not by something else, such as the patient's preexisting condition. However, the trial process is not likely to show in a favorable light a generalist who falsely claimed specialty status. The jury simply may not take kindly to the misrepresentation and may doubt the credibility of anything the defendant says during trial. The plaintiff may call as an expert witness a diplomate of the specialty, who might speak harshly of the performance of someone who falsely claimed specialty status. Most importantly, it may not be difficult for the plaintiff to prove that the generalist defendant did not meet the standards that would be expected of the ordinarily competent specialist.

Generalists who falsely claim specialty status also risk discipline by their state board of registration, such as suspension or revocation of the license to practice. State veterinary practice acts or regulations prohibit false, deceptive, or misleading public representations by licensees, and many specifically identify as a prohibited deceptive act representing that one is a "specialist" in a field in which one is not board-certified. For this reason alone generalists should never falsely state, indicate, or imply to any client or member of the public that they specialize in a field associated with a specialty officially recognized by the AVMA. False representations of specialty status can result in discipline by one's licensing board even if one performs in accordance with the standards of the specialty, or even if one's failure to so perform was not the cause of any injury or damage.

2. "Quasi-specialties"

Some veterinarians consider themselves "specialists" in areas not represented by AVMA-recognized specialty boards. Such expertise can relate to what can be called "quasi-specialties"—areas the profession does regard as separate fields practiced by doctors with advanced training or knowledge. These "quasi-specialties" can comprehend relatively broad areas of expertise that some day might be recognized as full-fledged specialties (e.g., aquatic medicine or radiation oncology) or areas within recognized specialties (e.g., gastroenterology, soft tissue surgery, or ultrasonography) regarded by many veterinarians as subspecialties in their own right even in the absence of officially recognized specialty status. Additionally, one can state that one has a specialty in any number of specific diseases, conditions, organs, procedures, or kinds of animals.

When a veterinarian claims special knowledge or expertise in a quasi-specialty or something else, he will be required to perform as would an ordinarily competent practitioner regarded as having such special knowledge or expertise. For example, a veterinarian claiming to "specialize" in lameness in race horses will be compared against practitioners who do have special knowledge or expertise in diagnosing and treating lameness in race horses. Similarly, a practitioner representing special expertise in transfusion medicine, nutritional problems of geriatric pets, or racing greyhounds will be required to perform as would the ordinarily competent veterinarian with special expertise in these areas.

Claiming that one has a "specialty" in an area within a recognized specialty can be dangerous for specialists as well as generalists. A board-certified veterinary internist who claims "special expertise" in liver diseases, for instance, might be held by a jury to higher standards than those that would be applied to the ordinarily competent internist —if there are internists who are regarded by their colleagues as knowing more about liver diseases than the average internist. This could result in his being held liable in a negligence lawsuit when he would not have been liable had he not claimed special expertise within his specialty. It may therefore be wise for specialists to state that they have an "interest" or "experience" rather than a "specialty" or "special expertise" in a subfield within their specialty. As dangerous as it can be for a generalist to claim special expertise in a recognized specialty, it can be even more dangerous for a generalist to claim expertise in a subfield within a specialty.

E. Relations between Specialists and General Practitioners

1. "Referrals" vs "Consultations"

There are two basic legal relationships between two or more veterinarians who are involved in the handling of a patient. In a referral, one doctor sends the patient to a second veterinarian and relinquishes all or a particular part of the case to the second doctor. In a consultation, the first doctor sends the patient to a second doctor for information or advice, but retains control over the case and makes the final decisions or takes the operative actions regarding treatment. In the paradigm "referral" the sending veterinarian has nothing to say about what shall be done to the patient with respect to the portion of the case for which the referral is made. In the paradigm "consultation" the receiving doctor merely offers an opinion which is or is not acted upon at the discretion of the sending doctor.

Relationships between generalists and specialists sometimes fall between these two poles. For example, if a specialist is located far from the client's place of residence the specialist may involve the referring generalist in the providing of specialty care by having the generalist administer part of the therapy or observe the progress of treatment. Nevertheless, it is useful to view one's legal obligations in terms of "referrals" and "consultations." In a referral, because the sending doctor relinquishes control over part or all of the case, he cannot be held liable for injury or damage that may occur as a result of the receiving doctor's handling of the case—unless he is negligent in making the referral, that is, unless he knows or should know that the receiving doctor cannot handle the case properly. (Such negligence will occur rarely in referrals by generalists to board-certified specialists. Absent specific knowledge by a referring doctor of a specialist's incompetence, that a doctor is a member in good standing of a specialty board should be sufficient to absolve a generalist of a charge of negligence in making a referral to this specialist.) In a consultation, because the sending doctor maintains control over the case only he can be held liable for negligence. Whenever the relationship between a specialist and generalist moves further from the paradigm of the pure referral, that is, the more both doctors participate actively in the treatment of a patient, the more they both can be held liable for negligence. In such a situation, the plaintiff may ask the jury to apportion the degree of liability between the participating veterinarians based on the proportion of their respective fault.

In sum, once a generalist makes a referral to a specialist, he is

typically out of danger from a lawsuit if anything goes wrong during or as a result of the specialist's handling of the case. Once a specialist begins referral care, he becomes subject to any legal liability, which is governed by the standards applicable to the specialty.

2. *Legal Duties of Generalists*

a. The Duty to Refer. The most important legal duty generalists have relating to specialization is the duty to refer. Generalists must refer a case to a specialist when any ordinarily reasonable, prudent, and competent generalist (in the relevant location invoked by one's state for standards of competence) would refer the case to a specialist. Put somewhat more concretely, a generalist should refer a case to a specialist when he knows, or when the average generalist would or should know, that a specialist's knowledge, expertise, techniques, or facilities are required for a proper approach to the case.

The duty to "refer" does not imply that the generalist must assure that the patient gets to a specialist. Absent an agreement by the generalist to transport the patient to a specialist, this is the client's responsibility. The duty to refer generally requires only that the generalist *advise* the client to employ the services of a specialist. Typically, it would seem reasonable (and therefore part of the duty to refer) for a generalist not only to advise the client to take the patient to a specialist but to suggest the name of a specialist, and in certain circumstances to contact the specialist before the client makes an appointment.

As in all other aspects of competent practice the requirement of reasonableness plays a central role in determining whether one has a duty to refer. Thus, a generalist with an emergency case in which treatment must be initiated immediately lest the patient die may be acting reasonably—and therefore without negligence—to initiate treatment, even though under nonemergency circumstances specialty care would be advisable for the patient's condition. (However, the generalist may be obligated in such a situation to refer to a specialist after beginning treatment or stabilizing the patient to a point where it can be taken to a specialist.) Whether it is reasonable to refer to a specialist can also depend on the availability of an appropriate specialist. There are parts of the country without members of some veterinary specialties. Where this is so, a generalist might not be faulted by a jury for failing to advise a referral. To be safe, however, a practitioner in such circumstances might tell the client that specialty care would be advisable, and leave it to the client to decide whether specialty care is too distant or inconvenient.

A generalist who fails to meet his duty to refer does not face auto-

matic liability for negligence. Under such circumstances the generalist will be required to perform as would the ordinarily competent member of the relevant specialty. The generalist cannot be held liable, if in fact he so performed, or if the client suffered no compensable damage as a result of the failure to refer. Nevertheless, a breach of the duty to refer can be disastrous. First, there will be circumstances in which generalists cannot perform as would ordinarily competent specialists, and in which the animal and client are harmed as a result. Second, state veterinary licensing boards have broad authority to discipline practitioners for negligence even in the absence of a causal connection between the negligence and any injury. It is negligent for a generalist not to refer to a specialist when he ought to, even if he happens to get it right or is lucky not to have been the cause of unfortunate results.

When a practitioner fulfills the duty to refer but the client declines, one should be exceedingly cautious. The law often permits animal owners to choose less than the best the profession can provide; indeed, they can *always* choose euthanasia instead of treatment. However, there are limitations to what a veterinarian may safely do when a client declines the advice to see a specialist. In one kind of situation, a generalist advises specialty care for a procedure he is not competent to perform, the client declines the recommendation, and the doctor performs another, albeit less than optimal procedure he is competent to perform, but one that would be regarded as acceptable in the profession. (An example would be doing a closed hip reduction when an open reduction by a specialist is preferable, but where the client cannot afford a specialist's services.) Provided the generalist performs competently, there should be no legal problem if the client has declined the referral after being presented with adequate information about the advisability of the referral and the risks of the procedure the generalist would perform. In such a case, one should carefully note in the patient's record one's advice that the case be referred, the information provided to the client, and the client's refusal. It might sometimes be advisable to have the client sign a statement indicating he was informed of the advisability of a referral but decided against it.

Such cases must be contrasted with situations in which a client declines the referral to a specialist and asks the generalist to perform a procedure that he is *not* competent to do or that violates standards of ordinary practice. A client who authorizes a doctor to perform an incompetent act can still sue the doctor for negligence. The law refuses to enforce such "authorizations," just as it refuses to enforce agreements by clients not to sue their veterinarian for negligence. One cannot, for example, attempt to remove a cataract if one has never done eye

surgery, or perform abdominal surgery without anesthesia, however strongly a client might want such things. It might be argued that generalists ought to be able to perform procedures they are not competent to do or that would ordinarily be inappropriate, if it would be *reasonable* to do so. For instance, it does not seem reasonable to prohibit a generalist from attempting a surgical procedure he would ordinarily not perform where the animal will die if it does not have the surgery immediately, there is no time to seek a specialist, and there is *some* chance the generalist can save the patient. However, if an action that normally would not constitute competent or appropriate care really is *reasonable*, it is probably incorrect to view it as an exception to the rule that veterinarians cannot be authorized by a client to act negligently. As has been discussed, the concept of negligence includes the element of acting reasonably. If veterinarians would find an approach such as that given in the example above reasonable, this approach would not be *negligent.*

b. Why the Duty to Refer Can Be Dangerous. It can now be appreciated why the recent and apparently continuing increase in both the number of specialties and the number specialists places significant legal burdens upon generalists.

First, with the establishment and flourishing of new specialties will inevitably come treatments that previously could not be offered to patients by generalists. Once such a procedure becomes available, it would appear that a generalist should at least discuss the possibility of a referral to a specialist who can provide it if there is some chance it could benefit the patient. For example, specialists in veterinary dentistry can now save by root canal therapy teeth that a generalist would previously have pulled without a second thought because there simply was no feasible alternative. Today, a generalist who fails at least to mention advanced dental—or orthopedic, oncological, or allergy—services to mention just a few other kinds of specialty care, might be negligent in failing to suggest a referral, even if the generalist thinks it unlikely that the client will choose specialty care.

Second, as the number of specialists within each specialty grows, and the distribution of members of any given specialty becomes more widespread, generalists will probably be expected to refer cases more frequently because of the increased availability of specialty care. It will become increasingly more difficult to justify not advising a referral on the grounds that there is no specialist accessible to the client.

Generalists should therefore keep current with developments in spe-

cialties relevant to the kinds of patients they see, so that they know what kinds of specialty care are available. They should also maintain an up-to-date list of members of these specialties within a reasonable distance of their practices so that the opportunity to refer is not missed because of ignorance of the availability of a member of a given specialty.

c. The Duty to Cooperate. Generalists also have a duty to cooperate with a specialist to whom a referral is made so that the specialist can perform competently. Generalists must, for example, provide adequate medical records to the specialist, respond promptly and accurately to questions regarding the patient's history, and follow appropriate directions regarding follow-up observation or care so that the specialist's treatment can be implemented. These duties—like all duties of generalists and specialists—are merely consequences of the overarching obligation to act reasonably.

d. The Importance of Medical Records. All practitioners must competently keep, maintain, and secure patient medical records. Because it can reasonably be anticipated that patients might someday be referred to a specialist it is essential that a generalist's records be in a form that is usable by any referral doctor. If a specialist is unable to provide appropriate care as a result of the generalist's incomplete, inaccurate, or illegible records the generalist may be held liable for negligence. Many state veterinary licensing boards have specific regulations regarding what must be contained in minimally adequate medical records. In making a medical record for any patient it useful to ask whether the record is in a form and contains information that would be required if the patient were referred for specialty care or were ever seen by another veterinarian.

3. Legal Duties of Specialists

Specialists too have legal duties regarding their relations with generalists. The most important of these duties is that of cooperation with the generalist in caring for the patient and serving the client. A specialist must, for example, see the patient promptly after accepting the referral where this is required by the patient's condition, explain clearly and carefully to the generalist the diagnosis and treatment provided if the generalist is to resume normal care of the patient, and provide adequate directions and explanations regarding any follow-up care to be provided by the generalist. All these legal duties—and others as well—follow from the requirement that specialists must act reasonably.

F. Specialty and General Standards: The Dynamics of Development

1. Developing Boundaries between General Practice and the Specialties

At the present time it is difficult to speak with great precision about where courts and juries would draw the line between what is reasonably done by generalists and what should be done only by specialists. The difficulty of locating the boundaries between general and specialty practice does not result from any desire on the part of the law to be antagonistic or unhelpful. What separates competent from negligent veterinary practice is determined by the veterinary profession itself. If the law is not clear about what is appropriately done by specialists rather than generalists, it is because veterinarians themselves are in the process of determining the boundaries between general and specialty practice.

Several specialties have been recognized for less than a decade. There appears to be no shortage of veterinarians who desire specialty recognition. Moreover, recognized specialties continually modify their standards of competence as their ability to provide care advances. In the case of new specialties this process may be in its infancy. It may also take some time before the profession sorts out what ought to be done by a specialist from what is appropriately part of ordinary general practice. For example, the American Veterinary Dental College and the American College of Veterinary Nutrition were recognized by the AVMA in 1988, the American College of Veterinary Emergency and Critical Care in 1989, and the American College of Poultry Veterinarians in 1991. In 1993, the AVMA Board of Veterinary Specialties approved the American College of Veterinary Behaviorists and permitted the American Board of Veterinary Practitioners (ABVP) to recognize a subspecialty of feline practice (Ames, 1993b). By 1995, the ABVP encompassed no fewer than five subspecialties: "avian practice," "canine and feline practice," "dairy practice," "equine practice," "food animal practice," and "swine health management" (AVMA Directory, 1995b). Many generalists practiced in these areas long before official recognition of these specialties, and many generalists will continue to practice in these areas without seeking certification in these specialties.[3] How much will these generalists or the profession regard as

[3]For example, as of the end of 1994 the subspecialty of swine health management listed three diplomates (AVMA Directory, 1995b). Clearly, there are far more than three veterinarians who regard themselves as swine practitioners or even "swine health management" practitioners.

appropriate only for members of these new specialties? Much of the answer will depend on what knowledge or techniques are developed by the new specialties.

Another problem in characterizing the legal boundaries between general practice and the specialties results from the flow of knowledge and techniques from the specialties into general practice. Like human medicine, veterinary medicine strives not just to increase knowledge and improve techniques but to make advances available to front-line general practitioners. The interest of generalists in offering new, advanced procedures is nurtured by the publication of reports of new treatments in professional journals, the participation of leading specialists in continuing education seminars where generalists are presented information about new advances, and the fact that most veterinary school faculty are board-certified and teach their students, many of whom will be generalists, about the latest developments in the specialties.

One example of how the flow of information from the specialties might obscure the borders between specialty and general practice is provided by cancer chemotherapy. Just a few years ago very few, if any, generalists would have undertaken the use of human chemotherapy drugs to treat lymphoma in cats or dogs. Today there are generalists who perform such treatment, sometimes with the consultation of a board-certified veterinary internist, sometimes on their own. Would a jury find it appropriate for a generalist to undertake such therapy? In this as in many treatments and procedures that flow from the specialties into general practice, the resolution of the proper borders between the two will depend upon consensus within the profession.

In sum, veterinary medicine has embarked upon what may be a lengthy legal voyage during which the profession will draw the boundaries between specialty and general practice. This journey probably will be more difficult for generalists than specialists. As the number of veterinary specialties increases it may be necessary for the typical general practitioner—accustomed to working in many areas—to become familiar with the locations of the developing borders between specialty and general practice in *all* these areas.

2. Legal Dangers Presented by Overlapping Specialties

One aspect of the continuing emergence of new specialties could prove especially problematic for generalists. Some of these newer specialties may represent areas that traditionally have been the province of other specialties. There is thus the possibility that two or more specialties will have or will claim expertise in the same areas. As noted

above there are already recognized specialties in canine and feline, equine, and food animal practice. Perhaps some day small ruminants and fish will also be represented by separate specialties. There are also areas of practice viewed by many in the profession as distinct fields that could conceivably become specialties in their own right, such as allergy, gastroenterology, geriatrics, and neonatology–pediatrics. All these already existing or potential specialties would cover areas represented by previously existing specialties.

Will practitioners certified in an existing or future specialty that overlaps a previously existing specialty be more skilled in treating certain conditions than members of the older specialty? For instance, is it now, or might it some day be the case, that board-certified feline practitioners can better handle feline urinary conditions than internists? A generalist who ought to refer a feline patient with a urinary problem to some kind of specialist will need to know the answer to this question, lest he be held liable for negligently referring to the *wrong kind* of specialist. If feline practitioners and internists (for example) come to disagree about their relative levels of expertise regarding certain conditions, generalists may be in a quandary as to which kind of specialist to seek. At the very least, the existence of a new specialty of feline medicine (for example) may require generalists to pay continual attention to how this specialty develops so that they know what kinds of treatment it may offer that exceed the capabilities of general practice, or of other specialties. The more the profession recognizes specialties that overlap previously existing specialties, the more questions are bound to arise regarding which specialty might be better capable of handling a particular kind of condition in a particular kind of patient. For the generalist, knowing the answer to these questions could mean the difference between competent practice and negligence.

My aim here is not to criticize the recognition of new specialties nor to assert that the specialty of feline practice, or any other particular new specialty, will inevitably raise insoluble legal questions for generalists. I wish only to warn that with every new specialty *must* come new questions about when cases should be referred to members of this specialty,[4] and that these questions can be difficult if the new specialty's areas of expertise overlap those of other specialties.

[4]The creation of a new specialty would not raise any questions relating to when generalists or members of other specialties should refer cases to that specialty only if that specialty offered nothing that a generalist or a member of an already existing specialty could do. But if this were so, there would be no need for the specialty, nor should the courts recognize it as such.

G. The Role of Specialists in Malpractice Lawsuits

Specialists play a major role in the litigation of veterinary malpractice lawsuits. A jury applying the standards of ordinary competence does not invent these standards but discovers them from the testimony of expert witnesses. These expert witnesses are veterinarians who either have participated directly in the events relating to the lawsuit or give their opinion regarding the performance of the defendant-veterinarian after reviewing the relevant facts. In the typical negligence case the plaintiff's expert witnesses disagree with the defendant's expert witnesses about whether the defendant-veterinarian fulfilled the standards of ordinary competence. It is the task of a jury of lay persons to determine whose expert witnesses are more credible regarding the appropriate standard of care and whether the defendant performed in accordance with this standard.

Attorneys for both plaintiffs and defendant-veterinarians often seek to find specialists to testify on behalf of their clients. The aim, indeed, is to find specialists who are regarded even by specialists as leaders in their field, such as authors of important textbooks and senior members of veterinary school faculties. It is believed that the testimony of such specialists carries great weight with juries, or will drive the opposition to settle the case out of fear of being beaten badly at trial. It is not unusual in lawsuits involving potentially large monetary judgments for each party to search the profession for specialists more prominent than those secured by the other side.

A specialist is permitted to testify in a negligence case involving a general practitioner, if the specialist's area includes the subject matter of the lawsuit and the specialist can claim familiarity with the standards of practice in the location that the state's law considers relevant in determining ordinarily competent practice. These conditions rarely prevent specialists from testifying. A specialist typically is called to testify because his specialty is relevant to an issue in dispute. And courts generally hold that specialists know the standards not just of their specialty but of general practice. In contrast, generalists typically are not viewed as having reliable knowledge of specialty standards. Therefore, it is difficult but sometimes possible for a generalist to be qualified by a trial judge to testify as an expert witness in a case involving a specialist when the subject of the lawsuit relates to the defendant's specialty.

If a specialist or generalist has performed some action that is an essential part of the subject matter of the lawsuit, he can be compelled to testify by one of the parties through a court-issued subpoena. This

can occur when the patient, having been treated by the veterinarian who is the defendant in the case, was seen by a second doctor, either because the patient was referred by the defendant or was taken by the client to the second doctor to undo the defendant's alleged mistakes. Under such circumstances, the second doctor will be unable to avoid testifying. However, courts rarely order a veterinarian to testify if he or she has not played a role in the history of a case. Thus, most specialists who testify as expert witnesses are retained by the plaintiff or defendant specifically for the purpose of presenting testimony. A practitioner who is approached by a party in a lawsuit should not agree to testify for that party without first reviewing the case, including all available medical records. Otherwise, one can be caught having to change one's mind about supporting that party during the case, which can seriously compromise the party one has agreed to assist. It is appropriate to require a fee to examine the case prior to making a decision whether to testify. It is also appropriate to charge the party for whom one testifies a fee based on time spent in preparation, travel, and appearance. Typically this fee is higher for trial preparation and testimony than for appearance at pre-trial oral depositions or planning sessions. Contrary to portrayals on television and in the movies, juries do not seem surprised to learn that expert witnesses are compensated for their testimony.

Although specialists play a major role in veterinary negligence litigation, there is an inherent problem in their testifying in lawsuits involving generalists. Specialists are called as witnesses because it is hoped they will impress juries with their distinctive knowledge and expertise. However, generalists are not required to perform as would specialists. This fact can get lost if opposing specialists battle about whether the case was handled as *they* would handle it. Trial judges, attorneys, and specialist witnesses themselves must be vigilant in focusing on the standards of practice that apply when a negligence lawsuit involves a general practitioner.

II. Ethical Issues Raised by Veterinary Specialization

Ethical issues relating to veterinary practice are often more difficult to resolve than legal issues. To be sure, legal concepts such as negligence are based on basic moral values, and veterinarians who accord due respect for the interests of patients and clients should be well on the way to protecting themselves from malpractice liability (Tannenbaum, 1993a). However, the law differs fundamentally from ethics.

Laws are objectively ascertainable rules created by legislators, regulators, and judges. When they are unclear or seem wrong-headed, they can be changed. In contrast, the fact that any group of people thinks that something is ethically correct does not make it so. Ethics involves values—what *ought* to be—and disagreements about how to resolve ethical questions are as old as civilization itself. This chapter therefore cannot attempt to settle all ethical issues raised by veterinary specialization, but surveys some of the most important of them.

A. Overview of Veterinary Ethics

1. Major Sources of Ethical Principles

There are three major sources of ethical principles relating to veterinary medicine.

a. Official Values. Official ethical standards are those that are articulated for veterinarians by the profession itself. The most important source of official standards is the AVMA *Principles of Veterinary Medical Ethics*, which contains general ideals and specific rules relating to various aspects of practice. The AVMA has also issued a large number of official ethical statements on topics ranging from low-cost spay and neuter clinics to the possession of wild animals as pets (AVMA Directory, 1995a). Official ethical standards present views that have been seriously considered and often express the profession's most central ethical beliefs. However, there are several reasons why official ethical standards are of restricted value.

First, official standards are not infallible guides to morality. That the *Principles* or an official AVMA position say something is right or wrong does not make it so.

Second, official ethical standards are created by veterinarians, often through a process of compromise among groups within the profession with differing points of view (Tannenbaum, 1995). Veterinarians, however, are not the only ones affected by ethical questions relating to veterinary practice. Clients and patients far outnumber practitioners. Neither clients nor the public participate in the fashioning of official ethical standards and thus cannot assure that their interests are factored into official standards.

Third, federal law severely restricts the authority of the AVMA to compel adherence to official moral rules. The United States Supreme Court has held that neither state licensing boards nor private professional groups can ban or restrict promotional activities by practitioners, unless such promotion is false, deceptive, or misleading (Bates

v. State Bar of Arizona, 1977). The Federal Trade Commission (FTC), which enforces federal antitrust laws, prohibits the professions from adopting ethical rules that could make it more difficult for practitioners to compete freely in the marketplace. These legal developments have resulted in the elimination from the *Principles* of ethical guidelines designed to prevent aggressive promotional attacks by veterinarians against each other, and business practices such as sale of nonprofessional products like pet toys and clothing, traditionally associated with commercial trades rather than the healing professions.

Finally, official standards are limited in their ability to encourage ethical behavior because the profession cannot compel compliance with official standards. The strongest penalty the AVMA can impose for violation of its ethical rules is expulsion from the AVMA. Practitioners threatened with such action can simply resign their membership.

b. Administrative Values. Administrative veterinary ethical standards are moral rules that are articulated and enforced by government bodies. Among governmental agencies whose function includes guiding veterinarians in ethical behavior are the Food and Drug Administration, the FTC, and the U.S. Department of Agriculture. The most important sources of administrative veterinary ethical standards are the state veterinary licensing boards. The practice acts and regulations administered by many of these boards contain ethical rules such as prohibitions against false advertising, claiming specialty status when one is not board-certified, and inhumane treatment of patients. A number of the boards have adopted full-fledged codes of ethics. Among prohibitions typically found in such codes are providing services not needed by the patient or client, guaranteeing a cure or result, violating the confidences of clients, and serving clients with conflicting interests, e.g., the buyer and seller of the same animal (Tannenbaum, 1995).

Like official ethical standards, administrative rules are not an infallible guide to morality, for they too can be as good or bad as those who make them. Unlike official standards, administrative ethical rules cannot easily be escaped. They have the force of law. One can be punished or disciplined by the government for violating them. A state veterinary licensing board, for example, can suspend or revoke one's license to practice because of violation of its ethical rules.

c. Normative Values. Philosophers use the term "normative ethics" to refer to the search for correct moral standards. Normative ethical standards are more important than official or administrative principles. Professional associations and government bodies strive to conform their ethical pronouncements to what is really right, good, or

just. Moreover, as has been noted above, veterinarians can no longer be compelled to advertise in a dignified and professional manner, nor can they be prohibited from aggressive competition against colleagues. There are thus large areas of behavior regarding which veterinarians must turn to their own individual sense of right and wrong for guidance (Tannenbaum, 1993a).

2. *The Basic Fact of Veterinary Ethics: Conflicts of Interest*

Unlike physicians, who serve the interests of their patients, whoever pays their fee, veterinarians serve two parties: the animal patient, and the client. Many questions in veterinary ethics are difficult precisely because the interests of the veterinarian's two masters sometimes conflict (Tannenbaum, 1993b). It may, for example, be in the interest of a patient to receive the best care the doctor can provide, but this may not be in the client's interest if he cannot afford the treatment. It may be in the interest of a client with a terminally ill animal to hold on to the animal as long as possible, but this may not be in the interest of a patient in severe pain or with no prospect of a good quality of life. Time and again, veterinarians are thrust into the middle of these conflicts, wishing to satisfy the needs of both patient and client but unable to do so. As will be discussed, the availability of specialty care sometimes exacerbates the intensity of these conflicts and makes their resolution even more difficult.

B. Ethical Issues in Relations between Specialists and Generalists

1. *Fee-Splitting*

An important principle of normative professional ethics which has long been expressed in the official codes of human medicine and dentistry (American Psychiatric Association, 1981; American Dental Association, 1989; American Medical Association, 1989) is that a referral doctor should never pay, and a referring doctor should never receive, a kickback, rebate, or commission for a referral. Prohibiting fee-splitting assures that referrals are made solely because they are in the patient's interest, and that a referral to a particular practitioner is made solely because it is in the patient's interest. The veterinary practice acts and regulations of many states prohibit fee-splitting under any circumstances. Fee-splitting had been prohibited by the AVMA *Principles of Veterinary Medical Ethics* until 1989, when a new provision appeared stating that "it is unethical for a veterinarian to partici-

pate in fee-splitting and rebating or to accept a fee in connection with referrals, without informing the client of the arrangement" (AVMA Principles, 1989). This is not an acceptable approach. It allows a referring doctor to be influenced by considerations other than the needs of the patient and client. Telling a client—who may trust the wisdom of whatever the veterinarian recommends—need not by itself extinguish any temptation on the doctor's part to be influenced by the kickback. In 1993 the AVMA Judicial Council reinstituted the pre-1989 prohibition against taking kickbacks from *nonveterinarians* (such as pharmaceutical companies) in return for a doctor's use of their product or service. The Council admitted that such kickbacks create a potential conflict between veterinarians' interests and those of patients and clients (Council Recommends Revision of Commission and Rebate Principle, 1993). But the provision of the *Principles* allowing kickbacks between *veterinarians* was left in place. This inconsistent approach to kickbacks illustrates why the profession's official ethical code is not always an infallible (or, in states in which fee-splitting is illegal, a safe) guide for moral conduct.

2. Retaining the Patient

Some specialists can provide not only specialty treatment but primary, routine care for which animals are usually brought to generalists. When might a specialist be justified in retaining the patient after specialty care is completed, instead of sending the patient back to the referring doctor? This is an ethical rather than a legal question because clients always have the legal right to determine which veterinarian to employ. If a client wants to stay with a specialist, the specialist has no legal obligation to the referring generalist to send back the patient even when there has been an understanding between the doctors that he would do so. Nor does the law prevent a specialist from actively seeking to become the patient's new primary care veterinarian.

Whatever the law may allow, there can be ethical problems in retaining referred patients. A generalist who has referred a patient for specialty care will rightly feel he has been treated badly, certainly if the specialist seeks the patient on a permanent basis, and even if the specialist merely accedes to the client's wish that the specialist become the new veterinarian. The generalist will complain with justification that a fair reward for helping the patient, client, and specialist is not losing the client's future business. Retaining referred patients is also ethically questionable because in the long run it can harm everyone. The inevitable result of a general practice of specialists' retaining

referred patients will be a diminution in referrals by generalists. This will deprive patients and clients of needed specialty care, specialists of appropriate income, and generalists of the trust of clients whose animals need referrals.

A case for retaining a patient can be made where there is some particular reason why the patient or client would be significantly better off than with the first doctor (Tannenbaum, 1995). For example, if a large proportion of the animal's care henceforth must be specialty treatment and it will be disruptive to the patient's care and inconvenient to the client to shift back and forth between generalist and specialist, it may be appropriate for the specialist to retain the patient.

The AVMA *Principles* state that "consultants must not revisit the patient or communicate in person with the client without the knowledge of the attending veterinarian" (AVMA Principles, 1994). This rule follows from the nature of a consultation, in which the first doctor is understood by the client and both doctors to be the attending practitioner. Regarding referrals, the *Principles* provide that the referring doctor "should not take charge of a case or problem without the consent of the client and notification of the referring veterinarian" (AVMA Principles, 1994). Basic courtesy requires that a specialist at least inform the referring doctor of the client's decision to change veterinarians so that the first doctor knows what has happened to the patient.

3. Criticizing the Generalist

A specialist might also be ethically justified in retaining the patient if the referring doctor has not handled or is not likely to handle the patient competently. It is difficult to maintain that good relations among generalists and specialists, or a specialist's gratitude for receiving a referral, outweigh a specialist's moral obligation to assure that the patient and client receive good veterinary services. Indeed, it can be argued that if returning the patient to the referring doctor is likely to harm it, the specialist has an ethical obligation to try to prevent this from happening.

In many years of speaking with veterinarians about professional ethics I have found that the ethical question relating to specialization that is of greatest concern to generalists is how a specialist may properly react when he believes that the referring doctor has made a mistake. Veterinarians rightly fear the dangers of criticism of their performance made by other doctors to clients—including the potential for a lawsuit by the client, loss of other clients if this client disseminates the other doctor's criticisms, damage to one's reputation, and emotional

distress that can be experienced by a client who believes that his veterinarian acted incompetently.[5]

The view of this issue embodied in the AVMA *Principles* until the Federal Trade Commission's inquiry into the code in 1988 was that it is always unethical for any veterinarian ever to criticize another doctor to a client (AVMA Principles, 1988). As I have argued (Tannenbaum, 1995), the code's old absolute prohibition against criticism would sometimes violate clients' moral and legal rights. Practitioners are not entitled to be paid for negligent services, and clients are entitled to compensation for many kinds of damage caused by veterinary malpractice. There will be situations in which a veterinarian has performed negligently, but the client will not be aware of it (thinking, perhaps that the patient's injury was unavoidable) unless another doctor who discovers the first practitioner's negligence tells the client what happened.

In response to the FTC's claim that the prohibition against criticizing colleagues stifled competition among practitioners, the *Principles* were changed to require that "findings and discussions with the client shall be handled in such a manner as to avoid criticism of the attending [or referring] veterinarian, if that criticism is false or misleading" (AVMA Principles, 1990).

Whatever the historical reason for its adoption, this new approach should in fact prevent most unfair criticisms. In discussing this problem with specialists and generalists, I have concluded that the major danger of criticizing a colleague's performance stems from the fact that criticisms have a high probability of being *incorrect.* The case a second veterinarian sees is rarely exactly the same as that the first doctor confronted because the patient's condition can change. A client's recollection about what the animal experienced or what the first doctor did, even if well motivated, can be mistaken or incomplete. Perhaps most importantly, there is sometimes more than one competent way to handle a case, something a second practitioner with a strong belief in his way may not always appreciate. If veterinarians follow the current approach of the *Principles* and avoid criticism that can be false or

[5]One practitioner who criticizes another can also be sued by the latter for defamation of character. This intentional wrong consists of the making of false statements, oral or written, regarding the character or competence of another that diminishes the latter's reputation in the community. However, the making of an adverse comment to a client does not in itself constitute defamation. To be defamatory, statements must be made to more than one person. Additionally, a veterinarian who sues another doctor for defamation generally must demonstrate that the latter's statements resulted in actual damage, such as loss of income.

misleading, they are likely, it seems to me, to *presume* that criticism is unjustified and then to make adverse statements only when there is a *compelling* reason to do so—which will be the rare case indeed. The *Principles* also wisely recommend that one contact a colleague one believes has acted improperly (AVMA Principles, 1994). Doing this before one says something to a client gives the colleague the opportunity to present his side of things, and will likely clarify the situation he faced when attending to the patient.

Specialists rarely lack the ability to learn more about what happened when a patient was seen by a referring doctor, for they will usually have the patient's records and can communicate with the referring doctor. Additionally, specialists usually have a strong economic motivation not to denigrate a referring doctor, lest they lose a source of referrals. On the other hand, some specialists might think that the approach taken by their specialty is the ideal way of doing things, the "best there is," and therefore "*the right* way of doing it." Sometimes specialists can do it better than generalists. But even when this is so, it does not follow that generalists fall short of the mark. An invidious comparison between how "*I* (the specialist) would have handled this" and the generalist's actions may lead the client to believe that the generalist erred or was incompetent, even though the generalist may have fulfilled the standards of general practice. Clients may be especially influenced by a disparaging remark coming from a *specialist.*

4. Specialization as a Competitive Weapon

The view of the veterinary profession and therefore of the law is that specialists function as an adjunct to primary general care, and that in the great majority of situations it is not better for a patient to see a specialist than a generalist. Any representation by specialists to the public that, in general, they provide better services than generalists, is false, deceptive, and misleading—and is therefore not just unethical but in violation of state practice acts.

There are Yellow Pages and newspaper advertisements stating that veterinarians are or have on their staff board-certified specialists. Patients are supposed to find their way to specialists through referrals from generalists. Generalists are supposed to know what specialists practice in their communities so that patients can be referred when a referral is advisable. It is therefore not clear why specialists would promote their specialty status directly to the public if they did not think that this fact alone would be significant in motivating people to employ their services. Some of these advertisements, especially those

seeking new clients with offers of general services not related to specialization, do seem intended to attract clients by touting the superiority of specialty care.

Because some people are likely to think that specialty care is always better care, for the protection of the public as well as generalists, state veterinary licensing boards should crack down on advertisements and other kinds of public and private representations by specialists that falsely proclaim the general superiority of specialty care.

Additionally, fairness to veterinarians who want to become specialists and to patients and clients that would benefit from the increased availability of specialty services requires that specialties not be permitted to exclude qualified doctors from certification. The AVMA has acknowledged the merit of complaints about overly rigorous requirements of some specialty boards that make it difficult for veterinarians in private rather than academic practice to sit for examinations (Ames, 1993a).

Fairness to the public and all veterinarians demands that specialties be recognized only when there is strong medical justification for doing so. Because of public perception that specialists have greater expertise and are therefore often entitled to higher fees, no group of veterinarians can be permitted to benefit from the "specialist" label unless their specialty offers expertise that is substantial and distinctive.

C. Ethical Issues Intensified in Specialty Practice

There are serious ethical issues that are not unique to specialty practice but can be made more difficult by aspects of specialty care. Because these issues are complex even outside the context of specialization the reader is referred elsewhere for more detailed discussions (Tannenbaum, 1995). Here, the relevance of specialization to these problems will be highlighted.

1. Overutilization of Services

It is unethical and prohibited explicitly by the regulations of several state veterinary licensing boards to provide services excessive in quality or amount to the patient's needs. Such overutilization of veterinary services wastes a client's money and can cause the patient unnecessary stress, discomfort, or pain. Examples of overutilization are doing laboratory tests that will only duplicate previous results, recommending invasive or expensive diagnostic tests on a patient clearly *in extremis,* and selling a client more medication than is needed to treat the patient's condition. Although overutilization is, by definition, unethical

because it involves excessive services, it is not always easy to determine what is excessive, particularly in the context of specialty care. Specialists sometimes do procedures that cannot yet, or may never, promise clear results. If, for example, the performance of a sophisticated diagnostic test such as C-T scanning or treatment such as chemotherapy presents some probability of improving the patient's condition, how great a probability is required before the effort can be considered appropriate? The answer will probably depend on the specific circumstances of the case, such as the age and general condition of the patient, its projected quality of life should the treatment succeed, the ability of the client to afford and provide follow-up care, and the knowledge and experience of the specialist.

Clients can be especially vulnerable to the recommendations of specialists. Most come to a specialist having already decided at least to consider advanced services. To some clients, a specialist is the last hope for success, and there can be a tendency to want whatever the specialist offers. Specialists should therefore take particular care to explain the probable benefits and risks of any procedure and to make certain that a client's authorization is truly informed. Above all, because of the vulnerability of some clients and a specialist's position of authority and credibility, specialists must be on guard against recommending excessive or unnecessary services in the first place.

2. The Cost of Veterinary Services

Many ethical problems faced in veterinary practice involve the inability of a client to afford appropriate services. Are veterinarians ever morally obligated to reduce their ordinary fee or to allow special payment arrangements to help clients pay for care? To what extent should doctors accompany advances in medical care with general approaches that might enable more clients to pay for these advances, such as setting aside a certain amount each year for the waiving of fees to needy clients or doing *pro bono* work through local humane society clinics? Some of these questions are difficult. They can involve balancing one's desire to help patients and clients against one's own interest in compensation commensurate with the value of one's services.

The problem of affordability can be especially difficult in specialty care. The fees of specialists and the costs of some advanced procedures are sometimes quite high. Clients might be prevented unnecessary distress if they have some understanding of what specialty services are likely to cost before specialty care becomes so attractive that it is difficult to decline. It is therefore appropriate for generalists to men-

tion the potential cost of specialty services when discussing with a client the advisability of a referral. For their part, specialists must always scrupulously inform clients about all potential costs. They should also be prepared to explain how their fees are based upon their training and expertise and the services that will be provided.

3. Euthanasia

When veterinary medicine could offer little hope for seriously ill or injured animals, putting the patient out of its misery was often the only thinkable approach. The more that can be done for animals, the more one must ask hard questions about whether, or when, euthanasia is appropriate. In one kind of hard case, it is in the best interests of the animal that it be treated, but the client cannot afford the treatment economically or emotionally. In another, it is in the best interests of the patient that it be euthanized, but the client cannot bear to part with it or refuses to accept the hopelessness of the situation. Whether euthanasia is morally preferable to treatment can depend on many factors, including the probability that a proposed treatment will work, the probable effects of the treatment on the patient, the likely quality of life for the patient if the treatment succeeds, the economic and emotional stamina of the client, and the likely results of other approaches, including doing nothing (Tannenbaum, 1995).

Specialization can intensify ethical dilemmas raised by euthanasia. As specialization develops treatments that were once unavailable, the more possible it will become to help animals in ways some clients cannot sustain financially or psychologically. The easier it will become to keep animals alive, suffering or in a vegetative state, for clients who want treatment to continue. The more it will be possible to provide procedures that might work, but that might also cause patients significant discomfort. Poignant examples of how specialized, advanced procedures raise new ethical problems are presented in veterinary intensive care units. There are animals in such facilities that are in pain, the prognosis for which is hopeless, the treatment of which will cost their owners thousands of dollars, or that are terminally ill and are being kept alive by artificial and sometimes heroic means. In the past, many of these patients would have been euthanized promptly.

Specialists can face particular difficulties dealing with these issues. Specialists are trained, they *exist*, to provide advanced procedures. A difficult medical case cannot only excite a specialist's intellectual curiosity (there is nothing wrong with this) but also provide a challenge to the specialist's acumen—perhaps even to the state of knowledge of the specialty itself. Thus, specialists can have a professional interest in

solving a problem rather than "giving up" with euthanasia. A client, who may enter the professional relationship with great regard for the veterinarian as a specialist (there is nothing wrong with this either) may find it difficult to press the case for less than specialty care, or for euthanasia.

Specialists should therefore be prepared to argue in favor of euthanasia, or less than specialty level care, when this is in the interests of the patient and client. I have seen cases in which a specialist has helped a client to make a fully informed decision by having the client talk first with the referring generalist. The generalist, the family veterinarian, is often more knowledgeable than the specialist about the client and his relationship with the patient and can sometimes provide an additional invaluable source of information and advice.

III. Some Legal and Ethical Issues in Innovative Therapies and Clinical Trials

A. Legal Liability and Ethical Standards for Innovative Procedures

All veterinarians are legally obligated to perform as would an ordinarily competent practitioner of the relevant category (generalist or specialist). This requirement has been interpreted by courts to imply that where there exists a customarily accepted approach to a given condition, a practitioner is expected to utilize it.

An "innovative" procedure or therapy can be defined as one that is not customarily accepted within the profession as appropriate.[6] It is

[6]Innovative procedures must be distinguished from "unconventional" or "alternative" treatments or approaches. The law recognizes the existence of "schools" of medicine, general approaches that are accepted by significant portions of a healing profession. A treatment modality is considered ordinary or customary if it is endorsed by a school that is itself generally accepted as legitimate by the profession. For example, Freudian psychoanalysis is an accepted school within psychiatry. Therefore, a psychiatrist who is sued for negligence will not be held liable if his approach would not be accepted by non-Freudians, if he performed as would an ordinarily competent member of the Freudian school. According to a 1988 AVMA policy statement, both holistic and homeopathic veterinary medicine are "unconventional" (American Veterinary Medical Association, 1988). Unconventional or alternative approaches can in time become accepted schools within a profession; the 1988 AVMA statement appears to view acupuncture as an acceptable approach. "Innovative" procedures as discussed here reflect generally accepted approaches and utilize a novel modality that would, if effective, not constitute so radical a departure from generally accepted approaches to be classified as unconvention-

impossible to characterize in the abstract when a procedure passes in the eyes of the law from being innovative to being customary. Nor will it always be a simple matter to determine when a modification of a customary approach would be so slight or inconsequential as to constitute a minor variation of the customary approach rather than a true innovation. A jury in a malpractice action will determine whether an innovative procedure has been used. In doing so the jury can be expected to consider a number of factors, including how often the therapy has been used and the view of experts regarding its novelty, effectiveness, and safety.

Specialists sometimes recommend innovative procedures because there may yet exist no approach that is accepted by a significant proportion of doctors. Indeed, it is safe to say that many veterinary procedures become customary by first being utilized and refined by specialists, who then recommend use by other specialists or general practitioners.

The subject of innovation in veterinary medicine has not been addressed extensively by the courts, but there are many court decisions and commentaries by legal scholars on innovative procedures in human medicine (Waltz and Inbau, 1971). Unfortunately, these decisions and discussions do not agree on an appropriate approach. The consequence is that if, as can be expected, courts would attempt to apply to veterinary medicine theories of liability relating to innovation that have been proposed in human medicine, veterinarians cannot be completely confident about how to protect themselves from liability.

Traditionally courts have ruled that a physician who departs from customary practice is *ipso facto* negligent and is therefore liable for any bad results (Brook v. St. John's Hickey Memorial Hospital, 1977; Zeller v. Greater Baltimore Medical Center, 1986). This approach has been criticized on the grounds that it discourages innovation and progress (Waltz and Inbau, 1971). Some urge that a physician may use an innovative therapy if it does "not vary too radically from . . . accepted methods" and if "usual and accepted" procedures have already been tried unsuccessfully (American Medical Association Law Department, 1957). Another approach recommends that physicians be permitted to utilize an innovative procedure only after it has already been tested

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al. Thus, it would be innovative but not unconventional to use in animals a cancer chemotherapeutic drug approved for use in humans but not yet generally established as effective for animals. Cancer chemotherapy is in general an accepted treatment modality in veterinary medicine.

experimentally on patients (Board of Medical Registration v. Kaadt, 1948). Others argue that a physician should be allowed to use an innovative procedure when it is *reasonable* to do so (Waltz and Inbau, 1971). In determining the reasonableness of an innovative procedure, this latter approach would consider such factors as the availability of customary treatments, whether there is time to try a customary procedure before utilizing an innovative one, and the expertise of the practitioner regarding the innovative procedure. All courts and commentators maintain that human patients on whom innovative procedures are done must first exercise an informed consent to such procedures (Trantafello v. Medical Center of Tarzana, 1986), and that if it violates a patient's legal rights for a physician to perform such a procedure in the first place even an informed consent cannot extinguish the physician's liability.

There is reason to think that a court faced with a malpractice suit involving innovative veterinary therapy would adopt the last view mentioned above, namely that a veterinarian may utilize an innovative procedure if doing so is reasonable. First, some courts and legal scholars appear to be moving toward this view regarding human medicine (Waltz and Inbau, 1971; Rhodes, 1989). Second, much of the law's concern about abuses of innovative therapy by physicians is based on the need to respect peoples' fundamental legal and moral right to decide what shall be done with their bodies. In contrast, veterinary patients are animals, which the law regards as property under the control of their owners. Animal owners may use their animals as means toward their own economic or psychological ends, provided the animals are not subjected to unjustifiable pain or suffering. It would be paradoxical to prohibit owners to authorize innovative therapy designed to benefit their animals if this is what owners want, and if a veterinarian would reasonably deem such therapy advisable for the patient. Moreover, because an owner's purpose in requesting innovative therapy is at least in part to benefit the animal, one might expect courts also to demand of veterinarians as they require of physicians that innovative procedures be offered to benefit the patient and not just to obtain scientific data.

I wish to propose the following legal principles for approaching innovative procedures in veterinary medicine. They constitute, I suggest, a reasonable attempt to reconcile the need for innovation with the legitimate interests of the veterinarian's patients and clients. These principles are recommendations only. They may or may not be accepted by a given court in a given jurisdiction.

1. A veterinary specialist should advise an innovative procedure for a condition only if there is no effective customary procedure for dealing with this condition.

2. A veterinary specialist should recommend an innovative procedure only if reliable data or information justifies the conclusion that the procedure might benefit the patient, and is no less likely to benefit the patient than a customary procedure.

3. Although data relevant to the scientific validity and effectiveness of the procedure can result from the recommended treatment, no innovative treatment should be performed in order to obtain such data. Benefiting the patient must be the operative motivation.

4. The client must exercise an informed consent to the procedure. This means that the client must understand the nature of the procedure, the nature and likely consequences of any other available procedures, the potential known risks of the procedure to the life and quality of life of the patient, the possibility of unknown risks if the consequences of the procedure are especially unpredictable, and the fact that this is an innovative procedure. Although the law never requires authorizations by clients to be in writing, the more innovative, complex, or risky the procedure, the greater the reason to have the client sign a written consent—not just to protect the doctor but to assure that the client's consent is made knowingly and voluntarily.

5. Except in extreme or unusual circumstances (e.g., in an emergency where a customary therapy would clearly be ineffective) generalists should not attempt innovative therapy without consulting with or referring the case to a specialist. As a rule, specialists are more likely than generalists to know whether there already exists a potentially effective innovative procedure and whether a proposed innovative procedure is likely to benefit the patient.

B. Issues in Academic Veterinary Medicine

Clinician members of veterinary school faculties, most of whom are specialists, sometimes confront situations in which the ethical dilemmas posed by innovative procedures are intensified. Academic clinicians often view as part of their function the finding of better, innovative procedures and the discovery of data regarding the effectiveness of innovative procedures. Academic veterinarians will sometimes see a patient as a candidate for a different medical or surgical approach, when a specialist in private practice who may await reports of innovative procedures by academic clinicians in the journals might not.

The development of new treatments and techniques by specialists

in academic practice is essential for progress in veterinary medicine. Therefore, academic veterinarians sometimes have a legitimate interest in recommending innovative procedures, and one reason it can be morally correct to perform such a procedure is that other animals and clients may benefit from it. Difficult ethical issues arise if the patient may experience distress or discomfort as a result of the innovative procedure, either because it is not clear any treatment will help or because the procedure itself might cause more distress than would an existing customary one. Also relevant is the fairness of a treatment that might cost the client more than a customary approach (such as euthanasia) and that is aimed at obtaining future benefits for *other* patients and clients.

For academic veterinarians engaged in the search for innovative procedures, I reiterate the five principles suggested above and would add the following remarks. Clinicians must resist the temptation to permit a desire to find innovative procedures or to evaluate a particular innovative procedure to lead them to ignore or disfavor a customary treatment that will likely be more beneficial to *this* patient. Clients should always be informed when part of the clinician's motivation for recommending an innovative approach is to gather data that could be beneficial to other patients and clients. Clients should be informed whether this procedure is likely to be more distressful to the patient or more costly than a customary procedure or another possible innovative procedure. Interns or residents who are required as part of their academic program to present or publish clinical research reports must never perform procedures based on their own educational interests rather than the needs of the patient and client.

C. From Innovative Therapy to Clinical Trials: The Patient as Means Rather Than End?

The use of an innovative procedure on a particular patient can eventuate in a clinical trial to test the procedure more rigorously by utilizing a statistically significant number of animals. Conversely, practitioners may begin using an innovative approach after the completion of a clinical trial of the approach. Clinical trials differ from innovative therapy in that the former involve, by definition, procedures that are part of a larger, systematic effort to test a scientific or medical hypothesis. Clinical trials, especially randomized clinical trials (RCTs) in which subjects are assigned at random to experimental and control groups, have led to considerable controversy in human medicine (Levine, 1986). Among the many ethical questions that can be asked about

clinical trials in human or veterinary medicine are when it is appropriate to begin a trial (e.g., whether a trial can ever be justified when there already exists an effective treatment); how far a trial should proceed when it becomes apparent that the treatment being evaluated will not work; whether a patient doing poorly should be removed from the experiment if some other treatment would benefit it more; whether subjects in control groups can be removed from the group and treated if it becomes clear that a treatment under investigation is effective; to what extent placebos may be ethically justifiable; and whether it is appropriate to charge clients for the trial or medical care necessitated by it.

Ethical issues raised by full-scale clinical trials are discussed in Chapter 5. This chapter focuses on legal and ethical questions relating to specialization faced more commonly in practice by veterinarians, few of whom engage in conducting clinical trials. Nevertheless, it is useful here to highlight the most serious ethical problem in clinical trials. As has been discussed, this problem is already faced by specialists contemplating innovative therapies. By looking at this problem in the context of clinical trials, one can appreciate more clearly why it is of crucial importance whenever an innovative procedure is considered.

The major ethical problem in clinical trials, and especially RCTs, is that helping the patient (and in veterinary medicine the client as well) is no longer the sole focus of the practitioner. The patient is also a vehicle for achieving another goal: the testing of a procedure, product, or scientific hypothesis. Philosopher Charles Fried has argued (Fried, 1974) that human RCTs deprive patients of one of the central values in the doctor–patient relationship: the "good of personal care." Medical patients believe that their doctor is loyal to them and their interests, and only to *their* needs and interests. As Fried observes, RTCs deviate from this assumption, even when laws require that physicians who conduct such trials inform patients that part of the purpose of the investigation is to gather information of potential benefit to others.

Like human medical patients, veterinary clients assume that their practitioner will be loyal to them (Tannenbaum, 1995). This is why it is profoundly unethical for a veterinarian to utilize an innovative procedure on a patient without informing the client, and without assuring both himself and the client that the procedure is appropriate *for this patient.* Innovative veterinary procedures pose the risk that the desire to obtain useful information will compromise the loyalty assumed by the client. Full-scale clinical trials pose this risk even more strongly because they involve treating each patient as a vehicle to obtain benefits for other patients or other clients.

However defensible they might sometimes be, clinical trials, like innovative procedures, raise a warning for all veterinarians: in the typical professional relationship, in which a client brings a patient to you for its care, both the law and the fundamental principles of normative ethics assume that your sole aim is to serve the patient and client before you. If you deviate from this goal without good medical justification, and without full disclosure to the client and informed consent by the client, you endanger the good of personal care and proceed at your legal peril.

IV. Conclusion: Recommendations for the Future

Legal and ethical issues raised by veterinary specialization cannot be eliminated or made easy. The more specialization can provide effective treatments, the more generalists will have to know when they should refer to specialists; the more specialists and generalists will have to cooperate in the overall care of patients; and the more specialists will be able to offer complex, expensive, and innovative procedures.

A key element in all these legal and ethical issues is the importance and value of animals. The law would not obligate generalists to refer patients to specialists unless people thought that animals can *merit* specialty veterinary care in the first place. Nor would anyone be troubled about the morality of subjecting animals to difficult advanced treatments unless it was believed that they *deserve* not to suffer unjustifiable pain or distress.

Specialization generates legal and ethical questions not just because specialists develop advanced procedures that themselves raise ethical questions. Specialization also plays a central role in the elevation of the value of veterinary patients. As specialization makes possible more effective veterinary care, clients become more eager to obtain such care. The very ability to help an animal one wants to value allows the emotional or economic bonds to strengthen and grow. These bonds nourish the demand for advanced veterinary services, which in turn, drives the profession to provide them. As veterinary patients are valued ever more highly, the more the law will seek to protect them and their owners, and the more doctors and clients will be compelled to confront difficult ethical dilemmas.

The following recommendations are offered to assist veterinarians to prepare for the inescapable legal and ethical problems associated with specialization.

1. All veterinarians must appreciate that advances in veterinary medicine, often developed by the specialties, generate difficult legal and moral questions. All veterinarians should make the continuing study of veterinary law and ethics an important part of their professional activity.

2. State veterinary licensing boards should prevent false, deceptive, and misleading representations about specialization by generalists and specialists. States that do not have explicit regulations prohibiting generalists from representing themselves as specialists should adopt and enforce such rules. The boards should also guard against statements by specialists or facilities employing specialists that falsely claim the superiority of specialty over general care.

3. Generalists should strive to keep current with advances in the specialties so that they know when cases should be referred and when a new or advanced procedure is appropriately done, or expected, in general practice. States that do not yet require continuing education as a condition of licensure should do so, and the licensing boards should encourage all doctors to attend education sessions that will keep them informed about developments in the specialties. Specialists who give instructional talks at continuing education classes or professional meetings should make clear when they are informing generalists about the latest developments in their specialty, when they are indicating conditions for which a referral is advisable, and when they are describing techniques appropriate for general practice.

4. The process of the recognition of new specialties must include consideration of legal burdens such recognition can impose on generalists. Qualified candidates for certification must never be subjected to exclusionary barriers that can have the effect of protecting someone else's income or professional status. To protect the public, animals, specialists, and generalists, groups of practitioners should be recognized by the AVMA as specialties only if they provide services that are distinctive in some way from those provided by generalists or existing specialties.

5. The veterinary profession should engage in serious discussion of legal and ethical problems presented by innovative therapies and clinical trials. There is nothing in the *Principles of Veterinary Medical Ethics* that addresses this topic. By contrast, there is an extensive and growing literature relating to experimentation in human medicine, and the medical profession has authored several official ethical codes and guidelines dealing with experimentation on human subjects (Capron, 1986; Annas and Grodin, 1992). The importance of their patients requires that veterinarians also deliberate seriously about how the

need to develop newer, more effective therapies can be reconciled with the interests of current patients and clients.

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[7]Physician who had completed one-third of his residency training held to the higher specialty standard in lawsuit alleging negligence arising out of defendant's performance of a circumcision operation, which led to gangrene and the necessity of removal of the patient's glans penis. The court held that the defendant had "held himself out as a specialist" by virtue of his having performed between six and eight hundred such operations and his own testimony that he considered his residency a program one in which he "specialized" in these procedures.

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