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STUDIES IN CANINE IMMUNOBIOLOGY WITH SPECIAL REFERENCE
TO MECHANISMS OF LYMPHOID CELL DEPLETION
IN VIRAL DISEASE

DISSERTATION
Presented in Partial Fulfillment of the Requirements for
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By

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FIELDS OF STUDY

Major Field: Veterinary Pathology

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Studies in Oncology and Neuropathology. Professor Adalbert Koestner
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CHAPTER I

NEW ASPECTS OF CANINE IMMUNOBIOLOGY

I. Introduction

During the past decade, dramatic advances in immunological research have increased our understanding of the role of immune mechanisms in disease processes. The dog has played a central role in these endeavors, particularly in relation to transplantation, hypersensitivity and autosensitivity.

This review is intended to summarize the current status of immunologic injury in dogs and to suggest potential directions for future research. The discussion is divided into 6 sections: development of immunity, serum proteins, hypersensitivity, autosensitivity, transplantation and tumor immunity. Space limitations prevent our citing all pertinent references, but we have tried to select the most timely and comprehensive reports where possible.

II. Development of Immunity

1. General Considerations

The lymphoreticular system is responsible for the development and maintenance of adaptive immune mechanisms (reviewed by Good and Papermaster, 1964). Its structural components may be divided into a
triptite developmental and functional hierarchy: the stem cell compartment (probably bone marrow), the so-called central lymphoid tissue composed of thymus and gut-associated lymphoid tissue (GALT), and the peripheral lymphoid tissue composed of spleen and lymph nodes. Current evidence suggests that bone marrow may seed pluripotential stem cells to the central lymphoid tissues which direct their differentiation, subsequent deployment to peripheral lymphoid tissues and maintenance as immunologically competent cells (reviewed by Daniels et al., 1968).

2. Lymphoid Tissue

In the dog, lymphoid tissues mature during the last third of fetal life. The thymus is present by the 40th day of gestation and lymphoid cells appear in the lymph nodes and spleen by the 48th and 54th days, respectively. At birth, Peyer's patches are present in the small intestine and population of the lymphoid tissues is essentially complete (Kelly, 1963; Hayes, 1968). The adult dog is reported to have about 1.5 to 2.8 x 10⁹ mobilizable small lymphocytes per kilogram of body weight or about 7 times the number found in the peripheral blood (Schnappauf and Schnappauf, 1968). These lymphocytes originate in peripheral lymphoid tissues and probably recirculate from blood to lymph (Benninghoff et al., 1968). Architecturally, mature canine lymphoid tissue resembles that found in other mammals (Miller et al., 1964). Senile changes occurring in the lymphoid tissue of ageing dogs include: involution of the lymph nodes and thymus, nodular hyperplasia
of the splenic white pulp and intrathymic epithelial cyst formation (Mulligan, 1963; Kruvskii, 1967).

3. Active Immunity

a. Humoral and Cell-mediated Responses

Immunologic responsiveness, in many species, to a series of antigens, appears to develop sequentially, rather than simultaneously (reviewed by Sterzl and Silverstein, 1967). Recent evidence indicates that the dog follows this pattern. For example, a humoral antibody response can be detected at birth in pups inoculated with bacteriophage ØX-174 on the 40th day of gestation (Jacoby et al., 1969). On the other hand, pups appear incapable of responding to an aqueous solution of bovine serum albumin (BSA) inoculated at birth unless it is first emulsified in complete Freund's adjuvant. Moreover, humoral responses to such antigens, as expressed by serum antibody titers, increase with age. Humoral responses in adult dogs vary with the nature and quantity of antigen used, as well as the route by which it is administered. In general, dogs are better producers of non-precipitating than precipitating antisera and respond better to particulate than soluble antigens (Patterson et al., 1963b; Jacoby et al., 1969).

Similarly, cell-mediated responses in dogs appear to be age-dependent (Dennis et al., 1969a). Allogeneic skin grafts placed on canine fetuses at the 40th or 48th day of gestation are rejected in a protracted fashion (mean survival time of 42 days), whereas neonatal
dogs are able to reject allogeneic skin from the same donor as rapidly as adult dogs (mean survival time of 9.5 days).

The senile changes in canine lymphoid tissue associated with ageing may portend a diminished capacity to respond to antigenic stimulation as has been demonstrated in mice (Hanna et al., 1967).

b. **Role of the Central Lymphoid Tissue**

The thymus and GALT appear to share control of the structural and functional development of the immune system (Cooper et al., 1967); the thymus governing the maturation of cell-mediated defenses and the GALT regulating humorally-mediated defenses. Consequently, removal of the central lymphoid tissue prior to full immunologic development should ablate, or at least diminish, immunologic responsiveness. Dogs thymectomized in utero on the 48th day of gestation and grafted with allogeneic skin at birth retain the grafts for prolonged periods (mean survival time of 40.8 days) (Dennis, et al., 1969b). It has been technically impossible thus far, to remove the thymuses of younger fetuses to determine when thymic influence on immunologic development begins. Predictably, thymectomy of neonatal and adult dogs does not cripple cell-mediated responsiveness (Van de Water and Katzman, 1964; Oliveras et al., 1963), because this aspect of immunologic competence is mature at birth. Nevertheless, thymectomized adult dogs whose lymphoid tissues have been destroyed by x-irradiation, fail to regain immunologic competence following bone marrow infusions (Chertkov et al., 1965), whereas their non-thymectomized counterparts recover (Samoylina
and Chertkov, 1966; Epstein, et al., 1967). These results indicate not only that the functional capacity of the thymus, although dormant, remains intact in adult dogs, but that lymphoid cell precursors subsist in the bone marrow.

The role of canine GALT in the development of humoral responsiveness is unknown. Some control over this phase of immunity in the dog has been preempted by the thymus, since thymectomy of fetal dogs diminishes humoral antibody production against bacteriophage ØX-174 (Dennis et al., 1969b). Chretein and associates (1967) have shown that Peyer's patches, in contrast to peripheral lymphnodes fail to hyper-trophy in dogs subjected to subtotal lymphadenectomy, thus implying that intestinal lymphoid tissue is distinct in some manner. The function of canine GALT might be clarified by comparing the immune responses of dogs who have recovered from tonsillectomy and ileocecouctomy coupled with x-irradiation to the responses of sham-operated x-irradiated dogs. One would expect, as has been shown in rabbits (Perey et al., 1968), that GALT is necessary for return of humoral responsiveness in animals so treated.

4. Passive Immunity

a. Prenatal Transfer

The hemochorial placenta of the dog acts as a selective unidirectional barrier which permits passage of specific serum proteins from dam to fetus while blocking passage of fetal antigens into the maternal circulation (Brambell, 1958; Jackson, 1967). The amount of
antibody absorbed by a fetus during pregnancy is small. Less than 300 milligrams of maternal plasma reaches each fetus daily (Whipple et al., 1955) and immunoglobulins represent only a fraction of this total. Gillespie and co-workers (1958) reported that about 3% of a dam's neutralizing titer to canine distemper virus is transferred in utero. It is not known whether prenatally-transferred canine antibodies are exclusively low molecular weight 7S globulins as reported in most species (reviewed by Brambell, 1966) or high molecular weight 19S globulins or a combination of both.

b. Postnatal Transfer

The bulk of passive immunity is conferred on pups during the first days of life through ingestion and absorption of colostral antibodies. Carmichael and co-workers (1962) found that maximal absorption of antibodies to infectious canine hepatitis virus occurred during the 12 hours after birth and was essentially complete by 72 hours. Gillette and Filkins (1966) reported that maximal absorption of antibodies to Salmonella pullorum occurred if puppies were fed 8 hours after birth and absorption was completed within 15 hours. Absorption was not detectable in pups fed antibody later than 24 hours after whelping.

The mechanism of absorption is obscure, but may involve recognition of antigenic determinants on immunoglobulin molecules by specific receptors on intestinal epithelial cells (Brambell, 1966). Factors associated with cessation of absorption are also ill-defined. Filkins and Gillette (1966) and Gillette and Filkins (1966) noted that whereas
ingestion of other food is not a major factor, pups from bitches treated 24 hours prepartum with hydrocortisone or adrenal corticotropic hormone had a significantly reduced capacity to absorb antibodies through the intestine. Moog (1953) produced the same response in mice and found that it coincided with an elevation of duodenal alkaline phosphatase activity.

Colostral antibody titers of immune dams may exceed their corresponding serum antibody titers at the time of whelping, but decline markedly within several days, thus correlating well with cessation of antibody absorption by puppies (Mason et al., 1930; Carmichael et al., 1962). Quantitative relationships have been established between the antibody titers in the bitch's serum and colostrum and the serum antibody titers of their progeny (Baker et al., 1959). These relationships have been used to predict the optimum time for active immunization of puppies since the presence of maternal antibody interferes with this process. Recent evidence indicates that canine colostral antibodies may represent a special class of serum proteins (IgA) selectively secreted into milk (Section III).

III. Serum Proteins

1. General Considerations

Canine serum contains about 6 grams of protein per 100 milliliters (Engle and Woods, 1960; Kozma et al., 1967, Irfan, 1967). Albumin constitutes about 50% of the total serum proteins and the remaining portion consists of a structurally and functionally heterogeneous group
of compounds called globulins. Paper electrophoretic analysis of canine serum usually reveals 1 peak in the albumin region and 4 to 6 peaks among the globulins; 2 peaks in the $\alpha$ region (10-20% of the total serum protein), 1 peak in the $\beta$ region (7-20% of the total serum protein) and 1 to 3 peaks in the $\gamma$ region (7-19% of the total serum protein) (Spector, 1956; Vesselinovitch, 1959; Engle and Woods, 1960; McKelvie et al., 1966; Irfan, 1967; Kozma et al., 1967). The wide range of values results from variations in technic, interpretation and animal populations used for study. Immunoelectrophoretic analysis of canine serum has revealed at least 20 precipitin arcs (Okoshi et al., 1967).

2. Immunoglobulins

Globulins migrating in the $\beta$ and $\gamma$ regions are immunologically active proteins with a half life of about 8 days (Dixon et al., 1952), which are synthesized by the lymphoreticular system in response to antigenic stimulation and are known collectively as immunoglobulins. In man, 5 classes of immunoglobulins have been recognized; IgA, IgD, IgE, IgG and IgM (alternatively $\gamma\text{A}$, $\gamma\text{D}$, $\gamma\text{E}$, $\gamma\text{G}$ and $\gamma\text{M}$)$^a$ (reviewed by Merler and Rosen, 1966). Canine analogues for 4 of these classes have recently been proposed as summarized in Table 1. This classification may change as additional information about canine immuno-

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<tr>
<th>Class</th>
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<th>Electrophoretic Mobility</th>
<th>Selected Properties</th>
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<tr>
<td>IgM</td>
<td>19 S</td>
<td>γ1</td>
<td>Cross reacts with human IgM, precipitating and agglutinating activity</td>
</tr>
<tr>
<td>IgG</td>
<td>7 S</td>
<td>γ2&lt;sub&gt;a&lt;/sub&gt;, γ2&lt;sub&gt;b&lt;/sub&gt;(γ2&lt;sub&gt;c&lt;/sub&gt;)</td>
<td>Precipitating and agglutinating activity, Arthus activity, mediates passive cutaneous anaphylaxis in guinea pigs</td>
</tr>
<tr>
<td>IgA</td>
<td>7 S</td>
<td>γ1</td>
<td>Nonprecipitating, inhibits precipitation of antigen by precipitating antibodies, present in colostrum at 4-80 X greater concentration than in serum, present in bronchial and salivary secretions, inhibits passive anaphylaxis induced by reaginic antibody, cross reacts with human IgA.</td>
</tr>
<tr>
<td></td>
<td>Intermediate S</td>
<td></td>
<td>(7S-19S, probably polymeric form)</td>
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IgE 7 S - 9 S \gamma_1 \quad \text{Reaginic activity, Prausnitz-Kustner reactions, passive transfer of local and systemic anaphylaxis}

IgD (unidentified)

\footnote{Tentative classification based on reports by Johnson \textit{et al.}, 1967a, 1967b; Rockey and Schwartzman, 1967; Patterson \textit{et al.}, 1968, 1969; and Vaerman and Heremans, 1968}
globulins becomes available.

Patterson and co-workers (1963b) had difficulty producing precipitating antibody against heterologous serum proteins in dogs. They found subsequently that 2 types of antibody were made against the same antigenic determinant; a precipitating antibody and a non-precipitating antibody (Patterson et al., 1964). Non-precipitating antibody appeared to interfere with the ability of precipitating antibody to precipitate antigen in the region of antibody excess and resulted in a delay in attainment of equilibrium which is characteristic of canine precipitin curves. Precipitating antibody migrates electrophoretically in the \( \gamma_2 \) region as 2 or 3 antigenically distinct proteins which are collectively designated as canine IgG (Johnson and Vaughan 1967a, 1967b; Patterson, 1968). Non-precipitating antibody migrates as a fast \( \gamma_1 \) protein and is referred to as canine IgA. Specific antisera to canine IgA do not react with canine IgM or IgG (Patterson et al., 1968), but some antisera against human IgA do cross react with IgA from canine serum and milk (Vaerman and Heremans, 1968). Canine IgM has been identified as a fast moving \( \gamma_1 \) (\( \beta \)) macroglobulin and Vaerman and Hermans (1968) have shown cross reactions between human and canine IgM in immunoprecipitation tests. Antibodies which mediate immediate type hypersensitivity reactions (i.e. allergic rhinitis, anaphylaxis) are known as reagins. A canine immunoglobulin with reaginic properties, but antigenically distinct from IgG, IgA and IgM, has recently been identified and is provisionally designated as canine IgE (Patterson et al., 1968; 1969). Whether
all reaginic activity in canine serum is restricted to IgE is not known.

3. Other Immunologically Active Serum Proteins

Complement activity resides in an integrated system of at least 9 serum components. They interact in sequence after antibody has bound to antigen and mediate a variety of immunologic reactions in vitro and in vivo including: immune cytolysis, phagocytosis and immune complex phenomena (reviewed by Muller-Eberhard, 1968). The individual components of canine complement have not been studied, but Larin and co-workers (1957) showed that canine and guinea pig complement possess similar levels of hemolytic activity. They also used canine complement successfully in place of guinea pig complement for determining complement-fixing antibody titers in immune sera against infectious canine hepatitis virus. Normal canine serum complement levels based on hemolytic potency have been reported by several groups of investigators and generally range from 17 to 55 CH50 units/ml (Baltch et al., 1962, 1966; Gewurz et al., 1966).

C-reactive protein is a non-antibody serum component found in man particularly during the acute stages of rheumatic disease and which forms a precipitate with the somatic C-polysaccharide of the pneumococcus. Dillman and Coles (1966) have isolated a protein from the serum of dogs subjected to several inflammatory stimuli that was agglutinated by an antiserum specific for C-reactive protein in man. The function of this serum fraction remains obscure.
Properdin, another physiochemically distinct normal serum protein which may participate in several immunological reactions such as virus neutralization and bacteriostasis has also been identified in canine serum (Baltch et al., 1962; Michaelson et al., 1966).

4. Serum Protein Alterations in Disease

Increased levels of serum $\alpha_2$ globulins have been reported in the dog in canine distemper (Gibson et al., 1965; Snow et al., 1966), infectious canine hepatitis (Beckett et al., 1964), renal disease (Moegle et al., 1956), miscellaneous inflammatory conditions (Boguth, 1953), tumors (de Wael, 1956), allograft rejection (West et al., 1960), and following cortisone administration (Bossak et al., 1955). This alteration seems to be non-specific although Howard and Kenyon (1965) were able to produce elevated $\alpha_2$ globulins in normal dogs by administering histamine.

Seemingly nonspecific elevations of $\beta$ globulin in the dog have been found in chronic skin disease, endometritis, eosinophilic myositis, liver necrosis and nephritis (Boguth, 1953; Moegle et al., 1956). De Wael (1956) has claimed that $\beta$ globulins may be elevated in certain malignant, but not benign tumors. Some of these reported elevations of canine serum $\beta$ globulin may be due to specific antibody production since $\gamma\delta M$ is known to migrate to this region of an electrophoretic field.

Serum gammaglobulins may be elevated in almost any infectious, neoplastic or nonspecific inflammatory disease (Boguth, 1953; de Wael,
1956; Beckett et al., 1964, Snow et al., 1966). They may also be increased in association with the rejection of transplanted organs (Chiba, 1966) and in some of the so-called autosensitivity syndromes. Most of these increases probably represent host responses to specific antigenic stimulation.

Another type of canine hypergammaglobulinemia is associated with neoplastic disease of antibody-forming cells, plasma cell myeloma. Osborne and co-workers (1968a) reviewed 22 cases of canine plasma cell myeloma from 21 published reports. Unfortunately, serum protein values were available for only 11 of these cases, but each of 8 sera in this group examined for either hypergammaglobulinemia or paraproteinemia (abnormal globulins with little or no antibody activity) were positive. Bence-Jones proteinuria was found in 4 of 8 cases. The reader is referred to the review of Osborne and collaborators for a detailed account of this interesting canine gammopathy. To our knowledge, other forms of congenital or acquired canine dysgammaglobulinemias have not been reported. Reviews by Alper and associates (1966) and Rosen and Janeway (1966) provide an introduction to this problem in man.

Amyloidosis must be mentioned here because it is characterized by intracellular deposition of an amorphous material (Trautwein, 1965) which may or may not contain immunologically-active serum proteins (reviewed by Cohen 1967). The condition is frequently associated with chronic inflammatory disease and plasma cell myelomas. In dogs, the renal glomerulus appears to be a predilection site for amyloid
deposition and in advanced disease amyloid deposits may interfere with renal function and lead to uremia (reviewed by Osborne et al., 1968).

IV. Hypersensitivity

1. General Considerations

Hypersensitivity can be defined as heightened reactivity following repeated contact with an antigen and which results in immunologically-induced injury to tissues. Two basic forms of hypersensitivity are recognized: immediate and delayed. Immediate hypersensitivity reactions usually occur within minutes after administration of an offending agent and are mediated by circulating antibodies which unite with antigen on or near cell surfaces to cause release of pro-inflammatory substances. Anaphylaxis, allergy, and immune complex reactions (Arthus reactions, serum sickness) are examples of immediate-type responses (reviewed by Austen, 1965; Cochrane, 1968). Delayed hypersensitivity reactions appear 12 to 48 hours after challenge with antigen and are mediated by sensitized lymphoid cells rather than circulating antibody. When sensitized cells contact antigen they appear to release mediator substances which are chemotactic for leukocytes and nonsuppurative inflammation ensues. The tuberculin reaction, contact dermatitis, allograft rejection and certain forms of auto-sensitivity disease are considered prototypes of delayed hypersensitivity (reviewed by Uhr, 1966; Benacerraf and Green, 1969).
2. Immediate Hypersensitivity

a. Anaphylaxis

In 1902 Portier and Richet described a syndrome in dogs characterized by bloody diarrhea, emesis, prostration, and death following attempts to immunize the animals with an extract of sea-actinia. Dogs were injected at 3-week intervals and the fatal reaction quickly followed the second exposure. These workers decided they had not produced immunity or prophylaxis, but rather hypersensitivity which they called anaphylaxis.

The target organ for anaphylaxis varies with each species. In the dog, the liver is the principle shock organ. Sensitizing antigen when administered intravenously appears to be bound by anaphylactic antibody fixed on liver mast cells (Akcasu and West, 1960; Csaba et al., 1963, 1966). The mast cells release histamine (plasma levels increase markedly) which causes contraction of the spirally-arranged musculature of intrahepatic throttling veins (Arey, 1941). Blood is retained in the liver, venous return to the heart decreases, cardiac output falls and hypertension and shock ensue (Chou, 1965). Vasoactive amines other than histamine have not been incriminated in canine anaphylaxis (Cirstea et al., 1966).

A variety of antigens may elicit anaphylactic reactions in dogs including homologous and heterologous serum proteins (Bliss et al., 1959; Patterson et al., 1963b; Peng and Pi, 1967), ragweed antigen (Patterson and Sparks, 1962) and endotoxin (Spink et al., 1964).
The microfilariae of *Dirofilaria immitis* have recently been found to provoke anaphylactic reactions following transfusion of homologous blood into infected dogs (Ota *et al.*, 1962). Godfrey and co-workers (1966) observed similar reactions in 75% of dogs with and 21% of dogs without demonstrable microfilariasis. Mantovani and Kagan (1967) have isolated an antigenic fraction of *D. immitis* which will react in skin tests or passive hemagglutination tests on dogs infected with the homologous parasite but not in dogs infected with *D. repens* or *Dipetalonema sp.* In lieu of these findings, it has been recommended that either blood donors be free of microfilaria or recipients desensitized to *D. immitis* (if time permits) so that these "transfusion reactions" can be minimized (Ota *et al.*, 1962).

b. Allergy

The term allergy is used to describe a heightened predisposition (often familial) to spontaneous local anaphylactic reactions frequently involving the skin, respiratory tract or gastrointestinal tract. The inciting agent is known as an allergen and the mediating antibody is known as a reagin. Dogs may develop allergies to a host of environmental allergens including animal flesh, cereal grains, milk, dust, feathers, tobacco, trees, mold and internal parasites (Brunner *et al.*, 1944; Cortez *et al.*, 1947; Baker, 1966; Walton, 1966, 1967).

Most significant among canine allergies, for conceptual and comparative value, is allergy to ragweed pollen, which closely resembles hay fever in man (Wittich, 1941; Patterson, 1960). Susceptible dogs in
the Northern hemisphere begin to show annually recurring signs of gen­eralized pruritis, conjunctivitis, rhinitis and, occasionally, asthma during late summer which continue until the onset of cold weather. In succeeding years, however, multisensitivities may appear in allergic animals, thereby delaying the amelioration of clinical signs (Schwartzman, 1965; Patterson, 1968). Sex, age or breed predilections have not been established for the condition, but a high incidence has been reported in terriers (Schwartzman and Rockey, 1967).

Sensitive animals usually have positive skin reactions to ragweed pollen (Schwartzman, 1965) and asthmatic responses may occur in some dogs if exposed to aerosolized ragweed antigen (Patterson, 1960). Hypersensitivity to ragweed pollen can be passively transferred from affected to normal dogs with serum (Patterson and Sparks, 1962). Passively sensitized dogs challenged with ragweed antigen may display signs ranging from skin reactivity to asthma and anaphylaxis.

Ragweed allergy in dogs closely resembles hay fever in man in that both are: 1) incited by ragweed pollen 2) of proven immunological basis 3) demonstrable through direct or passive (P-K reaction) skin testing 4) ameliorated by antihistamines and epinephrine 5) clinically similar, and 6) probably familial (Schwartzman, 1965). Patterson and associates (1963c) have reinforced the last point by establishing a breeding colony of dogs with spontaneous allergy to ragweed. One major difference between the conditions in the two species is that dermatitis of obscure etiology occurs in dogs, but not in man (Schwartzman, 1965).
Allergic reactions to ingested allergens in dogs consist primarily of gastrointestinal disturbances (emesis, diarrhea), but may also include cutaneous responses such as pruritis and urticaria which lead to self-inflicted trauma (Joshua, 1956; Walton, 1966, 1967). Skin testing has been used successfully to incriminate a variety of ingested allergens in suspected cases of canine gastrointestinal allergy (Walton, 1967), but the pathogenesis of these reactions has not been studied.

Treatment of canine allergies by desensitization has been encouraging. Success appears to depend in large measure on accurate identification of the offending allergen and proper administration of standardized allergenic extracts (Baker, 1969).

c. Antibodies in Anaphylaxis and Allergy

Spontaneous canine allergy, at least to ragweed pollen, appears to be mediated by a nonprecipitating, thermolabile, skin-sensitizing reaginic antibody (Patterson et al., 1963a, 1969). On the other hand, normal dogs actively immunized with ragweed pollen develop non-precipitating, thermostable antibodies similar to those elicited by immunization with heterologous serum proteins (Patterson et al., 1965; Arkins et al., 1967; Bukosky et al., 1968). The latter seem incapable of mediating either active or passive systemic anaphylaxis, but rather inhibit the reactions of dogs passively sensitized with canine reagin to challenge with ragweed extracts (Tennenbaum et al., 1963; Patterson et al., 1965). These so-called "blocking antibodies" are also pro-
duced in man by active immunization against allergens and appear to protect individuals against allergic reactions by neutralizing the offending allergen before it can bind to reagin fixed to cell surfaces (reviewed by Connell, 1969).

Anaphylactic and reaginic activity to ragweed antigen seems to be located in the same serum fraction, but whether they are immunologically identical is not definitely known (Patterson et al., 1963a). Anaphylactic reactions may occur during the course of active immunization to heterologous proteins (Patterson et al., 1963b), but antibody from immunized dogs cannot sensitize normal dogs for PCA reactions. Canine anti-hapten or anti-egg albumin antibodies can, however, mediate PCA reactions in guinea pigs (Ovary et al., 1964; Patterson et al., 1964). Hence, anaphylactic activity in canine serum does not appear to be invariably associated with reaginic activity and may represent a mixture of antibody types. The anaphylactic antibodies of mammals have recently been reviewed by Bloch (1967).

d. Injury Due to Immune Complexes

When antigen unites with precipitating antibody, under conditions of moderate to great antigen excess, soluble complexes are formed. These antigen-antibody complexes can precipitate in tissues, usually beneath the capillary endothelium, and initiate the release of pro-inflammatory factors from bound complement. A focal suppurative inflammatory process ensues accompanied by thrombosis, necrosis and hemorrhage when elicited in skin (Arthus reaction). Generalized immune-
complex injury (such as serum sickness) may involve any tissue, but is most common in the kidney where renal glomerular capillaries provide vulnerable sites for deposition of immune complexes. Thus membranous glomerulonephritis is considered to be the classic lesion of immune complex disease (reviewed by Cochrane, 1968).

Arthus reactivity (Patterson et al., 1964) and serum sickness (Iwasaki et al., 1967) have been produced in dogs, but evidence of spontaneous immune complex disease is scant. Nevertheless, the growing recognition of spontaneous immune complex injury in other animal species (Porter and Larson, 1968; Oldstone and Dixon, 1969) prompts consideration of its occurrence in the dog.

Viruses. Nongranulomatous uveitis and interstitial keratitis occurs in about 20% of dogs during convalescence from infectious canine hepatitis (ICH). Carmichael (1964, 1965) was able to reproduce these lesions by inoculating ICH virus-antibody complexes intraocularly or by passive or reverse passive sensitization of normal dogs. Carmichael suggests that ICH virus may persist in the uvea following viremic stages of the disease and initiate immune injury upon complexing with antibody in the uvea and cornea. Similarly, ICH virus may persist for prolonged periods in the endothelium of renal glomerular and interstitial capillaries (Wright, 1967a, 1967b), thus virus-antibody complexes may trigger some cases of interstitial nephritis or glomerulonephritis in dogs.

ICH virus-antibody complexes may affect the progression of hepatic lesions in ICH as well. Gocke and collaborators (1967) showed
that dogs that were partially immune to ICH could survive acute stages of the disease only to develop chronic liver damage in 7 to 8 months in the form of chronic nonsuppurative hepatitis with widespread fibrosis. The disease was invariably progressive despite the fact that ICH virus was demonstrable in hepatic tissues by immunofluorescence for only the first 7 days. These workers reproduced the syndrome by challenging passively immunized dogs with virulent virus. They speculated that excess virus and low levels of antibody may have formed immune complexes continuously, producing chronic hepatic injury. This report awaits confirmation by other workers.

Hottendorf and Nielsen (1966, 1968) suggested an immune complex pathogenesis for glomerulonephritis found in 20 of 29 dogs with mastocytoma. Although they did not speculate on the antigen, the filterable agent recently incriminated in canine mastocytoma (Rickard and Post, 1968) is a plausible candidate.

**Autoantigens.** A group of diseases in man and lower animals is characterized by sensitivity to antigens of normal body tissues. In at least one of these conditions, systemic lupus erythematosus (SLE), circulating nuclear antigen-antibody complexes are known to deposit in the renal glomeruli and contribute to the glomerulonephritis invariably associated with the disease (Koffler and Kunkel, 1968). Canine SLE closely resembles its human counterpart (Lewis et al., 1965a), but the demonstration of immune complexes in nephritic canine kidneys has not been reported.
3. Delayed Hypersensitivity

The following discussion is restricted to contact dermatitis, flea bite dermatitis and brief mention of delayed hypersensitivity in infectious disease. Autosensitivity and allograft rejection are covered elsewhere. (Sections V and VI).

a. Contact Dermatitis

Contact dermatitis is a delayed cutaneous reaction to direct repeated contact with an environmental incitant. The offending agent may be any of a multitude of natural or synthetic chemical compounds (Muller, 1967). These compounds need not be antigenic in themselves, since many have haptenic properties and elicit sensitivity after combination with carrier proteins of skin (reviewed by Baer and Harber, 1965).

Contact dermatitis in dogs is accompanied by severe pruritus and erythema, particularly on sparsely-haired regions of the body, which may advance to excoriative or ulcerative dermatitis when self-trauma is extensive (Walton 1965). Histologically, it appears initially as a nonsuppurative dermatitis with typical perivascular mononuclear cell infiltrations common to all delayed hypersensitivity responses, but the reaction may become suppurative upon entrance of bacteria. Hyperkeratosis, acanthosis and dermal fibrosis with mast cell infiltration characterize chronic lesions. The severity of disease is enhanced by repeated contact with the sensitizing agent. Suspected incitants may be identified by patch testing which evokes a local
dermatitis after a delay of 12 to 96 hours. Contact dermatitis cannot be passively transferred with serum, since it is cellullarly mediated, thus differentiating it from dermatitis associated with immediate hypersensitivity. Cure depends on elimination of the causative agent from the dog's environment.

b. **Flea Bite Dermatitis**

Summer eczema of dogs, long suspected of being a hypersensitivity dermatitis (Kissileff, 1938), is now thought to be caused by sensitivity to flea bites. The reaction is similar, symptomatically and pathologically, to contact dermatitis (Muller, 1961) and may be initiated in sensitized dogs by the bite of a single flea (Kissileff, 1962).

The pathogenesis of flea bite hypersensitivity has recently been examined in detail using guinea pigs sensitized to the bite of the cat flea (*Ctenacephalides felis felis*) (Benjamini *et al.*, 1960). Initial exposure to sensitized animals to flea bites or whole flea extracts produced a delayed skin reaction for the first 5 to 7 days, which evolved into a combination of delayed and immediate reactions lasting up to 7 weeks eventually predominated by immediate hypersensitivity, and progressed to a nonreactive state about 1 month later (Benjamini *et al.*, 1961). The initial hypersensitive state could, however, be sustained for up to 18 months following a single exposure to fleas. Histologically, skin lesions reflected the change from delayed to immediate hypersensitivity, being first characterized by mononuclear
cell infiltration and later by the presence of eosinophils (Larivee et al., 1964). It was then shown that an oral secretion from cat fleas contained one or more haptens which can combine with dermal collagen components during feeding to sensitize susceptible animals (Benjamini et al., 1963; Young et al., 1963; Michaeli et al., 1965, 1966). Thus contact dermatitis and flea bite dermatitis appear to be initiated by similar pathogenetic mechanisms. Although this work was done in guinea pigs there is little reason to suspect that a different mechanism is operating in dogs. Hudson and coworkers (1960) showed that sensitized guinea pigs reacted to several species of fleas other than the one inducing sensitivity, indicating that all flea saliva may contain a common allergenic hapten. Furthermore, Michaeli and Goldfarb (1968) desensitized dogs and cats to flea bite hypersensitivity by weekly injections of a hapten fraction isolated from the saliva of cat fleas.

c. Infectious Agents

This topic receives brief treatment, not to minimize its importance, but because so little is known about it in dogs. Both ocular and cutaneous delayed reactions have been elicited in dogs convalescing from leptospirosis (Torten et al., 1967; Ben-Efriam and Torten, 1968). Canine histoplasmosis and coccidioidomycosis (Ditchfield, 1968) are also associated with delayed-type cutaneous reactivity, but the role of hypersensitivity in the pathogenesis of these diseases requires further study. Conversely, the relative resistance of dogs to tuberculosis has not been adequately explained (Hobson and Ellett, 1968). The recent
development of in vitro correlates of delayed hypersensitivity (reviewed by David, 1967) should allow further examination of hypersensitivity in canine infectious diseases.

V. Autosensitivity

1. General Considerations

Considerable interest has been generated among immunologists by the recognition of spontaneous diseases of man and animals in which the host appears to evoke immunologic injury against its own tissues. Collectively these are known as autosensitivity (autoimmune, autoallergic) disorders.

From a theoretical standpoint, if one accepts the hypothesis that during immunological development an individual's lymphoreticular system is programmed for nonreactivity to unsequestered autoantigens (Burnet, 1959), then autosensitivity becomes, fundamentally, the manifestations of loss of tolerance to autoantigens. Mechanisms proposed to account for breakdown of tolerance to "self" include: 1) release of sequestered antigens normally concealed by physioanatomic barriers; 2) altered antigenicity of host cells by physical or chemical modification; 3) neoantigen formation following combination of autologous and exogenous (e.g. viral) determinants; 4) cross-reactivity between exogenous (e.g. bacterial) and host tissue antigens; and 5) genetic or neoplastic abnormalities of the lymphoreticular system (reviewed by Burnet and Mackay, 1963; Paterson, 1966).
Witebsky (1959) and Mackay and Burnet (1963), in the spirit of Koch, have tendered sets of postulates as guides to the categorization of spontaneous immunopathies as truly autosensitive (summarized in Table II). We have liberally interpreted these postulates in our discussion of canine autosensitivity to stimulate further investigation of conditions which may currently satisfy only one or two of them.

2. Hemopoietic Diseases and Systemic Lupus Erythematosus

a. *Autoimmune Hemolytic Anemia*

Canine autoimmune hemolytic anemia (AHA) was first reported by Miller and associates (1957) and later described in detail by Lewis and coworkers (1963, 1965b). The disease is marked by severe, recurring hemolytic anemia accompanied by a positive direct antiglobulin (Coombs) test, which, in contrast to man, becomes negative during remission in dogs. Clinical signs include pallor, weakness, icterus, hemoglobinuria, anorexia, fever and malaise. Splenomegaly, peripheral lymphadenopathy and tachycardia may be detected during physical examination. Clinico-pathologically, the anemia is macrocytic and normoblastic, with polychromatophilia, anisocytosis, poikilocytosis, spherocytosis and hyperplasia of the bone marrow. Most importantly, eluates of erythrocytes from affected dogs can passively sensitize normal canine erythrocytes for the indirect antiglobulin test, thus supporting an autoimmune pathogenesis for the condition. Nevertheless, the etiology and pathogenesis remain largely obscure. Corticosteroid therapy and splenectomy
<table>
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<th>Criteria for Autosensitivity</th>
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<td><strong>Witebsky (1959)</strong></td>
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1. Demonstration of circulating or cell-bound autoantibodies in patient.

2. Isolation and characterization of the antigen against which the antibody is directed.

3. Production of antibodies against the same antigen in experimental animals.

4. Appearance of pathological changes in the corresponding tissue of an actively sensitized animal that are similar to those seen in the spontaneous disease.

1. Demonstration of autoantibody in patient.

2. Hypergammaglobulinemia.

3. Deposition of denatured \(\gamma\)-globulin (i.e. in renal glomerulus).

4. Mononuclear cell responses in damaged tissues resembling delayed hypersensitivity.

5. Presence of multiple seemingly unrelated autoimmune processes.

6. Favorable response to immunosuppressive therapy.
has secured temporary remissions of AHA, but permanent cures have not been reported.

b. **Idiopathic Thrombocytopenic Purpura**

Canine idiopathic thrombocytopenic purpura (ITP) (Magrane, et al., 1959; Waye, 1960) is often, but not invariably, associated with AHA. The combined disease has been likened to Evan's syndrome in man and may herald the appearance of canine systemic lupus erythematosus (Lewis, 1968, Lewis et al., 1965b). About one-third of affected dogs are reported to show spontaneous hemorrhage, usually as melena, epistaxis, hematuria or petechiae and ecchymoses in the skin and mucous membranes. Megakaryocytes may be plentiful in the bone marrow but circulating platelet levels frequently fail below 10,000 per cubic milliliter. Splenomegaly, lymphadenopathy and pulmonary capillary thrombosis may also occur. Canine patients with ITP do respond to corticosteroids or splenectomy but the syndrome usually recurs. Thrombocytopenia has been produced experimentally in dogs by the administration of antiplatelet serum (Tocatins and Stewart, 1939) and by the isoimmunization of dogs with homologous platelets (Baldini, 1965). As with AHA, however, the mechanisms which evoke spontaneous disease remain open for investigation.

c. **Canine Systemic Lupus Erythematosus**

When canine ITP, AHA, and membranous glomerulonephritis occur simultaneously or sequentially in the same animal the syndrome is called canine systemic lupus erythematosus (SLE) (Lewis et al., 1965a,
Polyarthritis (Lewis and Hathaway, 1967), pleurisy and butterfly-shaped facial eruptions and hepatic necrosis have also been observed in canine SLE (Lewis et al., 1965a). The anemia and thrombocytopenia may respond to corticosteroid treatment or splenectomy, but renal impairment is usually irreversible and eventually fatal. Histologically, in addition to changes accompanying ITP and AHA, thickened glomerular basement membranes occur ('wire loop' lesions) identical to those in human SLE. Glomeruli also contain deposits of PAS-positive material and are often adhered to Bowman's capsule. Periglomerular lymphocytic and plasmacytic infiltrates occur and fibrinoid change can sometimes be found in small renal arteries, but perivascular fibrosis ('onion skin' lesions) of splenic arteries is not reported to occur, in contradistinction to human SLE.

Serologic aberrations variably associated with canine SLE include hypergammaglobulinemia and autoantibodies against gammaglobulin (rheumatoid factor), thyroid gland, erythrocytes (positive antiglobulin test) and nuclear material (antineuclear L.E. factor) (Lewis et al., 1965a). L.E. cells (lupus erythematosus cells, usually neutrophils which contain globoid intracytoplasmic bodies presumably representing nuclear material phagocytized after contact with L.E. factor) are found in the peripheral blood of nearly every patient with canine SLE, but hematoxylin bodies (nuclear material) are not seen in tissues. L.E. cells are occasionally found in other canine diseases such as idiopathic polyarthritis and leukemia indicating the possible participation of autosensitivity in these syndromes (Lewis, 1965).
The cause and pathogenesis of canine SLE are unknown. Attempts to produce the disease experimentally in dogs with hydralazine (a human anti-hypertensive agent) followed recognition of a lupus-like syndrome in man accompanying administration of the drug, but these have usually been unsuccessful (Comens, 1956; Dubois et al., 1957; alarcon-Segovia, et al., 1967).

Lupus glomerulonephritis of NZB mice (Mellors, 1968) and man (Koffler and Kunkel, 1968) is believed to involve deposition of antigen-antibody complexes with fixation of complement in the renal glomerulus. If the same phenomenon occurs in canine SLE immune complexes should be demonstrable in affected kidneys either immunohistochemically or ultrastructurally (McCluskey et al., 1966). One might also attempt to isolate such complexes from renal tissue or peripheral blood and determine 1) their antigenic constituents and 2) their ability to produce glomerulonephritis after intravenous injection into normal dogs (reviewed by Koffler and Kunkel, 1968). Isolation of complexes from blood may first require bilateral nephrectomy, since it is likely that the kidneys act as sponges for these complexes. Lewis (1968) has found that L.E. cells appear in the progeny of dogs with SLE as early as 4 months of age, thus giving support for genetic influences in canine SLE. Affected mice also develop lymphoma associated with virus particles, but the role of the virus in development of this multifaceted murine syndrome is unclear (Mellors, 1968). We do not know of any reports implicating immune aberrations in canine lymphoma.
3. Renal Diseases

a. Glomerulonephritis

Although spontaneous canine glomerulonephritis may be related to physical entrapment of immune complexes whose antigenic components are unrelated to kidney, glomerulonephritis has been experimentally produced in dogs by injection of nephrotoxic antikidney antibodies raised in homologous or heterologous species against canine renal antigens (Krakower and Greenspon, 1954; Stickler et al., 1956; Steblay, 1963; Robertshaw, 1967; Fukuda et al., 1968). This nephrotoxic serum nephritis appears to occur in two phases. Initially, nephrotoxic serum damages the renal glomerular basement membrane; later, deposition of electron dense material takes place beneath the glomerular capillary endothelium. These deposits are thought to be immune complexes comprised of either foreign serum protein antigen-antibody or autologous glomerular antigen-antibody, antigen in the latter complex being released during initial exposure to nephrotoxic serum (Movat et al., 1961).

The antigen responsible for evoking nephrotoxic antibody appears to be a soluble component of glomerular basement membrane (Shibata et al., 1966a, 1966b). It seems closely related to a nephrotoxic antigen of human glomerular basement membrane, since antihuman glomerular basement membrane produces glomerulonephritis in dogs (Steblay, 1963).
Heymann and associates (1962) attempted, unsuccessfully, to produce experimental autoallergic nephritis in dogs by the injection of homologous kidney antigen emulsified in Freund's adjuvant. Spontaneous autoallergic nephritis has not been reported in dogs. Hypothetically, immunologic injury could play a role in the pathogenesis of glomerulosclerosis, one of the common renal lesions of aged dogs. Guttmann and Anderson (1968) reported that low level x-irradiation accelerates development of glomerulosclerosis and IgG was found in the mesangium and capillary basement membrane of sclerosing glomeruli.

A canine homologue for human poststreptococcal rheumatic disease (reviewed by Cluff and Johnson, 1965) has not been documented. There are reports, however, of post-bacteremic glomerulonephritis associated with experimental Streptococcus mitis infection in dogs (Highman et al., 1958) and occasionally we have observed chronic membranous glomerulonephritis associated with longstanding streptococcal polyarthritis. Furthermore, the valvular lesions seen in hearts of aged dogs resemble similar lesions of rheumatic heart disease in man (Jones and Zook, 1965). It is not unreasonable to speculate that a portion of idiopathic canine membranous glomerulonephritis and endocarditides may be rheumatic sequellae to acute bacterial disease.

b. **Interstitial Nephritis**

Chronic interstitial nephritis (CIN) is the most frequently occurring yet least understood renal disease of dogs. It begins as an acute nonsuppurative interstitial nephritis but progresses inexorably
to the subacute and chronic stages. Glomerular lesions are usually not present early in the syndrome, but membranous thickenings, fibrosis and adhesions are evident in many chronic cases (McIntyre and Montgomery 1952; Anderson, 1968). The natural history suggests that immunologic injury may contribute to the pathogenesis of CIN.

The initial cause of interstitial nephritis is unknown although leptospiroae are sometimes associated with the acute and subacute phases (McIntyre and Montgomery, 1952). Recently, Anderson (1967) produced subacute interstitial nephritis in 3 of 5 dogs inoculated with Leptospira canicola. McIntyre and Montgomery (1952) proposed that these organisms first invade the tubules from the interstitium since glomerular lesions do not accompany acute leptospiral nephritis. If one assumes that leptospiroae or some other infectious agent produces primary damage to tubular portions of the nephron, how can one account for the chronic progressive nature of CIN?

Firstly, the offending agent may remain in the tissues (carrier state) and become a source of constant irritation, thus provoking a chronic inflammatory response. Leptospira (McIntyre and Montgomery, 1952) and ICH virus (Wright 1967a, 1967b) are capable of establishing carrier states in dogs. Moreover, delayed type hypersensitivity against leptospiral antigens can be elicited in dogs convalescing from acute leptospirosis and Anderson (1967) found that experimental leptospiral nephritis developed only in dogs capable of immune response to the organism. Thus immunologic injury to kidney in CIN may result from hypersensitivity to environmental antigens (i.e. leptospiroae) lodged in
renal tissues. (For further discussion of this hypothesis see Paterson, 1968).

Alternatively, the causative agent may precipitate release of kidney-specific antigens which can induce an autoimmune response against kidney by the lymphoreticular system. Anderson (1968) noted that the glomerular lesions of CIN are compatible with immunologic injury by anti-kidney antibody. In this connection, Edgington and associates (1967) isolated a renal tubular epithelial antigen from rat kidney that is capable of inducing experimental allergic glomerulonephritis in immunized rats. Dixon (1968) states that glomerular basement membrane antigens present in the urine of normal animals are nephritogenic. He suggests that renal damage from infectious agents may expose these antigens to immunocompetent cells and result in self-perpetuating immunologic injury to the kidney. If immunologic injury is a component of CIN then it may be possible to demonstrate nephrotoxic activity in serum or lymphoid cells of affected dogs by using in vitro correlates of immunologic injury such as cytotoxicity testing. Hypothetically, nephrotoxic activity could also be transferred to normal dogs by sensitized cells or cell fractions or immune serum, although if such activity were cell-mediated, histocompatible donor-recipient pairs should be used for testing.

The similarity of CIN to chronic pyelonephritis of man deserves mention. Both lesions are characterized by a chronic nonsuppurative interstitial inflammation, tubular atrophy and dilatation with intratubular casts and fibrosis. Human pyelonephritis often progresses in
the absence of detectable infectious agents (Angell et al., 1968) so chronic pyelonephritis and CIN may develop by similar mechanisms.

4. Endocrine Diseases

a. Thyroiditis

Musser and Graham (1968) found that more than 10% of nearly 1000 Beagle dogs in one colony had evidence of spontaneous thyroiditis. Most affected dogs descended from 1 female with thyroiditis and introduction of a new stud increased the incidence more than twofold. Beierwaltes and Nishiyama (1968) studied 67 Beagles from this colony and noticed histologic similarities between canine thyroiditis and human thyroiditis (Hashimoto's struma). Lesions in the canine thyroids ranged from focal lymphocytic infiltration without acinar involvement to acinar atrophy, macrophage infiltration and Hurthle cell changes of the follicular epithelium. Dogs with severe lesions had indications of subnormal thyroid function (low PBI and 24 hour $^{131}$I uptake values). Antithyroglobulin antibodies were demonstrable in the sera of affected dogs, but they did not correlate with the severity of thyroid lesions. In addition, control dogs, purchased independently, also had serum titers to canine thyroglobulin. The pathogenesis of this disease, other than its apparent familial component, is obscure. Attempts to produce the disease in normal dogs by passive transfer of serum or lymphoid cells from thyroiditic dogs have not been reported.
Experimental allergic thyroiditis has been produced experimentally by injecting dogs with homologous thyroid extracts emulsified in Freund's adjuvant (Terplan, et al., 1960). Antithyroid antibodies appeared in the blood of affected dogs but, as in spontaneous thyroiditis, their levels in serum did not correlate well with the severity of the lesions. Attempts to transfer this "autoimmune" thyroiditis to normal dogs with serum were unsuccessful.

b. Parathyroiditis

Lupulescu and collaborators (1968) induced parathyroiditis and hypoparathyroidism including a decrease in serum calcium concentrations by inoculating dogs with homologous parathyroid tissue emulsified in Freund's adjuvant. Affected animals had delayed skin reactions to intracutaneous challenge with parathyroid tissue and contained antiparathyroid antibodies in their serum.

5. Nervous Diseases

a. Postdistemper Demyelinating Leukoencephalopathy

Canine distemper, a spontaneous viral disease of dogs, often terminates in a demyelinating leukoencephalopathy weeks to months after clinical recovery from generalized infection (Innes and Saunders, 1962). The brain and cord lesions bear morphologic resemblance to several idiopathic and virus-associated leukoencephalopathies of man and experimental allergic encephalomyelitis (Greenfield and Norman, 1963, Paterson, 1966). Theories proposed to account for post-infectious
demyelination include: 1) the virus persists within central nervous tissue following recovery from systemic illness and damages or destroys the myelogenic capacity of glial cells (Waksman and Adams, 1962; Appel, 1969); 2) virus-brain cell interactions cause exposure of nervous tissue antigens to immunocompetent cells which then mount an autoallergic attack on the central nervous system (Sudduth, 1955; Pette et al., 1965, 1968); and 3) the preceding mechanisms participate either simultaneously or sequentially (Webb and Smith, 1966; Paterson, 1969).

Most current evidence supports the first theory since canine distemper virus can be detected in a substantial portion of brains from affected animals (Moulton 1956; Appel, 1969) and can induce demyelination of myelinated explants of canine cerebellum (Storts et al., 1968). Nevertheless, recent data suggest a role for immune mechanisms in post-distemper leukoencephalopathy. Alvord and associates (1968) demonstrated precipitins against proteins of human and guinea pig brain in the sera of dogs suspected of having distemper. Furthermore, Long and Koestner (unpublished data) discovered that certain sera from dogs with histologically-confirmed disease can demyelinate myelinated canine cerebellar explants. The myelotoxic factor present in these sera as well as the antigen(s) against which it is directed have not yet been identified.

Canine distemper virus belongs to a group of viruses (myxo- and para-myxoviruses) which may release host antigens from concealment or alter cell antigenicity during replication (Isacson, 1967). If post-distemper demyelinating leukoencephalopathy proves to have a virus-
triggered autoallergic component, mechanisms by which similar viruses could incite immunologic injury would be open to direct examination.

Experimental allergic encephalomyelitis is probably the most thoroughly investigated of the experimental autosensitivity diseases and has been produced in many species (reviewed by Paterson, 1966). It can be evoked in dogs by injection of homologous central nervous tissue emulsified in Freund's adjuvant (Thomas et al., 1950; Hughes et al., 1966). Clinical signs of encephalitis usually appear in 7 to 10 days accompanied histologically by perivascular infiltrations of mononuclear cells with demyelination in the central nervous system (Lazar et al., 1966). For unknown reasons, cold weather, increased humidity, rapid changes in atmospheric pressure or electrical discharges appear to predispose sensitized dogs to clinical and pathologic exacerbations of the disease (Maros et al., 1967). This finding remains unconfirmed by other workers. Demyelinating antibodies have been found in experimental allergic encephalomyelitis of rodents, but their role in the pathogenesis of the disease is unclear since only sensitized lymphoid cells from affected animals appear capable of transferring encephalitis to normal individuals (Paterson, 1966).

b. Coonhound Paralysis

Coonhound paralysis is a spontaneous polyradiculoneuritis which may occur in dogs 7 to 14 days after sustaining a racoon bite (Cummings and Haas, 1967). Clinically and histologically, the disease resembles the Landry-Guillain-Barre syndrome of man and experimental
allergic neuritis (the peripheral nerve counterpart of experimental allergic encephalomyelitis) (Waksman and Adams, 1955). Speculation that coonhound paralysis begins as a viral infection has not yet been substantiated. On the other hand, immunologic studies of the disease have not been reported, despite its similarities to experimental allergic neuritis.

6. Gastrointestinal Autosensitivity

a. Experimental Gastritis

Gastritis, gastric atrophy and achlorhydria have been produced in dogs following injection of autologous, homologous or heterologous gastric juice and stomach extracts, usually emulsified in Freund's adjuvant (Hennes et al., 1962; Langr et al., 1967). Autoallergic gastritis has been associated with antiparietal cell antibodies capable of reacting with autologous gastric juice as well as with delayed skin reactions to gastric antigens. Krohn (1968a, 1968b) found that absorption of vitamin B₁₂ from the gut of affected dogs was decreased and that canine anti-canine gastric juice antibody partially inhibited the action of human intrinsic factor on vitamin B₁₂ absorption. Pernicious anemia has, however, not been reported in connection with canine experimental autoallergic gastritis.

b. Experimental Colitis

Most attempts to induce immunologic injury in the colon of the dog as a model for ulcerative colitis in man have been unrewarding.
Acute colitis has been produced by infusions of heterologous anticanine colon antiserum, but chronic disease did not develop and serum injections occasionally provoked anaphylactoid reactions (Bicks and Walker, 1962).

7. Miscellaneous Conditions

Polyarthritis, resembling rheumatoid arthritis of man, occurs occasionally in dogs, usually in association with canine SLE (Lewis et al., 1965a; Lewis and Hathaway, 1967). Polyarteritis nodosa has also been reported in dogs (Lewis et al., 1965b), but its pathogenesis has not been investigated.

VI. Transplantation

1. General Considerations

The greater the antigenic similarity (histocompatibility) between two individuals, the less likely they will be to reject each other's tissues as foreign. Transplantation (histocompatibility) antigens exist on the surface of all cells in varying concentrations. They are expressions of closely linked histocompatibility genes which appear to behave as Mendelian dominants. The antigenic strength manifested by each gene varies so it is common to speak of "strong" and "weak" histocompatibility barriers between individuals. Transplanting across a strong barrier is generally more difficult than transplanting across a single or even multiple weak barriers (reviewed by Kahan and Reisfeld, 1969).
Should a transplanted tissue or organ be recognized as foreign by the host, rejection ensues. Rejection has been considered a cell-mediated event and, as such, a prototype of delayed hypersensitivity (Wilson and Billingham, 1967). Humoral antibodies are associated with the rejection process, but their significance is incompletely understood (reviewed by McDonald, 1966). Once rejection of a graft has taken place (first-set phenomenon), an individual is sensitized to antigens present in the graft and will reject subsequent grafts containing these antigens in an accelerated manner (second-set phenomenon). Furthermore, the sensitized state may be transferred from one animal to another by means of lymphoid cells. For further discussion of transplantation biology the reader is referred to the review of Russell and Monaco (1965).

The dog has occupied a central position in the development of organ transplantation, particularly at the level of clinical research. Accordingly, the literature is replete with articles on canine transplantation, so we have had to be selective in organizing this review. Current transplantation nomenclature given in Table III serves as an aid to the following discussion.

2. Transplantable Organs

Virtually every organ in the dog's body, except the central nervous system, has been transplanted at one time or another. Selected examples are listed in Table IV. Canine kidney transplantation has been most thoroughly studied, so we selected it to illustrate some aspects of transplantation immunology in dogs.
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<thead>
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<th>Old Term and Adjective</th>
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<td>Isograft</td>
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<tr>
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### TABLE IV
Examples of Transplantable Canine Organs

<table>
<thead>
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<th>Organ</th>
<th>Investigators</th>
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<th>Investigators</th>
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<td>Intestine</td>
<td>Lillehei et al. (1967)</td>
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<tr>
<td>Blood</td>
<td>Swisher and Young (1961)</td>
<td>Kidney</td>
<td>Mitchell et al. (1967)</td>
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<td>Arrocha et al. (1968)</td>
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a. Clinical Aspects

Primary renal allografts seldom survive more than 3 weeks in immunologically-unmodified recipients and are usually rejected within 9 days (Zukoski, 1968). Rejection is associated with oliguria terminating in anuria and uremia. A second kidney transplanted to a sensitized recipient is normally rejected in 1 to 2 days and may never function successfully at all. Thus the detection of imminent rejection at the earliest possible moment is of critical importance if it is to be reversed. Indications of rejection include proteinuria, elevated blood urea nitrogen, lymphocyturia, and evidence of tissue destruction upon biopsy (Koo et al., 1965; Vieth et al., 1967). Recently, elevated urinary or serum levels of renal lactic dehydrogenase, alkaline phosphatase and lysozyme activity have been found to correlate well with impending rejection (Koo et al., 1965; Najarian et al., 1965; Van Breda Vriesman et al., 1967).

b. Pathology of Rejection

During acute rejection of a primary renal allograft mononuclear cell infiltration of the renal cortex accompanied or preceded by endothelial damage to small intertubular vessels may be seen within 72 hours after transplantation (Kountz et al., 1963; Shorter and Hallenbeck, 1968). Damage to renal vessels progresses (Horowitz et al., 1965) and the blood supply to the kidney is compromised during the 3rd through 6th days. Thrombosis of peritubular capillaries or larger vessels
results in tubular destruction, infarction, hemorrhage and complete rejection over the next several days.

Immunological modification of the recipient (i.e. by immunosuppressive therapy) usually prolongs the course of rejection and chronic rejection is characterized by chronic inflammation. Histologically, recurring episodes of vascular damage accompanied by mild inflammation progress to generalized renal fibrosis and atrophy (discussed in detail by Porter et al., 1964; Sheil et al., 1968).

c. Cell-mediated versus Humoral Rejection

The relative importance of cell-mediated versus humoral immunity in canine transplantation is unclear. Cell mediated sensitivity against graft antigens probably dominates first set rejection (Govaerts, 1960, whereas second-set rejection, which is basically a vascular thrombotic phenomenon, may be mediated by circulating antibodies (reviewed by Perez-Tomayo and Kretschmer, 1965). Cytotoxic antibodies have been found in the serum of dogs during or following the rejection of tissue and organ allografts (Altman and Simonsen, 1964; Milgrom, 1966; Hampers et al., 1967; Dempster, 1968; Almgard and Svehag, 1968; Yamada and Kay, 1968; Clark et al., 1968) and it has recently been demonstrated that sensitization to canine renal allografts may be transferred by immune serum (Altman, 1963; Dubernard et al., 1968). Some investigators believe that renal antigens form immune complexes with circulating anti-kidney antibodies to produce vascular damage during rejection (Horowitz, 1965; Lowenhaupt and Nathan, 1968). The role of serum
factors is further amplified in xenograft rejection. Porcine renal xenografts transplanted into dogs are rejected peracutely (10 to 20 minutes) apparently due to endothelial damage from performed anti-porcine cytotoxic antibody present in dog serum with the participation of complement (Gewurz et al., 1966; Perper and Najarian, 1966; Nelson, 1966). In the end, both humoral and cell-mediated effector mechanisms may be found necessary for full manifestation of primary allograft rejection as suggested by Gewurz and associates (1966).

3. Modification of Rejection

Immunological modification of graft recipients is essential for successful transplantation of organs between allogeneic animals such as dogs. Two basic procedures have become popular for this purpose, suppression of host effector mechanisms (lymphoreticular system) and establishment of immunologic tolerance by pretransplantation treatment of the host with donor antigens.

a. Immunosuppression

Immunosuppressive therapy induces a generalized hyporesponsive state in the lymphoreticular system, and thus represents the crudest and perhaps most dangerous of the modifying regimens. Nevertheless, judicious use of chemical and physical immunosuppressants can prolong graft survival without compromising the host.

Chemical immunosuppressants used in canine transplantation include: azathioprine (Murray et al., 1964; Starzl et al., 1967; Stuart et al.,
1967; Diethelm et al., 1968), 6-mercaptopurine (Zukoski and Ende, 1965) methotrexate (Thomas et al., 1963; Storts, et al., 1968) cyclophosphamide (Storb et al., 1969) azaserine (Haxhe et al., 1967; Diethelm et al., 1968), actinomycin (Stuart et al., 1967), corticosteroids (Kountz and Cohn, 1967; Diethelm et al., 1968), and phytohemagglutinin (Calne et al., 1965; Gertner et al., 1969). Azathioprine (Imuran), a purine analogue, is currently considered the chemical immunosuppressant of choice for dogs and may be used alone or in combination with other immunosuppressive agents (Murray et al., 1964; Diethelm et al., 1968). Azathioprine may be hepatotoxic at pharmacologic levels so dose ranges and schedules must be tailored for individual dogs to attain maximum immunosuppression without untoward side effects (Starzl et al., 1967). Dosage levels can usually be cut in half for maintenance once a graft is well-established (Murray et al., 1964). Additional discussion of the pharmacology and use of chemical immunosuppressants is provided by Gabrielsen and Good (1967).

Antilymphocyte serum (ALS) is a promising new immunosuppressive agent, which, unlike most chemical immunosuppressants, does not depend on generalized depletion of lymphoreticular cells to be effective. Anticanine ALS has been produced in horses (Iwasaki et al., 1967), sheep (Fox et al., 1968), goats (Simons, 1968) and rabbits (Shorter et al., 1968) by immunization with lymph node cells (Russell and Monaco, 1967) thymocytes (Braf et al., 1967; Shorter et al., 1968) or thoracic duct lymphocytes (Herman and Schloerb, 1967). Unmodified ALS, which contains antibodies to many antigens, may cause undesirable side
effects such as anemia, anaphylaxis or serum sickness, so it is normally absorbed with canine erythrocytes and may be fractionated to anti-lymphocyte globulin before use (Iwasaki et al., 1967; Russell and Monaco, 1967).

The immunosuppressive potency of ALS in vivo does not correlate well with either its cytotoxic potency and leukoagglutinability in vitro (Russell and Monaco, 1967) or the degree of lymphopenia it produces in vivo (Jeejeebhoy, 1967). Thus the effectiveness of ALS must be determined through clinical trials (Russell and Monaco, 1967). ALS is usually administered in combination with other chemical immunosuppressants, such as azathioprine, beginning several days before a transplantation, for maximum effect (Murray et al., 1964; Starzl, 1967).

Long term side effects, such as intercurrent bacterial infection, are rare during ALS therapy (Fateh-Moghadam et al., 1967). Conversely, the development of distemper and hepatitis in vaccinated dogs given ALS has been reported (Abaza et al., 1966). ALS is also known to potentiate viral infections in rodents (reviewed by Hirsch and Murphy, 1968). The current status of ALS has been reviewed by James (1969).

Physical methods for immunosuppression include irradiation, thoracic duct drainage and thymectomy.

Whole body irradiation, at levels required for effective prolongation of graft survival, is usually lethal for dogs unless supplemented by allogeneic bone marrow infusions (Thomas et al., 1963a). Local x-irradiation of grafts (Wolf et al., 1967) and intraarterial implantation of Yrittium 90 pellets have been used in immunosuppressive therapy.
with equivocal results (Wolf and Hume, 1965). Extracorporeal irradiation of blood produces lymphoid cell depletion and may be capable of reversing early, but not late stages of rejection (Donahoo et al., 1967; Maginn and Bullimore, 1968). Hume and Wolf (1967) have reviewed the immunosuppressive effects of irradiation.

Thoracic duct drainage of lymphocytes has been reported to enhance survival of canine skin and kidney allografts (Singh et al., 1965; Parker et al., 1966) and, when used in combination with chemical immunosuppressants such as azathioprine, it may reduce the time and dose of drugs required for therapy (Vega et al., 1968). Thoracic duct cannulas are, however, difficult to maintain for extended periods and are esthetically unappealing (Kozuszek, 1967).

Thymectomy, although without detectable effect on immune responsiveness of adults by itself, has been used successfully with immunosuppressive chemotherapy to maintain grafts longer than if drugs alone had been employed (Kisken, 1966; Furuse et al., 1967).

b. Tolerance

Tolerance may be defined as specific immunologic unresponsiveness to antigenic stimulation. This definition predicts that an animal rendered tolerant to donor transplantation antigens, need not comprise its immunologic competence to all environmental antigens in order to maintain allografted tissue. Thus induction of specific immunological tolerance to tissue allografts remains a central goal in transplantation immunology.
Attempts to induce tolerance in dogs have taken 2 general forms, exposure of neonatal or adult dogs to antigen, frequently in massive doses, and exposure of immunosuppressed dogs to antigen followed by withdrawal of immunosuppressive therapy.

**Tolerance by Pregrafting Exposure to Antigen.** Some species, notably rodents, are immunologically immature at birth and can be rendered tolerant to many antigens provided exposure to these antigens occurs during the first day or 2 of life (reviewed by Hasek et al., 1961). Dogs are considered immunologically mature to most antigens at birth so, generally speaking, efforts to induce tolerance in canine neonates by injection of tissue extracts have failed (Fowler, et al., 1961; Grosjean and Otte, 1966b). Puza and Gombos (1958) and Gombos and associates (1962) were able to induce tolerance to skin and kidney allografts by giving exchange transfusions of donor blood to 3 to 12 day old puppies, but this technic seems rather heroic for general use. Anderson (1965) described a state of "immunological inertia" existing between a bitch and her progeny during the week after parturition, whereby maternal skin grafts were accepted by pups for exceptionally long periods (occasionally more than 1 year). These results could also be explained by assuming that favorable histocompatibility relationships existed between the dam and her litter.

Predictably, preexposure of adult recipients to donor antigens in order to induce tolerance has also met with little success. Furthermore, prolonged graft survival in these instances may be due to immunological enhancement rather than tolerance (see section VI. 5)
Tolerance Through Immunosuppression. Murray and associates (1964) in reporting the results of 1000 canine renal allografts, observed that most dogs rejected kidneys after being withdrawn from immunosuppressive therapy, but some grafts were retained for more than a year. These results could not be wholly attributed to harmony between donor and recipient antigenic profiles since a second kidney transplanted from the original donor could be rejected while the first one continued to function. Furthermore, persisting renal allografts were permanently accepted if retransplanted to original donors, indicating that expression of new antigens was not responsible for tolerance of the graft by the allogeneic host. Calne (1968) suggested that the results reported by Murray's group could be explained either by antigenic deletion occurring in the renal allograft or by masking of transplantation antigens on the donor kidney by antirenal antibodies formed in the recipient. The latter phenomenon resembles enhancement as mentioned above. Nevertheless, tolerance to allogeneic tissues in adult dogs appears easier to produce if recipients are initially given immunosuppressive therapy. This principle was further supported by Epstein and associates (1967) who produced permanent tolerance to allogeneic bone marrow in 2 lethally-irradiated adult male dogs.

Antigenic Specificity in Tolerance. Antigenically complex canine tissue extracts compound the difficulties of tolerance induction in dogs since unresponsiveness may ensue to some antigens in the mixture
only to be negated by sensitization to others (Calne, 1968). Thus injections of isolated and standardized major transplantation antigens could facilitate tolerance while reducing adverse responses to minor antigens. Solubilized transplantation antigens have been used to prolong survival of renal allografts in dogs (Wilson et al., 1969) but the biological and chemical characterization of these preparations has not been completed (reviewed by Kahan and Reisfeld, 1969). This approach to tolerance in transplantation is additionally promising, since minute quantities of solubilized protein fractions can be used to produce immunologic tolerance in adult animals (reviewed by Dresser and Mitchison, 1968). Some strains of bacteria also appear to contain antigens which can sensitize recipients against donor tissues, but whether they can substitute for canine transplantation antigens in the induction of tolerance remains to be investigated (reviewed by Zabriskie, 1967).

4. Histocompatibility

a. General Considerations

If immunologic modification of a graft recipient is to be minimized, then donor and recipient must be reasonably histocompatible. In other words, methods must be developed to identify and match the major transplantation antigens of donor-recipient pairs. Erythrocyte typing before transfusion is an example of this approach to donor-recipient pairing. It looked for a time as if similar typing for solid tissue grafting would be a hopeless task in genetically hetero-
geneous species but the discovery that leukocyte antigens can be used for histocompatibility testing has reduced the problem to manageable size (reviewed by Bach, 1968). Thus, what appears to be the major histocompatibility locus of man, has recently been identified (reviewed by Dausset and Rapaport, 1968).

Similar advances in histocompatibility testing of dogs have been painfully slow despite the widespread use of dogs in transplantation studies. Nevertheless numerous accounts exist of prolonged allograft survival in immunologically unaltered dogs. For example, Hurley and Kosek (1968) reported the 5 month survival of a cardiac allograft transplanted between littermates. Koo and associates (1966) studied a dog which had maintained a renal allograft for 123 days without immunosuppressive therapy when it died from unrelated causes. We have transplanted skin allografts between littermates in a closed inbred Beagle colony and recorded graft survivals of up to 125 days (Jacoby and Dennis, unpublished data). These results can be explained by assuming that donor-recipient pairs were more histocompatible than would be expected on the basis of random selection.

b. Canine Blood Groups

Swisher, Young and associates have pioneered the study of canine blood groups and their comprehensive reviews of the subject are only briefly summarized here (Swisher and Young, 1961; Swisher et al., 1962). The seven major canine blood groups, designated alphabetically A through G appear to be inherited as autosomal dominants. The A group
consists of two subgroups, A\textsubscript{1} and A\textsubscript{2}, and is expressed in about 63\% of a random dog population. Most clinically significant transfusion reactions follow infusion of A-positive blood into isoimmunized A-negative donors. Reactions may include tremors, emesis, fever, urinary and fecal incontinence, hemoglobinemia, hemoglobinuria, thrombocytopenia, asthmatic breathing, convulsions, hives, and transient prostration. Nevertheless, animals usually recover within 24 hours without the renal injury often associated with transfusion reactions in man. Infusion of blood from isoimmunized A-negative donors into A-positive recipients may cause similar reactions.

Swisher and associates indicate (1962) that only a small risk is involved upon primary transfusion of incompatible canine blood since less than 10\% of the dog population possess naturally-occurring isoantibodies to major blood group antigens. Cross-matching for A antigen, however, reduces the risk involved with either single or multiple transfusions. Beyond this, use of unsensitized A-negative donors is encouraged.

Incompatibility reactions may also occur in A-positive neonatal pups suckling isoimmunized A-negative dams who were mated with A-positive sires (Young \textit{et al.}, 1951). The syndrome is precipitated by absorption of colostral isoantibodies against A-factor and is characterized clinically by hemolytic anemia and a positive direct antiglobulin test. Transplacental passage of isoantibodies does not appear to be significant in this condition.
c. Canine Leukocytes in Histocompatibility Testing

It was hoped originally that canine blood group antigens could be used in histocompatibility testing for transplantation of other tissues. Enthusiasm for this prospect was dampened by reports indicating that little correlation existed between blood group matching and graft survival (Kasakura et al., 1964; Thomas et al., 1964). Rubinstein and associates (1968) have described a new class of erythrocyte antigens which appear to act as histocompatibility markers, but their findings are presently unconfirmed by other workers. Still, erythrocyte cross-matching of donor-recipient pairs is practiced by some investigators in order to reduce side reactions accompanying transplantation (Kasakura et al., 1964; Serre and Clot, 1968). On the other hand, present evidence indicates that canine leukocyte antigens will be useful histocompatibility markers.

Histocompatibility testing, as a general technic, can be divided generally into 2 categories, matching procedures and typing procedures. Matching tests give a rough estimation of the extent of antigenic similarity between two individuals, whereas leukocyte typing implies precise identification of specific antigens (reviewed by Russell et al., 1966; Bach, 1968). Among the matching tests applied to dogs are: 1) the normal lymphocyte transfer test, 2) the irradiated hamster test, and 3) the mixed lymphocyte culture test.

In the normal lymphocyte transfer test, recipient lymphocytes are injected into the dermis of donor skin. If the transformed cells
recognize donor tissue as foreign a delayed skin reaction occurs whose intensity and size may correlate with the duration of graft survival (Gray and Russell, 1965). Hornick and Sensenig (1968) found an inverse relation between reaction intensity and skin allograft survival among adult mixed breed dogs. Streilein and Barker (1967) and Hinchey and Bliss (1965) also observed delayed skin reactions in dogs, but did not correlate their findings with graft survival.

The **irradiated hamster test** is based on the fact that leukocytes of antigenically dissimilar individuals provoke a delayed inflammatory reaction if they are mixed and injected into the skin of an irradiated (immunologically unreactive) hamster (Ramseier and Streilein, 1965). This procedure has been used sparingly in dogs and with conflicting results. Cabasson and associates (1967) reported good correlation between results of the irradiated hamster test and survival of canine cardiac allografts in several animals, whereas Streilein and Barker (1967) found that mixed canine leukocytes produced only weak reactions in hamster skin and test results were not useful in selecting animals for renal transplants.

The **mixed leukocyte culture test** depends on the finding that leukocytes (lymphocytes) from antigenically dissimilar individuals have a reciprocal mitogenic effect upon one another when mixed in vitro (Bain and Lowenstein, 1964). After several days of incubation the intensity of reaction may be gauged histologically by counting blast forms in the culture or quantitated by determining the uptake by cells of radiolabeled amino acids from the culture medium. This test appears
to correlate well with the results of other matching procedures in dogs (Serre and Clot, 1968) but it has not been evaluated in clinical transplantation trials.

At least 3 major drawbacks are associated with leukocyte matching procedures. First, they are only semiquantitative, since multiple incompatibilities of weak loci may elicit the same results as a single incompatibility across a strong histocompatibility barrier. Secondly, they may not indicate the polarity of incompatibility reactions since donor leukocytes may provoke reactions if they recognize recipient leukocyte antigens as foreign. Thirdly, leukocytes from azotemic patients, such as those patients presented for kidney transplantation, do not respond well to antigenic stimulation (Mannick et al., 1960). Main and coworkers (1967) simplified interpretation of the mixed canine leukocyte test by converting it from a "two-way" to a "one-way" reaction. They first inactivate donor cells by x-irradiation or freeze-thawing, so that only recipient leukocytes in the mixture can undergo blastogenesis.

**Leukocyte typing** with specific antisera appears to hold great promise as a histocompatibility test (reviewed by van Rood and Eernisse, 1969) and several attempts have been made to design typing tests for dogs. Briefly, donor and recipient cells are exposed to immune sera. If agglutination or cytotoxicity is detected for both or neither groups of cells, they are assumed to be antigenically similar. If one group is affected and the other is not, they are assumed to be antigenically dissimilar. Epstein and associates (1968) prepared cytotoxic anti-
leukocyte antibodies of limited specificity by immunizing dogs with leukocytes from their littermates. Bone marrow donor-recipient pairs were then purposefully matched or "mismatched" according to the results of leukocyte "typing" tests using 4 antisera and then the recipients were x-irradiated. Bone marrow allografts survived significantly longer in "histocompatible" than "histoincompatible" recipients (Epstein et al., 1968; Storb et al., 1968). Mollen and associates (1968) prepared 12 cytotoxic antisera largely by reciprocal exchange of skin grafts and peripheral blood leukocytes between littermates. These antisera detected leukocyte antigens which seemed to be inherited as Mendelian autosomal dominants in a colony of closely inbred Beagles. Subsequent skin grafting experiments indicated that animals detected as being antigenically dissimilar from donors rejected grafts nearly twice as fast as animals who were antigenically similar to donors. Furthermore, typing of 3 donor-recipient pairs who had maintained either lung, kidney or bone marrow allografts for long periods, revealed their detectable leukocyte antigens to be identical.

The number of major canine tissue antigens that would require matching is unknown, but Thomas and associates (1963b) suggest, on the basis of survival rates of dogs after bone marrow allografting, that it may be small. Further encouragement is derived from knowing that only a single major histocompatibility barrier, albeit with multiple alleles, has been identified in each of the other species studied thus far (reviewed by Kahan and Reisfeld, 1969). Moreover, parents have restricted gene pools, so natural segregation of genes could result in
25% of the siblings in a litter being histocompatible with each other (reviewed by Amos, 1968).

VII. Tumor Immunity

1. General Considerations

Cells undergoing neoplastic transformation acquire tumor-specific neoantigens which may provoke an immune response by the host. When these antigens occur on cell surfaces they can act as weak transplantation antigens called tumor-specific transplantation antigens (TSTA). Thus responses to neoplastic cells may closely resemble responses to normal tissue allografts. In fact, Burnet (1968) hypothesizes that the evolutionary significance of cell-mediated immunity may be primarily related to surveillance and elimination of aberrant cells since allograft rejection responses are artificially elicited by human interference.

In virus-induced tumors, all cells transformed by a given virus have a common TSTA regardless of the morphology of the tumor or its host of origin, but the TSTAs of tumors induced by different viruses are immunologically distinct. Conversely, tumors induced by a chemical or physical carcinogen are usually antigenically heterogeneous so that on a given animal 2 tumors induced by a single carcinogen may be antigenically unrelated. Other tumor-specific antigens associated with virus-induced tumors include T-antigens (complement-fixing nuclear antigens) and virion-specific antigens. Although they may elicit immune
responses by the host, they probably have little influence on acceptance or rejection of neoplastic cells (reviewed by Klein, 1968 and Smith, 1968).

2. Transplantation of Canine Neoplasms

Tumor allografting in dogs presents formidable problems. First, dog populations are genetically heterogeneous so canine tumors must be transplanted across normal histocompatibility barriers as well as across tumor-specific antigenic barriers. Secondly, dogs are able to reject tissue allografts from the time of birth. As a result, most attempts to establish transplantable tumors in dogs without the aid of devastating immunosuppressive regimens have failed (Allam et al., 1956; Nielsen and Cole, 1961; Spencer and Leader, 1962). Successful transmission has been restricted to tumors of proven or suspected viral causation such as the oral papilloma (DeMonbreun and Goodpasture, 1932; LeBouvier et al., 1966), venereal sarcoma (Stubbs and Furth, 1934; Karlson and Mann, 1952), lymphosarcoma (Moldavanu et al., 1966; Kakuk et al., 1968) and mastocytoma (Lombard and Moloney, 1959; Lombard et al., 1963; Rickard and Post, 1968). Presumably tumor transplantation between histocompatible dogs with the help of selective immunosuppressive agents such as ALS (Phillips and Gazet, 1967) would prove more feasible than current "shotgun" methods to establish experimental tumor lines.

3. Tumor-specific Antigens

Tumor-specific antigens are most easily identified by trans-
planting tumor cells between syngeneic animals since only neoantigens will be recognized as foreign by the host. In dogs, tumor-specific neoantigens must be separated from normal antigenic constituents present on tumor cells, a task of considerable dimensions. Nevertheless, demonstration of antigenic identity among groups of canine tumors would implicate viral participation in the neoplastic process. Recently, several investigators have tried to identify neoantigens in canine tumors. McKenna and Prier (1966) detected a soluble antigen associated with 10 of 51 spontaneous canine neoplasms of different types. They also demonstrated antibodies against canine venereal sarcoma antigens by conglutinating complement-fixation tests in nearly all dogs carrying this tumor. Powers (1968) claims to have demonstrated specific antibody against venereal sarcoma in dogs by immunoprecipitation and passive cutaneous anaphylaxis. He did not, however, absorb sera with normal tissues so his results, while interesting, are not conclusive. Yurko and collaborators (1969) found that dogs may produce tumor-specific antibodies against authochthonous tumors. The antibodies did not react with either normal autologous tissues or embryonic tissues. Southam (1967) and Klein (1968) have reviewed the status of tumor-specific transplantation antigens in man, and their approaches to the problem apply as well to dogs.

4. Host Responses

Several types of naturally-occurring canine neoplasms including oral papillomas, venereal sarcomas and histiocytomas normally regress
spontaneously and affected animals are then immunologically resistant to further induction of tumor growth (Stubbs and Furth, 1934; Karlson and Mann, 1962; Chambers and Evans, 1959; Mulligan, 1963). Little correlation appears to exist between regression and the presence of circulating cytotoxic antitumor antibodies, at least in the case of solid tumors (Karlson and Mann, 1952; Chambers et al., 1960; Prier and Brodey, 1963). Powers (1968), however, was able to effect a decrease in maximal size and accelerate rejection of venereal sarcomas by passively immunizing dogs with serum from immune donors. Furthermore, if immune serum and tumor cells were transferred into a susceptible animal simultaneously, tumors failed to grow.

The role of cell mediated immunity in spontaneous regression of canine tumors has received only cursory attention (Chambers et al., 1960) despite the fact that tumors are frequently circumscribed or infiltrated by mononuclear cells. In vitro correlates of cell-mediated immunity could be used to clarify this issue. Thus it may be possible to demonstrate cytotoxic reactions of autologous lymphoid cells against monolayers of autochthonous tumor cells (Rosenau, 1968). Alternatively, autochthonous tumor extracts may specifically inhibit migration of autologous macrophages on glass (Kronman et al., 1969). These methods could also prove helpful in identifying tumor-specific antigens.

The question arises as to why all tumors are not rejected, if they contain antigens foreign to the host. There are many factors bearing on this aspect of the host-tumor relationship such as antigen concent-
tration on tumor cell surfaces, rate of cell proliferation versus rate of host response, vertical transmission (tolerance) of oncogenic viruses from mother to progeny enhancement (reviewed by Smith, 1968). Howard (1967) found that dogs with recurrent mastocytomas had defective immune responses to Brucella abortus strain 19 vaccine. Other workers have shown that leukemogenic viruses and chemical carcinogens are capable of immunosuppressive activity in mice (Ceglowski and Friedman, 1967; Stjernward, 1967). It has even been suggested that an oncogen can enhance its own pathogenicity by suppressing a host's lymphoreticular system while initiating neoplastic transformation (Schwartz and Andre-Schwartz, 1968). The implications of these findings for canine neoplasia remain to be studied. Conversely, Wilson and coworkers (1968) warn that neoplastic cells, inadvertently transferred to immunosuppressed patients during allografting procedures, may continue to proliferate.

Genetic predisposition to neoplasia may occur in dogs. The Boxer breed, for example, has a significantly higher incidence of tumors, particularly of the lymphoreticular system, than the random dog population and they are more liable to develop multiple, morphologically distinct tumors (Howard and Nielsen, 1965; Priester, 1967). It would be interesting to determine if the multiple tumors occurring in a Boxer dog shared common tumor-specific antigens, since this might suggest the existence of a multipotential oncogenic virus in this breed.
5. Immunization Against Neoplasia

Immunization against virus-induced canine tumors appears feasible since the antigenicity of tumor and virus would remain relatively constant from animal to animal. On the other hand, non-virus-induced tumors represent a problem, because each may be antigenically distinct and therefore unresponsive to a mass-produced vaccine (Smith, 1968). Minton and associates (1967) tried to circumvent this obstacle by immunizing dogs with autogenous tumor cells coupled to rabbit γ-globulin and emulsified in Freund's adjuvant. They succeeded in preventing recurrence of a partially excised hemangiopericytoma and thyroid adenocarcinoma in 2 dogs 10 and 8 months after "vaccination" respectively. There is risk associated with immunization particularly against solid tumors, since tumor growth may be accelerated rather than abrogated. This enhancement phenomenon probably results from masking of tumor cells by antibody which is produced during injections of tumor material. The masked cells go undetected by the host's immune mechanisms and continue to proliferate (reviewed by Kaliss, 1965).

VIII. Conclusions

Immunological injury deserves increased attention from veterinarians as an effector of canine disease. Hypersensitivity, autosensitivity, tissue allograft rejection and tumor regression in dogs are now well recognized phenomena and offer useful models for study in both
canine medicine and comparative medicine.

Although many types of immunological injury are mediated by humoral antibody an equal, if not greater variety of such lesions are probably evoked by sensitized immunocompetent cells. Hence, many experimentally-induced hypersensitivity states can be transferred from affected to normal animals only with cells and not with serum. Furthermore, passive transfer of sensitivity with cells frequently constitutes the critical demonstration of the existence of hypersensitivity.

Unfortunately, dogs, being genetically and therefore antigenically heterogeneous, are not amenable to cell transfer experiments. This point is dramatically demonstrated by the failure of canine tissue allografts to survive for prolonged periods without substantial immunosuppressive chemotherapy. Consequently, a single problem hampers research of hypersensitivity states and allotransplantation in dogs: lack of knowledge about canine histocompatibility antigens. It is now time for a cooperative effort by veterinarians to characterize the histocompatibility antigens of dogs and develop reliable tissue typing procedures for donor-recipient pairing in clinical and investigative canine medicine. To this end, workshops could be organized by veterinary immunologists, as has been done by physicians, to facilitate comparison and standardization of typing tests. Moreover, selective in-breeding of closed dog colonies should increase the chances of attaining monovalent typing sera in a relatively short time.
CHAPTER II

THE EFFECT OF ADRENALECTOMY ON THE LYMPHOID LESIONS OF EXPERIMENTAL CANINE DISTEMPER

1. Introduction

Lymphopenia and cellular depletion of lymphoid tissues often accompany myxovirus infections of man and animals (Jubb and Kennedy, 1963; Gibson et al., 1965; Gresser and Lang, 1966). Studies with gnotobiotic dogs in our laboratory established that experimental infection with a canine myxovirus (canine distemper virus) resulted in generalized lymphoid depletion including severe atrophy of the thymus (Gibson et al., 1965). The pathogenesis of the lymphoid lesions is commonly attributed to a direct effect of the myxovirus on host leukocytes, since canine distemper virus (CDV) can be found in lymphoid cells (Lui and Coffin, 1957; Cornwell et al., 1965).

Endogenous adrenal corticosteroids released during stress, however, can cause similar changes in lymphoid tissue in the absence of infection (Selye, 1946). Because virus infection itself may constitute a stress (Beisel and Rapport, 1969), the lymphoid lesions developing during myxovirus infection may be mediated in part by the adrenal glands. If this hypothesis is true, lymphoid cell depletion should be less severe in animals whose adrenals have been removed before the animals are exposed to virus. To test this possibility, adrenalectomized and sham-operated dogs were inoculated with CDV and the lesions were compared during the acute phase of disease.
2. Materials and Methods

**Dogs.** Twelve puppies from two mixed-breed litters were delivered into germ-free isolators and maintained as gnotobiotes (Griesemer and Gibson, 1963) until they were 9 weeks old. They were subsequently transferred to specific-pathogen-free (SPF) isolation units for the remainder of the experiment.

**Adrenalectomy.** When they were 10 to 12 weeks old the 12 dogs were randomly divided into 2 groups. The 6 members of one group were adrenalectomized according to the procedure described by Markowitz and associates (1964). The remaining 6 dogs underwent sham-operations including manipulation of both adrenal glands.

Adrenalectomized dogs were injected intramuscularly with 5.0 mg of cortisone acetate (Cortone-acetate, Merck, Sharp & Dohme) and 0.5 mg desoxycorticosterone acetate (Percorten acetate, CIBA) preoperatively. Postoperatively their drinking water was replaced by 0.85% saline solution and they were given daily intramuscular injections of 0.3 to 0.5 mg desoxycorticosterone acetate. The efficacy of electrolyte feeding and steroid replacement therapy were determined by measuring serum concentrations of glucose by the o-toluidine method (Hyvärinen and Nikki, 1962) and potassium by flame photometry at regular intervals. The former were considered normal at 70 to 100 mg/100 ml and the latter at 3.6 to 4.6 mEq/liter (Kronfeld and Medway, 1969). Sham-operated dogs continued on a water ration. The dogs were allowed 10 days for recovery after surgery.
**Virus.** Lyophilized samples of canine distemper virus, Snyder Hill strain, were donated by Dr. Max Appel, Veterinary Virus Research Institute, Cornell University. The virus was reconstituted in Hanks' balanced salt solution and passaged sequentially in 2 SPF Beagles to obtain a virus pool for inoculation. Splenic tissue collected from the second dog passage on the 8th post-inoculation day was ground to make a 20% suspension in Hanks' solution and stored at -90 C. Splenic tissue from a normal SPF Beagle was collected in an identical manner for use as control inoculum.

The virus content of the stock virus was assayed in canine pulmonary macrophages as described by Appel and Jones (1967). Each ampule of virus for animal inoculation was tested for bacterial contamination on blood agar and in thioglycollate broth at 25 C. and 37 C. in both aerobic and anaerobic atmospheres. Cultures were uniformly negative.

**Inoculations.** Four adrenalectomized and 4 sham-operated dogs were inoculated intraperitoneally with 1.0 ml of 20% splenic tissue suspension containing $1 \times 10^6$ TCID$_{50}$ of CDV. Two adrenalectomized and 2 sham-operated dogs were given identical volumes of control suspension prepared from CDV-free spleen.

**Clinical evaluation.** The following parameters were monitored daily in each dog beginning 2 days before inoculation of virus: body weight, rectal temperature (at 12 hour intervals), total and differential leukocyte count, erythrocyte count, hematocrit and hemoglobin. Every fourth day, eosinophils were counted in a hemocytometer chamber and
leukocyte size distributions were determined using a model B Coulter-Counter (Coulter Electronics, Hialeah, Florida).

Serum 17-OH corticosteroid (17-OHCS) concentrations were determined by Dr. Phillip W. Murdick, College of Veterinary Medicine, The Ohio State University, using the radio-stereo assay described by Murphy (1967). The presence in serum of desoxycorticosterone given as replacement therapy to adrenalectomized dogs did not interfere with the determination of endogenous 17-OHCS levels.

**Virus recovery:** Samples of thymus and spleen were collected from each dog at necropsy and ground to make a 20% suspension in Hanks' balanced salt solution. The suspensions were freeze-thawed 3 times and clarified by centrifugation at 2000 rpm for 10 minutes. Serial ten-fold dilutions were inoculated into 24-hour cultures of canine pulmonary macrophages to detect the presence of CDV by the method of Appel and Jones (1967). Control cultures consisted of macrophages exposed to: 1) supernatant prepared from splenic tissue of an SPF Beagle 2) supernatants of splenic tissue from infected dogs which were incubated at 37 C. for 60 minutes with either hyperimmune canine anti-CDV serum or serum from a normal dog and 3) hyperimmune canine anti-CDV serum or serum from a normal dog.

**Antibody determination:** Serum neutralizing antibody against CDV was determined in embryonated eggs by Dr. Appel (Gillespie et al., 1958).

**Necropsy:** Dogs were killed by electrocution and necropsy performed. Samples of the following tissues were fixed in 10% neutral buffered formalin: thymus, spleen, peripheral lymph nodes (mandibular, retro-
pharyngeal, mediastinal, bronchial, prescapular, axillary, mesenteric, sublumbar and popliteal) tonsil, ileum, cecum, stomach, duodenum, colon, pancreas, liver, kidney, adrenal gland (region normally containing adrenal gland in adrenalectomized dogs), gall bladder, urinary bladder, heart, lung, gonads, thyroid, parathyroid, brain, spinal cord, skin and eyes. The pituitary gland was fixed in glutaraldehyde and processed for electron microscopy. The tissues were embedded in paraffin, sectioned at 6 μ and stained with hematoxylin-eosin, Giemsa or Schorr's triple S stain.

3. Results

Clinical signs: All 8 dogs given CDV developed signs of distemper. Typically, these included: 1) a biphasic fever with elevations 1 to 4°F above baseline values beginning on the second and sixth post-inoculation days and lasting 24 to 48 hours; 2) anorexia and malaise coinciding with periods of fever; 3) progressive weight loss; 4) progressive dehydration evidenced by dry, inelastic skin and sunken eyes and 5) diarrhea, mucopurulent nasal and ocular discharge, coughing and dyspnea coinciding with the second temperature elevation.

The only striking difference in clinical response between the 2 groups exposed to CDV was the appearance of severe hypoglycemia in adrenalectomized dogs on the 8th to 9th postinoculation-day. Blood glucose levels fell below 50 mg/100 ml in all 4 dogs and were accompanied by retching, emesis, malaise, lateral recumbency, convulsions, opisthotonus, extensor rigidity and coma. Oral and parenteral administration of dextrose caused only transient remission of clinical signs,
so the dogs were killed when death appeared imminent.

Neither adrenalectomized nor sham-operated control dogs (unexposed to CDV) developed signs of disease.

**Clinical pathology:** Adrenalectomized dogs had no detectable serum 17-OHCS either before or after exposure to CDV within the acceptable margin of error (± 0.2 mg/100 ml) for the assay. Sham-operated dogs given virus had 17-OHCS levels 2 to 6 times above preinoculation values (Figure 1).

Transient leukopenia developed in adrenalectomized and sham-operated dogs given virus. It usually began on the second post-inoculation day, reached about equal intensity in both groups and lasted approximately 96 hours. All control dogs without virus unexpectedly developed leukocytosis (Figure 2).

Absolute lymphopenia accompanied leukopenia in infected dogs and persisted until at least the 8th post-inoculation day. In contrast, adrenalectomized but not sham-operated controls developed absolute lymphocytosis (Figure 3).

Mild transient neutropenia with a slight left shift occurred in all animals given virus, whereas all controls developed persistent absolute neutrocytosis (Figure 4). Eosinophil counts were higher in adrenalectomized dogs but were lower in both groups exposed to CDV compared to their respective non-inoculated controls (Figure 5). Monocyte values varied from day to day but were markedly elevated in several infected dogs late in the course of the disease. Size distribution plots of peripheral blood leukocytes failed to reveal significant
**Fig. 1.** Serum concentrations of 17-OH corticosteroids in 8 dogs inoculated intraperitoneally with $1 \times 10^6$ TCID$_{50}$ of CDV compared to 4 control dogs given diluent. Each pair of bars represents one animal. Solid bar indicates concentration just before inoculation, while open bar indicates post-inoculation concentration on day shown atop bar.
Figure 1
Fig. 2. Means of total leukocyte counts of 4 adrenalectomized and 4 sham-operated dogs inoculated intraperitoneally with $1 \times 10^6$ TCID$_{50}$ of CDV (solid lines) compared to 2 adrenalectomized and 2 sham-operated control dogs given diluent (broken lines). Stipled area represents baseline values ± S.D.
Figure 2

![Graph showing cell counts over days for sham-operated and adrenalectomized groups, with standard deviations indicated.]

SHAM-OPERATED

ADRENALECTOMIZED

Days

10^3 Cells PER mm^3
Fig. 3. Means of total lymphocyte counts in adrenalectomized and sham-operated dogs during experimental distemper infection. See legend of figure 2 for explanation of depiction.
Figure 3
Fig. 4. Means of total neutrophil counts in adrenalectomized and sham-operated dogs during experimental distemper infection. See legend of figure 2 for explanation of depiction.
Figure 4
Fig. 5. Means of total eosinophil counts in adrenalectomized and sham-operated dogs during experimental distemper infection. See legend of figure 2 for explanation of depiction.
Figure 5

SHAM-OPERATED

ADRENALECTOMIZED

$^{+} \text{S.D.}$

$-3-$

$-2-$

$-1-$

$-0-$

$10^3 \text{CELLS \ PER mm}^3$

DAYS

DAYS
changes between infected and non-infected dogs.

**Virus recovery:** CDV was recovered from the thymus of 6 dogs and the spleen of 5 dogs (Table 1). No virus was detected in tissues of control dogs. Canine anti-CDV serum, but not normal canine serum, inhibited virus activity in macrophage cultures.

**Antibody titers:** Preinoculation sera from 10 of 11 dogs tested had no titer to CDV. Antibody against CDV was detected in post-inoculation sera of 6 infected dogs tested but in none of 3 controls (Table 1). Anti-CDV activity in the serum of dog M-49 before inoculation with virus can not be explained. This dog developed signs and lesions of dis-temper after exposure to CDV.

**Gross lesions:** All thymuses of both adrenalectomized and sham-operated dogs infected with CDV were atrophic (Figure 6). In addition, thymuses from the sham-operated infected dogs were jelly-like in consistency due to interlobular edema. Aside from occasional hemorrhages, the remaining lymphoid organs in infected dogs appeared normal. Seven of 8 dogs given CDV had mucopurulent rhinitis and tracheobronchitis but pneumonic foci were found in the lungs of only 4 dogs. One adrenalectomized dog had small amounts of free blood in the lumens of both small and large intestine. No lesions were found in non-inoculated control dogs.

**Microscopic lesions:** All lymphoid tissues from infected dogs contained the following lesions: 1) lymphocytokaryorrhexis and lymphoid depletion; 2) reticuloendothelial cell hyperplasia; 3) presence of multinucleated giant cells; and 4) pale eosinophilic intracytoplasmic and intranuclear
TABLE V

Virus Reisolation and Serum Antibody Determinations in Dogs Inoculated Intraperitoneally with $1 \times 10^6$ TCD$_{50}$ of CDV Compared to Control Dogs Given Diluent.

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Treatment</th>
<th>P.I.D.$^a$</th>
<th>Virus Recovery</th>
<th>Serum Antibody (Log$_{10}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Spleen</td>
<td>Thymus</td>
</tr>
<tr>
<td>M-49</td>
<td>Sham</td>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-42</td>
<td>+</td>
<td>9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M-47</td>
<td>Virus</td>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-53</td>
<td></td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M-45</td>
<td>Adrenalectomized</td>
<td>8</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-41</td>
<td></td>
<td>9</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M-48</td>
<td>+</td>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-50</td>
<td>Virus</td>
<td>9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M-38</td>
<td>Sham +</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M-51</td>
<td>Diluent</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M-43</td>
<td>Adrenalectomized</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M-52</td>
<td>+ Diluent</td>
<td>12</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Postinoculation day

$^b$ Not determined
Fig. 6. Weight of thymus compared to total body weight in dogs with experimental distemper. Each bar represents one animal. Number atop bar indicates post-inoculation day.
Figure 6

THYMUS PER CENT BODY WEIGHT

SHAM & VIRUS

9

11

9

8

9

9

ADR-X &

12

12

SHAM & DILUENT

12

12

12

Figure 6
CDV inclusions.

Atrophy of thymic lobules occurred in all dogs given virus but depletion of cortical thymocytes in sham-operated dogs was so extensive that discrimination between cortex and medulla was impossible. In contrast, thymocyte depletion in adrenalectomized dogs was much less severe, so that cortico-medullary differentiation remained clear (Figures 7,8,9). Other thymic lesions in both groups of dogs were identical to those previously described by Gibson and coworkers (3) in dogs with distemper.

The tonsils and Peyer's patches of all infected dogs lacked discernible germinal centers. Foci of small lymphocytes, ostensibly follicular remnants, were seen in tissues of adrenalectomized dogs whereas only reticuloendothelial cells were present in corresponding tissues of sham-operated dogs.

Small lymphocytes were depleted from the splenic white pulp of sham-operated dogs, whereas the white pulp of adrenalectomized dogs resembled that of non-infected control dogs. Germinal centers were absent from the spleens of all infected dogs.

Lymph nodes from either adrenalectomized or sham-operated infected dogs were generally found to be equally depleted of lymphocytes, regardless of their anatomical location. The single exception was a more pronounced depletion of the mesenteric complex in infected dogs. Although cortical and paracortical regions of all nodes were decimated of lymphocytes, cortical lymphocytic foci were seen in nodes of adrenalectomized infected dogs. They were rarely found in nodes of sham-operated infected dogs. Germinal centers were absent from the nodes of
Fig. 7. Thymus from a control dog. Notice medullary region of lobules surrounded by broad collar of cortical thymocytes. Interlobular septae are of normal width. Magnification 50X; stained with hematoxylin and eosin.
Fig. 8. Atrophic thymus from a sham-operated dog 8 days after inoculation with $1 \times 10^5$ TCID$_{50}$ of CDV. Notice complete absence of cortical thymocyte collar, whereas reticuloepithelial cell framework of lobule remains. Interlobular septae are markedly widened and edematous. Magnification 50X; stained with hematoxylin and eosin.
Fig. 9. Thymus from an adrenalectomized dog 8 days after inoculation with $1 \times 10^6$ TCID$_{50}$ of CDV. Broad interlobular septae indicate atrophy, but cortical thymocyte collar is largely intact. Multiple ring-shaped foci in cortex are areas of cell necrosis and giant cell formation. Magnification 50X; stained with hematoxylin and eosin.
all infected dogs and moderate numbers of plasma cells were found lining medullary sinuses.

Non-infected adrenalectomized and sham-operated control dogs were free of lesions, although the tonsils and lymph nodes of the former appeared hyperplastic compared to the latter.

4. Discussion

The experiment indicates that adrenalectomy prevents full expression of cell depletion in lymphoid tissues of dogs experimentally infected with canine distemper virus, but has negligible effect on the intensity and character of the associated leukopenia. Thus the lymphoid lesions associated with distemper are mediated, in part, by the adrenal glands, most likely through adrenocorticosteroid secretion triggered by the stress of viral infection.

The data indicate that CDV infection constituted a stress since serum 17-OHCS levels in sham-operated dogs exposed to CDV increased 2 to 6 times over pre-infection levels. One dog given CDV failed to show a post-inoculation elevation of serum 17-OHCS, but it had recovered from clinical disease when the blood sample for assay was obtained. In contrast, both infected and non-infected adrenalectomized dogs had no significant serum concentration of 17-OHCS. Neither gross nor histologic evidence of adrenal tissue was found in these pups. Electron microscopy of anterior pituitaries from adrenalectomized dogs revealed hypertrophy and hyperplasia of adrenocorticotrophs suggesting increased production of ACTH in response to decreased circulating levels of corticosteroids. (C. C. Capen, personal communication)
The changes observed in lymphoid tissues were manifested in 2 ways: leukopenia and lymphoid depletion in situ. Leukopenic responses were similar in sham-operated and adrenalectomized dogs given CDV. We did not attempt to recover virus from blood, but other workers have demonstrated the presence of CDV in circulating leukocytes during acute infection (Lui and Coffin, 1957; Cornwell et al., 1965). Although stress-induced release of 17-OHCS causes lymphopenia (Selye, 1946) CDV may have destroyed enough circulating cells to mask the lymphocyto-karyorrhetic effect of corticosteroids in sham-operated dogs. Alternatively, at least in rodents, circulating lymphocytes are long-lived cells derived from paracortical regions of the lymph node (Everett and Tyler, 1967). Since paracortical areas were markedly depleted of lymphocytes in both sham-operated and adrenalectomized infected dogs, a virus attack on long-lived lymphocytes in situ could have contributed to the lymphopenic response.

Lymphocytosis, commonly associated with adrenal ablation, occurred in the adrenalectomized controls, but a neutrophilic response ensued in all control pups. This may have been caused by reaction to cellular debris or excitement provoked by daily handling and venepuncture.

Lymphoid cell depletion was more extensive in sham-operated than adrenalectomized dogs with distemper. This contrast was most striking in the thymus. The small thymic lymphocyte (thymocyte) is thought to be a "short-lived" cell which is more susceptible to lysis in the presence of 17-OHCS than the "long-lived" lymphocyte (Miller and Cole, 1967, Esteban, 1968). This may explain the severe depletion of thymocytes in thymuses of sham-operated pups. Since thymuses from adrenalectomized pups were also atrophic and since thymuses from both groups of infected
dogs contained CDV, virus-thymocyte interactions probably contributed to the development of the lesion.

Depletion of small lymphocytes from splenic white pulp of sham-operated dogs could also be due, in part, to the effects of corticosteroids, since evidence exists that they are "short-lived" cells (Esteban, 1968).

The absence of germinal centers in lymphoid tissues during distemper has been reported previously (Lauder et al., 1954). Adrenalectomy did not prevent this change, so it is likely due directly to viral activity. Lui and Coffin's (1957) immunofluorescent studies showed that CDV antigen was concentrated in lymphoid follicles of ferrets 3 to 4 days following inoculation. Many viruses, including myxoviruses, require lymphoid blast cells for multiplication, as found in germinal centers (Dunne et al., 1958, Miller and Enders, 1968). Conversely, lymphoid blast cells are apparently less susceptible to lysis by corticosteroids than small lymphocytes (Dougherty and White, 1945). This may explain why foci of small lymphocytes persisted in follicular areas only in the lymph nodes of adrenalectomized dogs given CDV.

Antibody production against CDV seemed unimpeached despite lymphoid depletion. Lymphoid follicular destruction occurs in other virus diseases during the act of immunologic response to the replicating agent (Mims, 1964). Mims (1964) views this process as a "race" between virus growth and antibody production, the outcome determining the recovery or demise of the host. Antibody levels were similar in adrenalectomized and sham-operated pups but insufficient numbers of animals were tested to draw conclusions about this point. Detrimental
secondary bacterial invaders are, however, commonly associated with canine distemper. Since a related myxovirus (measles virus) can suppress immunoreactivity to Mycobacterium antigens (Star and Berkovich, 1964), CDV could induce a similar transient immunodepressive effect during its replication in lymphoid tissue, thereby enhancing susceptibility of the host to secondary invaders.

The regular onset of hypoglycemia in adrenalectomized but not sham-operated dogs with canine distemper suggests that intact dogs are protected from hypoglycemia during periods of anorexia by gluconeogenic metabolic pathways under adrenal cortical influence.
5. Summary

The lymphoid lesions produced in dogs following inoculation with virulent canine distemper virus were characterized in adrenalectomized and sham-operated animals. Adrenalectomy had little influence on the intensity, character or duration of the ensuing leukopenia but did diminish the extent of small lymphocyte depletion in all lymphoid tissues. Serum concentrations of adrenal 17-hydroxycorticosteroids were substantially elevated in sham-operated animals receiving virus. It is suggested that lymphoid tissue alterations in canine distemper result from an additive effect between direct viral insult to lymphoid tissue and lymphocytokaryorrhetic activity by adrenal corticosteroids released in response to the stress of virus infection.


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**Chapter II**


