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AVIAN ONCOGENIC VIRUSES IN CHICKENS AND TURKEYS:

PATHOGENESIS AND IMMUNOSUPPRESSION

By

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ABSTRACT

AVIAN ONCOGENIC VIRUSES IN CHICKENS AND TURKEYS:
PATHOGENESIS AND IMMUNOSUPPRESSION

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Abdel Rahman Khogali Elmubarak

The virologic, immunologic and pathologic effects of the Georgia (GA) isolate of Marek's disease virus (MDV), an FC126 isolate of turkey herpesvirus (HVT) and a Rouse-associated virus-1 (RAV-1) strain of a lymphoid leukosis virus (LLV) were studied in chickens and turkeys.

A total of 436 chickens and 404 turkeys were used in four comparative trials. Each of the two species was divided into inoculated and uninoculated groups. Animals were inoculated with viruses at 1 day of age.

Chickens and turkeys had tumors and died due to MDV infection. Tumors in turkeys occurred most frequently in the liver, followed by spleen and nerves. In chickens, tumors occurred most frequently in nerves followed by liver and gonad. Both species had significant weight loss. Virus concentrations in blood were higher in chickens than in turkeys. Virus titers rose gradually and peaked at week 5 in turkeys, while in chickens the peak was seen in the fourth week.

Marek's disease virus depressed the relative weights of bursa and thymus and increased the relative spleen weight in chickens and turkeys. The effect was more pronounced in chickens than in turkeys.

The cellular immune response was depressed in chickens during tumor formation. The impairment of this response in infected turkeys was detected at week 4. The humoral response was suppressed in both but was preceded by a transient enhancement in turkeys.

The HVT vaccination in chickens resulted in a high degree of protection against challenge with MDV, while similar vaccination was ineffective in preventing MD in turkeys. Most HVT-vaccinated and MDV-challenged chickens were not viremic for MDV, and the titers were very low in those that remained viremic at week 5. In the vaccinated turkeys, but not the unvaccinated, MDV viremia disappeared by week 5.

Neoplastic transformation of bursal cells was detected at week 14 after infection in chickens and turkeys infected with RAV-1. Lymphoid and lymphoreticular proliferation and infiltration were detected in many organs in RAV-1 infected chickens and turkeys early after infection and before bursal transformation occurred. These lesions were more frequent in the turkeys than in the chickens. Gross tumors in the bursa and visceral organs occurred in chickens but were not detected in turkeys. Rous-associated virus-1 was isolated from infected chickens at weeks 2 and 10 after infection and from turkeys at week 2 only. Bursal weights were significantly increased in infected chickens due to tumorous involvement. Spleens were significantly enlarged in chickens but were decreased in turkeys. The humoral immune responses were significantly decreased before bursal transformation in chickens. Humoral responses in turkeys were not affected. During late stages of infection, antibody response in chickens was significantly elevated. Mitogenic response of whole blood lymphocytes was not significantly affected in chickens but was depressed in turkeys.

Chickens and turkeys infected with HVT had mild microscopic lymphoproliferative lesions in many organs. Although these lesions were clearly detectable and had neoplastic components in chickens, they were minimal and lacked detectable neoplastic components histologically in turkeys.

Chickens and turkeys were viremic, but the turkeys had a higher viral titer at peak levels. A significant suppression of body weight during early stages of infection occurred in turkeys but not in chickens. Bursal and splenic weights were significantly increased in chickens and turkeys. Turkey herpesvirus was more lytic for bursal cells in turkeys than in chickens. Thymus to body weight ratios increased more in chickens than in turkeys. Humoral response was significantly reduced by HVT infection in chickens but not in turkeys. Mitogenic response of whole blood lymphocytes was depressed in turkeys but was not significantly affected in chickens.

DEDICATION

To my mother, my late father,

my wife, Afaf,

and my son and daughter,

Ayemen and Nuha

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LIST OF ABBREVIATIONS

ALC	avian leukosis complex
AMB	avian myeloblastosis virus (an avian leukosis virus)
B cells	bursa derived cells
BF	bursal follicle
BH	Bryan high titer strain (a Rous sarcoma virus)
C/E	chicken embryo fibroblast resistant to subgroup E virus
CEF	chick embryo fibroblasts
CMI	cell mediated immunity
Con A	Concanavalin A
COFAL	complement fixation for avian leukosis
CZAV	Carr-Zilber strain; an LLV of subgroup A
DEAE	diethylaminoethyl
DEF	duck embryo fibroblasts
DMSO	dimethylsulfoxide
GA	Georgia isolate
gs	group specific
FAV-1	Fujinami strain; an LLV of subgroup A
HVT	herpesvirus of turkeys
IP	intraperitoneal
IV	intravenous
LL	lymphoid leukosis
LLV	lymphoid leukosis virus
MATSA	Marek's disease tumor associated surface antigen
MAV	myeloblastosis associated virus
MSB-1	Marek's disease lymphoblastoid cell line
MD	Marek's disease
MDV	Marek's disease virus
PFU	plaque forming units
PHA	phytohemagglutinin
PM	phenotypic mixing
QEF	quail embryo fibroblast
RAV	Rous-associated virus
RIF	resistance inducing factor (an avian leukosis virus)
RPL 12	Regional Poultry Laboratory strain of leukosis virus (Olson tumor transplant)
RPRL	Regional Poultry Research Laboratory in East Lansing, Michigan
RSV	Rous sarcoma virus
R-Q	Rous virus quail cells

SE	sheep erythrocytes
SPAFAS	specific pathogen-free avian supply
T cells	thymus derived cells
TSSA	tumor specific cell surface antigen
WTHV-1	Wisconsin turkey herpesvirus

INTRODUCTION

Three groups of oncogenic viruses induce neoplasms in poultry. The first group contains Marek's disease virus (MDV), the cause of the most important neoplastic disease of chickens. This group also includes turkey herpesvirus (HVT), which is largely apathogenic for chickens, although chickens infected with HVT develop Marek's disease (MD) tumor associated surface antigen (MATSA) positive cells and immune responses directed against MD lymphoma cells. The second group is the leukosis/sarcoma group, which causes lymphoid leukosis and other related neoplasms. The third group causes tumors composed of reticuloendothelial (RE) cells. The work reported here deals mainly with MDV, HVT and lymphoid leukosis virus (LLV).

Marek's disease is a lymphoproliferative neoplastic disease of chickens which until recently caused devastating losses in commercial flocks throughout the world. It is a viral disease caused by a group B herpesvirus and is characterized by infiltration and proliferation of lymphoid cells in the peripheral nerves, visceral organs, skin and iris. The infection produces a suppressive effect on the humoral as well as the cellular immune functions of the host.

Although MDV has been studied extensively in chickens, the response of turkeys to this virus is not well understood. Studies directly comparing the early pathogenesis and tumor production by the virus in chickens and turkeys have not been reported.

Herpesvirus of turkeys (HVT) is a cell-associated nonpathogenic herpesvirus that occurs naturally in turkey flocks and has a close antigenic relationship with MDV. It is used worldwide as a vaccine to protect chickens against the development of MD tumors. In chickens, HVT was shown to cause mild microscopic lymphoproliferative lesions in the nerves and gonads. These cellular components appeared to have morphologic properties of neoplastic cells. Recently, MASTA was demonstrated in HVT-vaccinated chickens. Almost all turkeys reared under natural conditions become infected with HVT at an early age and harbor this virus probably for life. It is not known if HVT also initiates neoplastic transformation in the turkey and if immune surveillance plays a role in the host-virus relationship.

Lymphoid leukosis (LL) is a neoplastic disease of chickens originating in the follicles of the bursa of Fabricius and metastasizing to other organs such as the liver, spleen, kidneys, and gonads. It is caused by ribonucleic acid (RNA) viruses of the leukosis/sarcoma group.

Lymphoid leukosis viruses (LLV) have not been studied in turkeys. Information on lesion induction, immunosuppression and viremic responses would be of comparative interest. Particular emphasis was on whether LLV suppressed T-cell or B-cell immune responsiveness, on the pattern of early viremic responses, and early lesion induction in turkeys.

The interest in this research was stimulated by the increasing importance of avian tumor viruses in cancer research, the recent rapid increase in lymphoproliferative neoplastic diseases in turkeys with mortality exceeding 20% in some flocks, and the increase in condemnation in the processing plants due to "turkey leukosis."

The cause of these diseases has not been established, and it is not known if any of the oncogenic viruses in chickens are involved in epizootics in turkeys.

The principal objectives of this study were (a) to compare viremic responses and early lesions in chickens and turkeys caused by selected oncogenic viruses (HVT, MDV and LLV), (b) to compare immunosuppression of T and B cell systems in chickens and turkeys caused by these viruses, and (c) to determine if turkeys offer any unique advantage in studies on oncogenic virology, immunology or immunosuppression.

LITERATURE REVIEW

Introduction

Avian oncogenic viruses cause a variety of neoplastic diseases. Some have considerable economic importance to the poultry industry. "Others have served as highly suitable models for studying the various forms of neoplasia; indeed, medical research has found avian oncology an abundant resource" (Calnek, 1978). Many types of tumors are found, but the lymphoproliferative neoplasms are by far the most prevalent of this group of diseases, and these make up the majority of what was known as the avian leukosis complex. Olson and Bullis (1942) found that the leukotic diseases accounted for 66% of the neoplasms found in chickens. Two years earlier, Gross (1940) reported that 19.5% of deaths in chickens were caused by neoplasms and that, of the neoplasms, leukosis comprised 90%. In 1941 Hutt et al. reported an even higher percentage and stated that approximately 95% of these deaths were attributed to neoplasms caused by lymphomatosis of one kind or another.

The incidence of leukosis in young chickens (most of which is now considered to be Marek's disease) increased dramatically during the ten-year period beginning in 1961 and was only reversed because of the wide application of MD vaccine. In the late 1960s, annual losses in the United States and Britain, for instance, were about $200 and $40 million, respectively (Payne et al., 1976; Calnek, 1978).

The term leukosis, which was proposed by Ellerman and Bang in 1908, has largely replaced the term "leukemia", which was used for the first time by Virchow in 1845. The reason is that this common avian neoplastic disease manifests itself as an extravascular infiltration of malignant blood cell precursors in the visceral organs, which causes enlargement and death from functional interference (Campbell, 1969).

There was considerable confusion in classification and nomenclature of the known transmissible neoplasms, because many virus strains appear to have multipotent characteristics and certain of these viruses induce some lesions indistinguishable from those induced by another unrelated virus. Calnek (1978) stated that the problem is compounded because most flocks and many birds (including those for experimental purposes) are infected with more than one agent and it is virtually impossible to examine one virus strain without observing the effects of a second, unrelated tumor virus. Because of these factors, an abundant supply of synonyms evolved. This problem was resolved by a committee (Jungherr, 1941) of cooperators of the Regional Poultry Research Laboratory, East Lansing, Michigan. They proposed two categories based on pathology rather than etiology: avian leukosis complex (ALC) and other neoplasms. The ALC was divided into lymphomatosis (visceral, neural, ocular or osteopetrotic) and leukosis (erythroblastic or granuloblastic). The various forms were understood to occur in subleukemic or leukemic varieties. Other tumors included myelocytomas and sarcomas.

Later, as more information was accumulated, there was a need to separate avian leukosis complex into fowl paralysis and other forms of leukosis. The first Conference of the World Veterinary

Poultry Association (Biggs, 1964) recommended, largely due to discussions by Campbell (1961) and Biggs (1961), that leukosis in its three forms (lymphoid, myeloid and erythroid) should be a separate entity from Marek's disease. It was also agreed that the sarcomas, endotheliomas, and the viral sarcomas were related to leukosis.

Today, the terminology most commonly employed is based on that originally adopted by the World Veterinary Poultry Association, with some modifications, i.e., categorization of diseases or disease complexes by agent-type instead of by pathologic manifestation and subdivision within agent-type diseases as determined by the pathologic expression (Calnek, 1978). The leukosis-sarcoma group includes lymphoid leukosis, erythroblastosis, myeloblastosis, myelocytomatosis and several other etiologically related conditions, such as sarcoma, nephroblastoma, endothelioma and osteopetrosis. Tumors of these conditions are caused by ribonucleic acid (RNA)-containing viruses.

Reticuloendotheliosis (RE) is caused by RNA-containing viruses unrelated to those of the leukosis-sarcoma group. The RE viruses cause lymphoid neoplasms in turkeys and other conditions in ducks and chickens (Purchase and Witter, 1975). Marek's disease is caused by a deoxyribonucleic acid (DNA) virus (group B cell-associated) that affects the nerves ("classical form") or viscera and nerves ("acute form") (Payne et al., 1976).

Marek's Disease

Marek's disease (MD) is a lymphoproliferative neoplastic condition affecting the peripheral nerves and visceral organs. It was first described by Marek in 1907 (cited by Calnek and Witter, 1978) who, thinking it to be an inflammatory disease, called it "polyneuritis." More recent studies (Payne and Biggs, 1967) indicated that MD was characterized by a neoplastic-like proliferation of lymphoid cells in the nerves and other organs.

Marek's Disease Virus (MDV)

Although the viral origin of MD was suspected for a long time, it was not until 1967 that Churchill and Biggs (1967) in England and Solomon et al. (1968) and Nazerian et al. (1968) in the United States independently isolated a herpesvirus in cell cultures from diseased chickens. They presented good circumstantial evidence that the virus was the cause of the disease. The proof that a herpesvirus is the cause of MD was due to the discovery that feather follicles are the site of maturation of the virus (Calnek et al., 1970a; Nazerian and Witter, 1970; Purchase, 1970). The first attenuated vaccine against the disease was developed by Churchill et al. (1969a,b). Shortly thereafter Okazaki et al. (1970) discovered that a closely related herpesvirus of turkeys (HVT) was also effective in preventing MD. Marek's disease virus is serologically distinct from other avian herpesviruses such as laryngotracheitis virus (Okazaki et al., 1970; Purchase et al., 1971a, 1972).

Marek's disease virus belongs to the cytomegalovirus or group B cell-associated herpesviruses. Enveloped virions from the feather follicles are 200 to 400 nm in diameter. Most infected culture cells contain filled or empty nucleocapsids of this herpesvirus which measure 85 to 100 nm. Besides DNA, virions contain lipids and at least 8 proteins, including probably 2 glycoproteins. Very infrequently virions measuring 130 to 170 nm may be observed budding from the inner nuclear or cytoplasmic vesicles. All infectivity of blood cells and tumor cells, and over 99% of the infectivity of cells in tissue cultures, is destroyed by treatments which remove viable cells from the inoculum, i.e., freezing and thawing, sonification, lyophilization, high-speed centrifugation and filtration. Tissue culture supernatants are devoid of infectivity.

Infection with MDV may result in two distinct virus-cell interactions. The first causes a "productive" infection of the cell in which virus DNA, virus induced enzymes and antigens are produced and results in fully infectious virus as occurs in feather follicle epithelium. Infection of cells may result in production of virus antigens and noninfectious virions are produced as in other epithelial cells in the chicken and in cultured cells. In infectious and noninfectious virus production, infection results in cell death. The second type of cell-virus interaction is encountered in the lymphoid cell system. The virus is present in multiple copies and is not usually expressed as viral antigens or virions but causes extensive proliferation and tumor formation (Nazerian et al., 1976).

Several different strains of MDV have been isolated. Virulent isolates include JM (Sevoian et al., 1962), HPRS-16 (Biggs et al., 1965), GA (Eidson and Schmittle, 1968), and Cal-1 (Bankowski et al., 1969). The nonpathogenic or mildly virulent isolates include HPRS-B14 (Biggs and Payne, 1963), Conn-A (Chomiak et al., 1967), and HPRS-27 (Biggs and Milne, 1972).

The virus for *in vitro* studies is readily obtained from whole blood, buffy coat or tumor cells from infected chickens. The proximal tips of feather shafts or homogenized skin from feather tracts of infected chickens provide cell-free virus (Calnek et al., 1970a; Purchase, 1970). For initial isolation, chicken kidney cells, duck embryo fibroblasts and cells from many other avian species are suitable (Purchase et al., 1971a; Sharma, 1971). Infected cell cultures develop discrete focal lesions of rounded, refractile, degenerating cells.

Continuous passage of MDV in tissue culture results in loss of oncogenicity and ability to spread from chicken to chicken. The virus also loses its "A" antigen but protects chickens against the disease; however, prolonged passage (over 100 times) renders it incapable of protection (Churchill et al., 1969a; Purchase et al., 1971a; Okazaki and Purchase, unpublished data).

The role of avian leukosis viruses in MD has been investigated by many workers. Frankel et al. (1974) reported a higher incidence of tumors in chickens infected with MDV and Rous-associated virus-2 (RAV-2) than in those infected with MDV alone. Another study by Peters et al. (1973) using a DNA probe from RNA of RAV-2 reported a higher level of RAV-2 specific RNA in dually infected chickens than in chickens infected with MDV alone. However, these studies could not be confirmed by other investigators. Witter et al. (1975a) produced typical MD lesions in chickens inoculated with a known avian leukosis-free stock of MDV. Chickens used in this study were free of the expression of endogenous avian virus (RAV-0). Witter's findings were later confirmed by Calnek and Payne (1976), thus indicating that MDV alone is capable of disease production.

Pathology and Pathogenesis

Chickens are the most important natural host of MDV. The virus was isolated from normal turkeys (Witter et al., 1974) and from a quail with ocular lesions (Kenzy and Cho, 1969). Lesions suggestive of MD have been observed in a variety of avian species, including pigeons, ducks, geese, canaries, budgerigars, swans, Ceylon jungle fowl, red jungle fowl, Japanese quail and great horned owls. The disease has been artificially induced in turkeys and pheasants. Ducks and Japanese quail were infected but did not develop the disease. Sparrows and various mammalian species were refractory to infection (Calnek and Witter, 1978).

Although ovarian transmission of MDV has been described (Sevoian, 1968), considerable evidence has accumulated against it (Witter and Solomon, 1971). For example, chickens reared in isolation have no evidence of MDV infection (Drury et al., 1969; Grunder et al., 1975).

Natural environment may be contaminated by saliva, feces and dander from infected chickens. The virus in the chicken house environment in infected litter may remain infectious for over 16 weeks at room temperature (Purchase, 1974). The virus spreads readily over long distances and infection of new hosts is thought to occur primarily by inhaled aerosols of infectious dander. Nearly all flocks of chickens are infected by the time they reach sexual maturity (Seigmund et al., 1973).

Although most chickens become infected with MDV, the percentage of chickens with signs of MD is quite variable (Chubb and Churchill, 1968; Witter et al., 1970a). Fifty percent of chickens in an acute outbreak may die between 10 and 20 weeks with multiple visceral tumors, while in the classic disease less than 1% may be affected at any one time (Purchase and Biggs, 1967).

The incubation period of MD depends on the virus strain, dosage and route of infection, age, genetic strain, and sex of the host (Calnek, 1978). Chickens infected at 1 day of age start to excrete the virus at about the second week after inoculation (Kenzy and Biggs, 1967). Maximum shedding occurs between the third and fifth weeks (Witter, 1972b). Lymphoid tissue degeneration occurs within 6 to 8 days and mononuclear infiltration of other organs about 2 weeks after infection (Payne and Rennie, 1973). Using high dosage of the virus and susceptible chickens, microscopic lesions can be induced in less than 2 weeks and clinical signs, gross lesions and mortality may occur by 3 weeks (Payne and Biggs, 1967). The incubation period and virus excretion in contact infection are about equal to the time required for virus to be excreted from inoculated birds (Biggs and Payne, 1967). Under natural conditions, mortality usually starts between 8 and 16 weeks (Witter et al., 1970a). "The induction of tumors within 10 to 14 days after inoculation of cellular material is suggestive of a transplantation response" (Calnek, 1978).

In the field, the clinical signs are seen most commonly in 12- to 24-week-old birds and exceptionally as late as 18 months or as early as 3 weeks. The classical signs are lameness or paralysis of the legs, wings, neck, eyelids or other parts of the body and occasionally dyspnea, crop dilatation, muscular atrophy, diarrhea, loss of weight and maybe anemia. Blindness may result from involvement of the iris. Young birds affected by the acute form may show signs of anorexia and depression for 1 or 2 days with early and high mortality (Biggs and Payne, 1967; Darcel, 1973). A temporary paralysis syndrome for 1 to 2 days followed in only a few weeks by recovery was described by Kenzy et al. (1973).

Nerve lesions are the most conspicuous feature of MD. Affected nerves are enlarged, grayish white and have no striations. In 99% of MD cases, nerve lesions may be found in celiac, cranial, mesenteric, brachial and sciatic plexuses, the nerve of Remak or the greater splanchnic nerve. Of these, the celiac plexus is most often involved (Goodchild, 1969). Changes in the brain and spinal cord are minimal, the neural form of the disease being essentially a disease of the peripheral nerves (Wight, 1962). Visceral organs, particularly the gonads, liver, lungs, heart, muscle and skin, may have diffuse or focal grayish-white lymphoid tumors. The thymus and bursa of Fabricius are usually atrophic when affected. A diagnostic feature in MD is the diffuse involvement of the bursa as opposed to the nodular tumor characteristic of lymphoid leukosis (Eidson and Schmittle, 1968; Payne and Biggs, 1967; Purchase and Biggs, 1967).

Early lesions in peripheral nerves are typified by an inflammatory change with edema and infiltration of lymphocytes, plasma cells, macrophages, heterophils and monocytes. Sometimes there is demyelination and Schwann cell proliferation. These lesions were classified as type B and a milder version as type C (Payne and Biggs, 1967) or types I and II, respectively (Wight, 1962). Type III (Wight, 1962) or A type (Payne and Biggs, 1967) are considered neoplastic with a predominance of large lymphocytes mixed with pleomorphic lymphoid cells and other nerve changes as in the inflammatory type. Nerve lesions may also contain large cells with very basophilic, pyroninophilic and vacuolated cytoplasm and a nucleus with little or no detail (Payne and Biggs, 1967). These are referred to as "Marek's disease cells" and are thought to be degenerating blast-type cells, a conclusion consistent with electron microscopic observations in which MD cells were found with intranuclear herpesvirus particles (Ubertini and Calnek, 1970). In the central nervous system, the lesion when present is a nonpurulent encephalomyelitis with perivascular cuffing with lymphocytes and focal gliosis. In the eye, the most consistent change is a mononuclear infiltration of the iris. The eye muscle may be involved. Ocular lesions have been reproduced experimentally (Sevoian and Chamberlain, 1963; Smith et al., 1974).

Visceral lesions are more uniformly proliferative and similar to type A nerve lesions, consisting of lymphoid cells, MD cells and activated and primitive reticulum cells (Payne and Biggs, 1967). Plasma cells are rare (Purchase and Biggs, 1967). Electron microscopic observation showed one cell type, an immature undifferentiated pleomorphic lymphocyte, as opposed to neural lesions which had a mixture of cell types (Doak et al., 1973; Frazier, 1974). Skin lesions are mainly inflammatory but may also be lymphomatous and are usually around feather follicles, arising as perivascular lymphoid aggregates. There is a disturbance in maturation of the keratinizing epithelium lining the follicles, which undergoes cloudy swelling and hydropic degeneration (Nazerian and Witter, 1970; Lapen et al., 1970, 1971; Purchase, 1970).

An acute cytolytic infection occurs in lymphoid tissues, particularly in the bursa, thymus and spleen, which subsides in a 2-week period of time. Infection also provokes acute inflammation with granulocytic invasion and increased reticulum cells and macrophages. These are accompanied by regressive changes in the bursa and thymus with weight loss. In the spleen there is lymphoid hyperplasia and an increase in weight (Payne et al., 1976).

There may be an increase of lymphocytes in the blood with the majority being T cells (Evans and Patterson, 1971). The bone marrow lesions include multiple tumor nodules (Sevoian and Chamberlain, 1964) or aplasia (Jakowski et al., 1970). In one study, bone marrow was normal (Purchase and Biggs, 1967).

About 75% of the cells in the lymphomatous lesions are T cells and most of the others are B cells. Focal proliferation of vascular adventitial cells is also involved (Kardevan et al., 1973; Payne et al., 1974; Sevoian and Chamberlain, 1964). Many lymphoma cells are nonproductively infected. They contain viral DNA and no viral antigens or virions but are infective (Nazerian et al., 1973). A tumor-specific antigen has been detected on a minor proportion of lymphoma cells and on all cells of lines developed from MD lymphomas. The cell lines are lymphoblastoid, of T-cell origin, and these cell lines carry the MDV genome. The B cells and some of the T cells present in lymphomas are believed to be immunologically reactive (Payne et al., 1976; Powell et al., 1974; Witter et al., 1975b).

Immunity and Immunosuppression

Hens infected with MDV transfer maternal antibody to chicks via the yolk sac (Chubb and Churchill, 1969), although the virus itself is not egg transmitted (Solomon et al., 1970). Antibody-bearing chicks are more resistant to development of MD lesions and mortality is lower. The onset of mortality is delayed and the latent period to death is increased. Tumor formation is reduced and acute destructive lesions in lymphoid and hemopoietic tissues are suppressed (Calnek, 1972b; Payne and Rennie, 1973). Passive antibody prevents bursal atrophy and lengthens the latent period for antigen and cell-free virus production in the feather follicle epithelium (Burgoyne and Witter, 1973). However, humoral immune response is not required for resistance to MD tumor development, since bursectomized birds may survive infection (Sharma and Witter, 1975). This does not diminish the importance of early presence of antibody in suppressing destructive infection of immunologic organs required for subsequent resistance.

Virus-specific antibodies develop 1 to 3 weeks after infection of chickens with MDV and can be detected by immunodiffusion (Chubb and Churchill, 1968), immunofluorescence (Purchase, 1969), virus neutralization (Calnek, 1972a; Sharma and Stone, 1972), indirect hemagglutination (Eidson and Schmittle, 1969) and complement-fixation (Marquardt and Newman, 1972). Infected birds possess persistent but fluctuating levels of antibodies. Antibody concentration was higher in birds that survived clinical MD lymphoma than in those that died early during the course of the disease (Witter et al., 1971; Calnek, 1972a; Purchase and Burgoyne, 1970; Sharma and Stone, 1972; Payne and Rennie, 1973). Precipitating, virus neutralizing, hemagglutinating and complement-fixing antibodies have been monitored during the course of the disease. Concentration of virus neutralizing antibodies was found to correlate with survival in genetically resistant birds (Calnek, 1972a; Sharma and Stone, 1972). High antibody concentration may not have been directly related to survival, since survival may have resulted from sparing of the bursal-dependent system in resistant birds (Smith and Calnek, 1973; Higgins and Calnek, 1975a,b).

Cell-mediated immunity in MD was reported by Fauser et al. (1973) and Byerly and Dawe (1972). Payne (1972) and Payne and Rennie (1973) postulated that resistance may be related to the thymus-dependent system because there was an increased incidence of lymphomas in thymectomized birds. Later Sharma et al. (1975) found that "age resistance" was severely compromised due to thymectomy. On the other hand, deficiency in the humoral response did not seem to influence age resistance (Sharma and Witter, 1975). Recently Sharma and Coulson (1977) and Powell et al. (1977) detected specific cytotoxicity of lymphocytes from MDV-infected chickens against cells of the MSB-1 lymphoblastoid cell line. This reactivity is probably directed against the tumor-associated antigen. Ross (1977), using the plaque inhibition test, showed that antibody to viral antigens may act as a target for sensitized lymphocytes and thus may have a role in cellular-mediated immunity to MD.

Several lines of chickens with genetic resistance were defined (Cole, 1968; Stone, 1975). Two mechanisms were used in the selection for resistance: (1) progeny testing and selecting chickens whose progeny were most refractory to MD inoculation, and (2) selection relying on the close relationship between resistance and certain alleles, especially B^{21} of the B blood group locus (Hansen et al., 1967). Other factors may also be involved. For example, line 6 and line 7 chickens of the RPRL are both homozygous at the B locus for another allele (B^2), but they differ markedly in MD susceptibility (Crittenden et al., 1972). Unlike resistance to RNA tumor viruses of avian leukosis, genetic resistance to MDV is not expressed at the cellular level (Spencer, 1969; Sharma and Purchase, 1974). Genetic resistance was fully expressed in agammaglobulinemic chickens (Sharma, 1974).

Marek's disease lymphoma can be induced by neonatal infection of maternal antibody-free chicks of a resistant strain, but not in older birds, suggesting that genetic resistance may be related to age resistance (Calnek, 1973; Sevoian and Chamberlain, 1963; Biggs and Payne, 1967; Witter et al., 1973). Genetic resistance and age resistance are associated with lower virus titers in blood and higher concentration of virus neutralizing antibodies that occur in young birds of the same strain. Age resistance differed from genetic resistance because there is lesion development and regression in the former but not in the latter (Sharma and Witter, 1975; Sharma et al., 1973a, 1975).

The immunosuppressive effects of MDV infection are well documented and reviewed by Payne et al. (1976) and Sharma (1979). The degenerative and proliferative changes in the bursa of Fabricius, thymus and spleen caused by MDV infection may be associated with impairment of the immune responses. Humoral and cellular responses to antigenic challenge were found to be deficient in infected chickens. The most consistent observation in the humoral system has been a depression of antibody response. This was observed for both primary and secondary responses to bovine serum albumin (Purchase et al., 1968) and for the primary response to *Salmonella typhi* and *Brucella abortus* antigens (Payne, 1970). A reduced response has also been noted to sheep erythrocytes (SE), *Salmonella pullorum* and *Mycoplasma synoviae* antigens (Burg et al., 1971; Jakowski et al., 1973; Evans and Patterson, 1971; Kleven et al., 1972). The degree of immunosuppression may be associated with the severity of the disease. Jakowski et al. (1973) reported that reduced antibody to SE in infected chickens was associated with reduced anti-SE IgG but not to reduced IgM levels. Humoral response impairment was found before and during overt MD and the severity of impairment correlated with the depletion of bursa-dependent follicles from the spleen rather than from thymic or bursal damage (Evans et al., 1971). The immunological responsiveness was related to gammaglobulin levels. Infected birds that were able to mount an antibody response to *S. pullorum* antigen were hypergammaglobulinemic, as compared with uninfected controls, whereas unresponsive birds were hypogammaglobulinemic. However, the high concentration of IgG in responsive birds was not specific against the *S. pullorum* antigen, which in these birds stimulated an IgM response. Two studies indicated that in MD-affected chickens the antibody response to certain antigens was not depressed (Box et al., 1971; Kermani-Arab and Davis, 1976).

Cell-mediated immune response is also depressed during acute MDV infection. This has been demonstrated *in vivo* as well as *in vitro*. Marek's disease virus infection resulted in delayed skin graft rejection and a depression of delayed type hypersensitivity to tuberculin in sensitized chickens (Payne and Rennie, 1973; Purchase et al., 1968). The graft-versus-host reactivity of blood cells was found to be either normal or increased. Purchase et al. (1968) reported an enhanced response, while Payne (1970) showed the response to remain unaffected. Biggs et al. (1968) observed that MDV infection may increase susceptibility to certain protozoan parasites, thus substantiating the reported cell-mediated immunity depression in *in vivo* studies.

The activity of lymphocytes from infected chickens has been examined by *in vitro* mitogen stimulation. Many sequential studies were done using the mitogen phytohemagglutinin (PHA), which specifically stimulated T-cells to proliferate and undergo blastogenesis (Greaves et al., 1968). A depression in the mitogenic response is considered a sign of decreased immunoresponsiveness. Spleen cells from infected chickens bearing gross tumors showed a reduced response to PHA *in vitro* as compared with cells from controls and from nontumor-bearing infected chickens (Burg et al., 1971). This hyporeactivity to PHA reported by Burg et al. (1971) was confirmed in subsequent reports (Lee et al., 1978b; Lu and Lapen, 1974; Schat et al., 1978). Also, MD lymphoma cells were shown to be unresponsive to PHA (Alm et al., 1972) in spite of the presence of immunologically uncommitted bursa and thymus dependent lymphoid cells in the lymphoma (Payne and Roszkowski, 1972). Lu and Lapen (1974) and Theis et al. (1975) demonstrated a correlation between mitogenic depression and the presence of gross lymphoma. Marek's disease virus-infected chickens without detectable tumors at the time of the test had much less depression of mitogen response. Depressed responsiveness to PHA appears to be influenced by many factors, including severity of the disease, presence of gross tumors, time period after infection, prior vaccination with HVT, and genetic makeup of chickens. Splenomegaly and reduction in mitogen response were found to be associated so that the depression was probably due to the invasion of the spleen by nonreacting lymphoid cells. The response of spleen cells to concanavalin A (Con A) was found to be impaired less than to PHA and pokeweed mitogen (Lu and Lapen, 1974).

Kinetic studies showed that chickens infected with MDV or HVT had an initial transient drop in mitogenic response within 1 week after infection in resistant as well as in susceptible chickens. A few days later there was a detectable recovery in mitogenic response which was again depressed in chickens that developed severe clinical disease. There was no depression in chickens that survived infection, and in some there was even enhanced response (Benda et al., 1978; Lee et al., 1978b; Schat et al., 1978). Studies showed that spleen cells from infected birds were not only hyporesponsive to mitogen stimulation but also transferred this hyporesponsiveness to normal spleen cells (Lee et al., 1978c; Theis, 1977). These studies showed that in cell mixture experiments the PHA response of normal spleen cells could be inhibited by splenic cells from chickens with MD in the absence of direct contact between the two types of cell populations, because the inhibitory effect could be transferred by the supernatant of these spleen cells. Lee et al. (1978c) attributed the inhibitory effect of the splenic cells to the presence of suppressor macrophages, while Theis (1977) did not find any evidence for macrophage involvement.

Vaccination

Marek's disease can be prevented by vaccination. Immunity in MD could be directed against viral-associated or tumor-associated antigens (Kaaden and Dietzschold, 1975; Lesnik and Ross, 1975; Powell, 1975). Resistance to tumor formation may be induced by exposing chickens to HVT (Okazaki et al., 1970; Purchase et al., 1972), to attenuated MDV (Churchill et al., 1969b; Biggs et al., 1970, 1972), and to nonpathogenic MDV (Rispens et al., 1972a,b). In all types of resistance, chickens become infected with MDV but have less viral antigen than do susceptible birds. The lesions that develop are either very mild or are overcome by a resistant host (Sharma and Stone, 1972; Sharma, 1973; Sharma et al., 1973a).

The successful use of material free of DNA and RNA (Kaaden and Dietzschold, 1975) eliminates interference phenomena and vaccinal virus blocking of host cell-virus interaction. Humoral response does not seem to be of major importance in vaccinal protection, since chickens rendered agammaglobulinemic by surgical bursectomy and x-irradiation were protected by vaccination (Else, 1974). Chemical bursectomy with cyclophosphamide was found to negate vaccinal immunity (Purchase and Sharma, 1974), but cyclophosphamide causes a temporary depletion of thymocytes (Linna et al., 1972; Sharma and Lee, 1977), and thus thymic suppression may have produced a state of tolerance to the vaccine virus. These experiments point out the immunologic mechanism as a basis for vaccinal protection and cell-mediated response to be of principal importance in this regard.

Turkey Herpesvirus (HVT)

HVT in Turkeys

A syncytial type of cytopathic effect and the development of intranuclear inclusions were observed in kidney cell cultures prepared from apparently healthy turkeys. The virus isolated from these cultures was designated WTHV-1 (Wisconsin turkey herpesvirus) (Kawamura et al., 1969). In the same year Witter et al. investigated the prevalence of MDV in 3 turkey flocks in Indiana and Georgia.

They isolated a virus apparently identical to WTHV-1, showed that it was antigenically related to MDV, and gave it the acronym HVT (Witter et al., 1970b). Deshmukh et al. (Paul et al., 1972), in 1970, isolated HVT from *Mycoplasma*-free turkey flocks and from flocks with leukosis. All of these isolates appeared to be similar in their production of plaques in chick kidney and chick embryo fibroblast cultures and their lack of pathogenicity. The HVT plaques are distinguishable from those produced by the attenuated high passage and the virulent low passage strains of MDV. In addition, antibody to HVT could be distinguished from antibody to MDV by an immunofluorescence antibody test.

Turkey herpesvirus is ubiquitous and infects turkeys at a young age. The turkey is the only known natural host. Infected turkeys develop a persistent viremia and the virus can be readily isolated *in vitro* (Nazerian et al., 1976). Turkey herpesvirus is nononcogenic for avian (Witter and Solomon, 1971; Witter, 1972a) and mammalian species (Sharma et al., 1973b).

Airborne transmission of HVT was experimentally demonstrated between turkeys, from turkeys to chickens, and from chickens to turkeys. This horizontal transmission was also demonstrated in naturally infected commercial turkey flocks where HVT infection spreads very rapidly throughout the flock and persists at a high frequency for at least 20 weeks. The role of egg transmission of HVT in turkeys has been studied, and the evidence is against embryo transmission of HVT (Witter et al., 1970b; Witter and Solomon, 1971; Paul et al., 1972). It is regularly shed from the feather follicle epithelium of turkeys, in which the virus behaves epizootiologically in a manner similar to MDV in chickens. Commercial turkey poults are hatched free of infection, but soon acquire virus and the resulting antibody against it (Payne et al., 1976).

HVT in Chickens

Turkey herpesvirus has been studied extensively in chickens and is used worldwide as a vaccine to prevent lymphoproliferation caused by MDV. It does not usually replicate in the chicken feather follicle epithelium and so is not shed in the environment. When chickens without maternal antibody are given large doses of HVT, however, virus shedding may occur (Cho et al., 1971).

The development of resistance in vaccinated chickens is rapid and generally persists for life. When day-old chicks received an adequate dose of vaccine virus, they were protected against MDV challenge 3 weeks later (Okazaki et al., 1970). Experimentally only 3 PFU gave a 50% protection at 3 weeks (Purchase et al., 1972), but a dose as high as 9,000 PFU was not protective against challenge 1 week later (Okazaki et al., 1973). In the field, 500 PFU of HVT inoculated at hatching was effective in preventing MD. However, it was found necessary to vaccinate as soon after hatching as possible to insure protection under natural conditions (Purchase et al., 1971c,d; Okazaki et al., 1971; Eidson and Anderson, 1971). If exposed, vaccinated chickens also become infected with MDV. Dually infected chickens shed MDV but not HVT into the environment. Marek's disease virus shed by such vaccinated birds is unaltered in virulence and may produce disease in susceptible chickens (Purchase and Okazaki, 1971a,b; Nazerian et al., 1976).

HVT as a Vaccine

Three types of vaccines have been used against MDV (Purchase et al., 1971c). These are attenuated MDV (Biggs et al., 1970; Churchill et al., 1969a,b), naturally apathogenic strains of MDV (Rispens et al., 1972b; Zander et al., 1972), and the herpesvirus of turkeys (Okazaki et al., 1970; Purchase et al., 1971c, 1972). In 17 field trials including 27 replicated experiments, HVT offered greater than 50% protection against MD in all trials. The incidence of MD in unvaccinated birds reared in the same pen was greater than 2% (Purchase et al., 1971c, 1972). Protection exceeded 80% in 3/4 of these replicates. In 1970 the vaccine was licensed for use in Michigan, and in March 1971 it was licensed all over the United States. Turkey herpesvirus is in use worldwide. An important consideration not in favor of the other 2 vaccines is the possibility that altered viruses from chickens could become pathogenic after a period of time, especially in those strains that spread horizontally.

Turkey herpesvirus is the only MD vaccine that is readily obtainable in cell-free form from infected tissue culture after disruption of cells (Calnek et al., 1970b). The cell-free HVT may be lyophilized for storage. In the United States, the cell-associated vaccine which consists of HVT-infected tissue culture cells preserved in dimethylsulfoxide (DMSO) in liquid nitrogen is also used. Cell-free vaccine has some advantage over cell-associated vaccine, because it does not require similar exacting conditions of storage; on the other hand, maternally derived antibody in chicks may interfere with vaccination and the neutralization effect is greater against cell-free HVT than against cell-associated vaccine (Calnek and Smith, 1972; Patruscu et al., 1972). Maternal antibody associated with vaccination of the dam with HVT did not interfere with the effectiveness of chick vaccination unless the breeding stock was vaccinated repeatedly (Eidson et al., 1972). Recently, however, it was found that HVT inhibited induction of viremia and the antiviral antibody response was delayed and less uniform. The combined effects of HVT-maternal antibody and early exposure (1 week vs 2 weeks) to MDV resulted in tumor development in chickens given a low dose of HVT vaccine (Eidson et al., 1978). It was speculated that the practical importance of the influence of maternal immunity on vaccination comes from the fact that the frequency of MDV infection remains high because most flocks are exposed. Most chickens in exposed flocks are infected and are permanent carriers of the virus. This is aggravated by the high density of chickens not only within the houses but also within farms and entire geographic areas (Eidson et al., 1978).

Vaccination failure may be due to failure of establishment of infection by HVT because of neutralization by maternal antibody, failure of development of adequate vaccinal immunity because of early exposure to MDV, or to failure to inoculate an adequate dose of HVT because of faults in vaccine manufacture, storage, or inoculation methods (Payne et al., 1976). Vaccination efficiency is also influenced by host genetic factors (Spencer et al., 1974; Zanella et al., 1975; Cho, 1977).

Because of excessive losses from MD after the usual single vaccination at 1 day of age, many studies were done to evaluate revaccination to increase immunity (Spencer et al., 1974; Zanella et al., 1975; Cho, 1977; Ball and Lyman, 1977). In the field there was no significant difference between day-old vaccination and repeated vaccination. If exposure were delayed until after revaccination such as in experiments where chickens were kept in isolators (Spencer et al., 1974), then revaccination might be beneficial.

The widespread use of HVT vaccine against MD in the United States since 1970 has resulted in more than an 80% reduction in the number of chickens condemned from MD in poultry processing plants. A similar reduction in mortality during the growing period has been observed in vaccinated egg-laying chickens, and the vaccinated chickens lay approximately 4% more eggs than unvaccinated chickens. Indirectly, from this information one must conclude that subclinical infection with MD may result in a suboptimal performance of the chicken (Purchase, 1976b).

Mechanism of Vaccinal Immunity

The mechanism of vaccinal immunity is not fully understood. However, the observation that immunosuppression due to administration of large doses of cyclophosphamide to vaccinated chickens will compromise the protection offered by HVT suggests that a major role is played by the immune system (Purchase and Sharma, 1974). "Humoral neutralizing antibody, cellular antiviral immunity and interferon, although present and partly effective, do not fully account for the protection observed." Therefore, immunity is mediated mainly through immunologic reaction against the cells that form the tumor. The most likely mechanism appears to be cell-cell interactions involving "killer" or suppressor thymus-derived cells and virus-infected cells (Purchase, 1975).

Kaaden et al. (1974) and Kaaden and Dietzschold (1975) found that membrane fractions of HVT-infected cells, when used as a vaccine, protected chickens against MDV infection. These fractions lacked MDV when tested in tissue cultures.

Witter et al. (1976) observed that chickens vaccinated at hatching with high doses of HVT developed viremia. Titers peaked around the 12th day and gradually declined. They also noted that HVT infection induced mild microscopic lymphoproliferative lesions in the nerves and gonads. The lesions were most prominent around the 12th day and then regressed. These lesions were also induced by HVT in cyclophosphamide-treated chickens. This may suggest that the lesions were T-cell dependent. The presence in these lesions of large immature cells suggested that HVT may be able to cause neoplastic transformation. It was postulated that the infiltration of mature lymphocytes and rapid regression of these lesions might represent a cell-mediated immune response of the host to the specific tumor or viral antigens on the transformed cells. This study also showed that maternal antibody reduces these early lesions, a fact that could be taken as an explanation for some vaccine failures if these lesions were essential in vaccinal immunity.

Powell et al. (1974) and Witter et al. (1975b) postulated that the lymphoblastic cells in HVT lesions may contain MATSA, an antigen seen in MD tumor cells and on cell lines derived from tumors. They further postulated that an immune response to MATSA may develop in vaccinated chickens and that this response might protect them against subsequent lymphoma formation by MDV. The first evidence that a T-cell response against MATSA-bearing lymphoblastoid cells of MSB-1 develop in chickens inoculated with HVT as well as other MD vaccines was presented by Sharma et al. (1978). They inoculated chickens with HVT or with apathogenic or attenuated vaccine strains of MDV and tested spleen cells in a 4-hour ^{51}Cr-release assay for cytotoxic response against the MSB-1 cell line. They found that the cytotoxic cells generated by the vaccine viruses had characteristics similar to those noted for MSB-1 affected cells generated by MDV. Thus, they postulated that immunity associated with anti-tumor antigen may play a role in the mechanism of vaccine protection against lymphoma development.

The Leukosis/Sarcoma Group of Viruses

Introduction

"Lymphosarcomatosis" in chickens was first recorded by L. Roloff in Europe in 1868. In 1896, Caparini described fowl leukemia in Italy. The first clearly reported cases of lymphoid leukosis (LL) were described as "aleukemic lymphadenosis" by Butterfield and Mohler in 1905 in the District of Columbia and Michigan. Later Warthin used the term "lymphocytoma" and was the first to recognize the aleukemic and leukemic conditions as 2 forms of the same disease process and to consider both to be malignant neoplasms (Burmester and Purchase, 1979). It was not until later years that these neoplasms were known to be caused by viruses.

This group of viruses induces in chickens, and to a lesser extent in other avian species, a variety of transmissible benign and malignant neoplasms. The most widespread of these is LL, but erythroblastosis, myeloblastosis, endothelioma, nephroblastoma, hepatocarcinoma, fibrosarcoma, and osteopetrosis may also occur. In the past many names were used to describe LL and much confusion existed due to the fact that Marek's disease and LL could not be etiologically separated. Descriptive names such as lymphosarcomatosis, aleukemic lymphadenoma, visceral lymphomata, lymphatic leukosis or simply leukemia were used by pathologists and "big liver disease" by laymen to describe what is now LL (Purchase and Burmester, 1978; Burmester and Purchase, 1979).

The transmissibility of these neoplasms was first demonstrated by Hirschfield and Jacoby in 1907 and later confirmed by Buchard in 1912 and Magnusson in 1916 in Germany, and in the United States by Schmeisser in 1915, as reported by Burmester and Purchase (1979). They also reported the first successful transmission with a filterable agent in Copenhagen by Ellermann and Bang in 1908. In spite of these reports, many well known investigators over a period of 30 years considered LL nontransmissible because of the negative results of transmission experiments with various materials from affected chickens. These investigators held that the intravascular forms of leukosis, i.e., erythroblastosis and myeloblastosis, were transmissible but the extravascular form, i.e., LL, was a nontransmissible tumor (Burmester and Purchase, 1979).

Furth (1933) provided good experimental evidence that a transmissible filterable agent was the cause of LL, but conclusive proof that a virus causes LL was provided by Burmester (1947), Burmester and Cottral (1947), and Burmester and Denington (1947).

In 1911 Peyton Rous of the Rockefeller Institute showed that avian fibrosarcoma was transmissible with cell-free filtrate--a breakthrough in cancer research that led Rous to be awarded a Nobel Prize. This tumor and its virus were considered completely separate from leukosis. The close relationship between leukosis and sarcoma was proved by Harry Rubin's discovery in the 1960s of the resistance inducing factor (RIF) and Rous-associated virus (RAV), which were found to be LL viruses. He also established their role as "helpers" to Rous sarcoma virus (Burmester and Purchase, 1979).

Viruses

The avian leukosis/sarcoma group of viruses contain RNA, are ether sensitive, and have other characteristics of the myxoviruses. They have been included in the genus oncornavirus C, members of which are biophysically and biochemically indistinguishable from one another except for some minor differences associated with the type of cell from which the virus originated. They replicate through reverse transcription from viral RNA to a DNA intermediate and are thus placed in the family *Retraviridae*. Viruses of LL have in common certain properties of the virus genome, the most important of which is a group-specific (gs) complement-fixing antigen, and are classified into at least 5 subgroups and types according to their host range in genetically different chicken embryo fibroblast (CEF) cultures, their interference pattern with members of the same and different subgroups, and viral envelope antigens identified by neutralization. In chickens the subgroups are A, B, C, D and E. Viruses of ring-necked and golden pheasants belong to subgroups F and G, respectively, but recent results suggest that subgroup G may belong to a new class of RNA viruses. Viral host range or genetic cellular resistance to infection is controlled by at least 4 major genetic loci in the chicken (Vogt and Ishizaki, 1966a; Duff and Vogt, 1969; Burmester and Purchase, 1970; Fujita et al., 1974; Crittenden, 1975; Hanafusa, 1975; Purchase and Burmester, 1979).

The replication of leukosis viruses is probably similar to that for the sarcoma virus, although the time may be longer than for the sarcoma virus. The DNA from infected cells can be isolated and will transfect cells leading to transformation, virus production, or both (Vigier, 1974). Sarcoma virus particles are formed and released from infected cells by "budding" from cell membranes and cytoplasmic vacuoles and freed into the extracellular space (Beard, 1973). After adsorption, penetration and uncoating, reverse transcription from RNA to DNA forms the DNA provirus that contains the gene for virions and for transformation. Viral RNAs, viral proteins and virions are then formed. Replication activity associated with DNA provirus replication and with neoplastic transformation is lost (Temin, 1971; Vigier, 1974; Hanafusa, 1975).

The mutations that occur in sarcoma viruses are either conditional or nonconditional (Vogt et al., 1974; Hanafusa, 1975). The conditional mutants produce sarcoma when injected into chickens. The nonconditional mutants are either transformation defective or replication defective. The transformation defective mutant viruses cause leukosis when injected into susceptible chicks (Biggs et al., 1973). Leukosis viruses appear in all stocks of nondefective sarcoma viruses. Replication-defective mutants, such as Bryan high (BH) titer strain of Rous sarcoma, have been isolated from native and mutagenized stocks of avian sarcoma virus. These produce noninfectious progeny and the morphologically altered cells from which they are produced have been termed nonproducer (NP) or converted nonvirus producer (CNVP) cells (Purchase, 1965). Avian leukosis viruses or nondefective sarcoma viruses, i.e., "helper viruses", complement the replication of defective virus when they coinfect the same cell to provide the missing component in the viral genome. The helper viruses present in defective RSV stocks are referred to as

Rous-associated viruses (RAV). Activation of nonproducer cells can be used as an assay for leukosis viruses because the sarcoma virus activated from nonproducer cells by a leukosis virus can be detected directly in cell culture. This also offers a method for preparation of "tailor-made" Rous sarcoma viruses with envelope properties identical to the helper virus, i.e., the leukosis virus. Determination of envelope properties is easier with the sarcoma virus than with the leukosis virus (Purchase and Burmester, 1978).

In 1964, Okazaki and Crittenden noted that avian leukosis virus gs antigen without infectious virus was present in certain line 15_1 embryos and, in 1968, Payne and Chubb reported that gs antigen was inherited as a single gene. These observations gave a strong impetus to Huebner's oncogene and Temin's protovirus hypotheses, which state that all the information necessary for the formation of tumors or tumor viruses is present in every normal cell but is repressed. Various factors act to derepress this information, after which tumors result. These concepts were the bases for the discovery of endogenous viruses. Thus, all chicken cells apparently carry viral genome required to produce a complete leukosis virus (Burmester and Purchase, 1979).

There are regulatory genes controlling gs antigen and envelope antigens. Some or all regulatory genes are in the recessive state, which represses partial or full virus expression, although active infections have been detected in certain inbred lines (Crittenden, 1975; Weiss, 1975; Robinson et al., 1976). When complete endogenous virus is produced, it has envelope glycoproteins characteristic of subgroup E. Some birds have only partial repression of the endogenous viral genome and thus gs antigen, detected in the COFAL test, may occur or envelope glycoproteins may be produced in cells. These are called chick helper factors (chf) because they can complement RSV which are defective in certain envelope functions. Like exogenous viruses, endogenous viruses are capable of horizontal transmission in chickens but, because chickens are genetically resistant to infection with subgroup E, these viruses do not usually spread. There is no evidence that endogenous viruses are oncogenic (Motta et al., 1975).

The leukosis/sarcoma viruses infecting the same cells mix phenotypically, producing progeny with coat proteins of parental types but containing the genome of only one parent. However, the nucleoid determines the transforming ability of the virus which depends on the genome in the particular virus particle (Vogt, 1967). Using this property of phenotypic mixing (PM), Okazaki et al. (1975) developed a test to detect the presence of leukosis virus in selectively resistant cells. Cells are infected with a sarcoma virus of a particular subgroup. In the presence of a leukosis virus of another subgroup, a progeny virus with envelope properties of both viruses develops and can be identified. Phenotypically mixed virus is genetically unstable; thus, the progeny of a single infection is of the one parental type only. Genotypic mixing which is stable may also occur (Vogt et al., 1974).

There are many strains of LL viruses. The finding that the Bryan high (BH) titer strain of RSV was defective led to the classification into subgroups on the basis of interference, cross neutralization and host range patterns (Hanafusa, 1975). The first helper virus strain isolated from BH-RSV stock virus was designated Rous-associated virus-1 (RAV-1). It was recognized by its interference with RSV transformation. Rous-associated virus-1 was classified within subgroup A of LL viruses (Vogt and Ishizaki, 1966a). Rous-associated virus-2 (RAV-2), also isolated from BH-RSV stock, was identified as a member of a different subgroup of avian leukosis viruses (Hanafusa, 1975) and was called subgroup B (Vogt and Ishizaki, 1966a). As more leukosis viruses were isolated, the numbers of strains included in subgroups A and B increased. Besides RAV-1, subgroup A contains RPL-12, RAV-3, RAV-4, ALV-F42, RAV-5, MAV-1, FAV-1 and most of the LL field isolates. Subgroup B contains RAV-2, RAV-6, MAV-2 and AMV-B (Vogt and Ishizaki, 1966a,b; Vogt, 1970). Subgroups C and D were created to accommodate newly isolated helper viruses capable of growing in chick embryo fibroblasts (CEF) resistant to subgroups A and B (Duff and Vogt, 1969). Subgroup C contains RAV-7 and RAV-49 and subgroup D contains RAV-50 and CZAV. The endogenous viruses which were placed in subgroup E are RAV-0 and RAV-60 (Vogt and Ishizaki, 1966b; Vogt, 1970; Vogt and Friis, 1971; Smith et al., 1974). While all 5 subgroups contain LLV strains that grow in CEF, no member from groups B and D was shown to be able to grow on cells from turkeys (Hanafusa, 1975; Vogt, 1970).

Assay Procedures

The LL viruses may be assayed by chicken inoculation. Burmester and Gentry (1956) injected the RPL-12 strain of virus intra-abdominally into day-old susceptible line 15I chicks and were able to obtain a LL response 200 to 270 days later. Later Burmester and Fredrick (1964) used the procedure for initial isolation of the virus from the field. The time was shortened to 43 days when embryos were injected intravenously (Piraino et al., 1963). All sources of virus caused erythroblastosis, osteopetrosis, hemangioma and fibrosarcoma. Many routes were used for assaying sarcoma virus in chicks, but the subcutaneous injection into the wing web is most commonly used for titrations of Rous sarcoma virus (Bryan, 1956). The intra-abdominal route or intramuscular route is used for isolation and propagation of the virus (Purchase and Okazaki, 1964). Intracerebral inoculation may lead to death, which occurs between 7 and 35 days postinoculation (Groupe et al., 1956).

The first *in vitro* method of assay for LL viruses was that developed by Groupe in 1956 for RSV in CEF. The RIF test was used to confirm many observations, such as egg transmission, which formerly required a more laborious *in vitro* test. The RIF test was later replaced by the COFAL test (Sarma, 1964) and the PM test (Okazaki et al., 1975). These tests are now used to detect and assay for leukosis viruses.

Pathology and Pathogenesis

Lymphoid leukosis is now recognized as a lymphoblastoma caused by LL virus. It originates in the bursa of Fabricius and spreads to other organs with enlargement of the liver and tumorous involvement of other organs.

Lesions in young chickens were described by Calnek (1968) after infecting day-old chickens with RPL-12 strain of LL virus and examining them 4 to 10 weeks later. He observed gross lymphoproliferative lesions in the spleen, heart and testis and microscopic lesions in the liver and other visceral organs as well as in the dorsal root ganglia. The spleen was enlarged and mottled, and the testis and heart had small grayish translucent areas. Microscopically there were small discrete foci or large diffuse areas of lymphoblasts. These lesions were transitory since they disappeared grossly and were markedly reduced microscopically after 10 weeks of age.

The neoplastic lesions first occur in the bursa of Fabricius at 5 to 8 weeks after inoculation at 1 day of age. Bursal follicles become engorged with lymphoblasts (Gross et al., 1959; Purchase and Sharma, 1973). By 16 weeks, abnormal follicles are present in the bursas of most infected chickens. The abnormal follicles expand and encroach upon adjacent normal follicles. There is coalescence of adjacent neoplastic follicles into tumors which metastasize. These tumors were visible grossly at 16 to 24 weeks and were seen in almost every chicken dying with LL (Dent et al., 1967; Cooper et al., 1968; Burmester, 1969). In the bursa, there is loss of distinction between cortex and medulla. The lymphoblasts filling the follicles are uniform in size with a pyroninophilic cytoplasm. A few scattered macrophages give a "starry sky" appearance. Tumor cells have large vesicular nuclei with margination and clumping of the chromatin and prominent nucleoli. The use of special stains helps in differentiation of malignant cells in LL from normal cells and MD tumor cells (Siccardi and Burmester, 1970).

The bursa of Fabricius plays a central role in the pathogenesis of LL. The first evidence came from bursectomy experiments, which indicated that surgical bursa removal completely prevented LL (Peterson et al., 1966) and that LL occurs in birds chemically bursectomized and transplanted with viable bursal cells (Purchase and Gilmour, 1975). The disease was also prevented by hormonal treatment (Burmester, 1966), feeding of androgen analogs that have little or no androgenic effect (Kakuk et al., 1977; Romero et al., 1977), chemical bursectomy with cyclophosphamide (Purchase and Gilmour, 1975; Romero et al., 1977), and infection with infectious bursal agent at 2 or 8 weeks (Purchase and Cheville, 1975). The role of the bursa is also illustrated by immunofluorescence studies, which indicated that cells of LL tumors, transplantable tumors and lymphoid cell lines have B cell markers (Cooper et al., 1974; Payne and Rennie, 1973). Neoplastic changes in the bursa can be observed 4 to 8 weeks after infection regardless of the age at which infection occurred (Purchase, 1976a). Thymectomy has no influence on the disease (Peterson et al., 1964).

Whether the earliest change in the bursa starts in the cortex or medulla is not yet fully documented. Rarely, a cortex engorged with tumor cells surrounding a normal medulla indicates that cortical cells transform first; but sufficient cases have not been observed to confirm this hypothesis (Payne, 1976). There is evidence that some of the transformed follicles regress as target cells are transformed in the bursa of most birds, but few birds develop the disease (Cooper et al., 1968). When the bird reaches sexual maturity (16 to 24 weeks of age) and when normal bursas are rapidly regressing, the transformed cells burst through follicular walls into blood vessels and metastasize to the visceral organs, particularly the liver and gonad. It was reported that the target cells are post-stem cells because partial chemical bursectomy destroys the target cells before destroying the stem cells which provide for immune response (Purchase and Gilmour, 1975). Bursectomy up to 5 months of age prevents the disease (Peterson et al., 1966), thus indicating that target cells are resident in the bursa. When poststem cells are transformed, the maturation is arrested, thus producing only IgM (Cooper et al., 1974).

The tumor in visceral organs may take 3 different forms: discrete, miliary or diffuse. In all forms the tumors are multicentric, and even in the diffuse form the microscopic pattern is one of coalescing foci. With proliferation of neoplastic cells, the organs are compressed rather than being infiltrated. Liver nodules are usually surrounded by fibroblast-like cells that are remnants of sinusoidal epithelial cells (Gross et al., 1959). Similar foci of lymphoblasts separated by bands of fibroblast-like cells are seen in bursas with advanced tumors. Occasionally there may be fibrosis and sometimes infiltration of pleomorphic lymphoid cells in chronic cases, probably an immunologic response. A few plasma cells may be scattered around the nodules (Payne, 1976). In the spleen the neoplastic follicles are well circumscribed and closely associated with small blood vessels. The disease is essentially extravascular; leukemia is not common, although terminal metastasis in bone marrow, pancreas, thymus and gonads may occur (Darcel, 1973). Electron microscopy reveals an abundance of ribosomes in clusters and scattered strands of rough endoplasmic reticulum. Occasionally there is vacuolation and budding virus particles are seen in tumor cells (Dmochowski et al., 1964; Cooper et al., 1974).

Many tumors of the leukosis/sarcoma group are homotransplantable. Transplantation of cellular material from cases of LL and transplantable lymphoid tumor strains has been established. The most famous of these is the Olson tumor transplant, also identified as RPL_{12} at the RPRL, which has been maintained by a long series of passages since 1941 (Olson, 1941). When cell suspensions of transplantable tumors are injected into young chicks, a tumor develops to a palpable size at the site of inoculation within 5 to 10 days. A rapid and extensive metastasis occurs and is followed soon by death. The primary virus-induced tumors develop 4 to 5 months after inoculation of 1-day-old chicks. Histologically the transplants are more uniform and more anaplastic than the primary tumors. The transplant may contain the virus as in the Olson transplant, which causes erythroblastosis and osteopetrosis in addition to LL.

The fate of donor cells can be determined by the sex chromosome technique in the opposite sex (Vogt, 1965). Some tumors like the Olson transplant can be transplanted indefinitely, but others are converted into neoplasms dominated by cells of the recipient host (Ponten and Burmester, 1967).

Tumor transplants injected intravenously grow mainly in the liver, spleen, kidney, bone marrow, gonads and, occasionally, the thymus (Okazaki and Romero, unpublished). In early passage, tumor transplants are either diffuse or nodular; the nodular form seems to be a property of the less rapidly dividing tumor cells before adaptation to continuous growth. Once established, the diffuse form predominates. Transplantable tumors only rarely involve the bursa (Purchase and Burmester, 1978).

Chickens are the natural host for all viruses of the leukosis/sarcoma group. Besides the chicken, the only other avian species from which a virus of this group was isolated is the pheasant. Experimentally, some viruses have a wide host range. Rous sarcoma has the widest host range, which includes turkeys (Dunkel et al., 1964). Osteopetrosis can be reproduced in turkeys (Holmes, 1964).

Isolates of leukosis viruses in experimental chickens were found to produce more than one type of response, but under standard sets of conditions in the laboratory, successive passages produced tumors of relatively similar characteristics. These strains are not genetically pure, although some investigators consider that a single viral entity is the cause of all the various tumors with variability determined by the response of the host systems involved. Thus, erythroblastosis and lymphoid leukosis are responses of hematopoietic and lymphopoietic systems, respectively. Other investigators consider that each pathologic manifestation is induced by a single viral entity and that most strains are a mixture of viruses. For example, under standard conditions RSV causes predominantly sarcomas at the site of inoculation, BAI strain A produces predominantly myeloblastosis, and strain R produces erythroblastosis.

The dose and route of inoculation have an effect on the spectrum of tumor produced. Burmester et al. (1959) reported induction of primarily erythroblastosis with a high dose of RPL 12 and lymphoid leukosis with a low dose. Intramuscular inoculation of RPL 26 favored the induction of sarcomas, whereas intravenous inoculation produced mainly erythroblastosis and hemorrhages (Fredrickson et al., 1964). These differences may reflect the amount of virus reaching the target organs.

The leukosis/sarcoma viruses are transmitted vertically or horizontally. The latter is slow and inefficient, while the former is considered the major means of persistence of the virus from one generation to the other. Transmission through the egg was reported by Cottral et al. (1954) and Burmester and Waters (1955) and was later confirmed by many investigators. Viruses transmitted through the egg are predominantly of subgroups A and B. Congenitally infected chickens may be immunologically tolerant of the virus and thus fail to develop antibodies (Rubin et al., 1962), but a few develop antibodies to the original or superinfecting viruses and may remain viremic (Meyers, 1976) or may overcome the viremia (Rubin et al., 1961). The viremic birds transmit infection through saliva and feces until they reach sexual maturity, when they may become continuous egg intermittent transmitters. Antibody develops in chickens infected by contact exposure. Some of these overcome infection, but many remain infected and become intermittent transmitters of the virus through the egg. Large quantities of gs viral antigen and of infectious virus were detected in egg albumen (Spencer et al., 1976). The virus may infect the germinal cells just after leaving the ovary or the zygote in the proximal part of the oviduct. The male plays little role in the epizootiology in leukosis virus infection, since he acts only as a carrier of the virus and as a source of contact infection for other birds (Rubin et al., 1961; DiStefano and Dougherty, 1968).

Immunity and Immunosuppression

Maternal antibody is detected in most day-old chicks, but the efficiency of passive transfer is low. The titers increase to undetectable levels between 4 and 7 weeks, after which most chicks become infected from penmates or the environment. Infection leads to transient viremia and antibody production that persists through the life of the bird. Leukosis virus infection leads to protection against several sarcoma viruses (Meyers et al., 1972). The immunity is probably mediated through group-specific cell surface antigens or tumor specific cell surface antigen (TSSA). The mechanism of immunity to LL is not understood (Crittenden, 1975).

Natural resistance of chickens to neoplasm development increases with age. Neoplasm development is more rapid after a natural route of exposure than by the parenteral route (Burmester et al., 1960a,b). Males are more resistant than females (Burmester and Nelson, 1945).

The effect of LL viruses on the immune functions was reviewed by Payne (1970) and Sharma (1979). A depression of humoral immune response has been reported by many but not all investigators. Peterson et al. (1966) noted a depression in primary antibody response in early stages of the disease before neoplastic transformation. Purchase et al. (1968) reported a decrease in the primary response to bovine serum albumin (BSA) at 14 and 22 weeks after neonatal infection and again at 18 and 26 weeks after neonatal infection. Dent et al. (1968) found no effect from antigenic stimulation with rabbit erythrocytes up to 32 weeks after infection at hatching but reported a depression in the primary response to bovine serum albumin and *Brucella abortus* at 12 but not at 17 or 26 weeks in birds which were ultimately destined to die of the disease. This finding suggests an early immunologic impairment in birds with the progressive disease. Also, Fadly (1979) found no significant difference between infected and control birds when challenged with *B. abortus* and SE. However, normal levels of antibodies to *Salmonella typhimurium* H were reported by Cooper et al. (1974).

The depression of humoral response appears to be related to the stages of active viremia prior to neoplastic transformations of the bursal follicles and tumor metastasis (Dent et al., 1968; Peterson et al., 1966).

The results of investigations on the cellular immune functions were inconsistent. Graft versus host response was found to be depressed in one strain of chickens but not in another (Purchase et al., 1968). Dent et al. (1968) reported no effect even in the chicken strain reported to be affected in Purchase's study. Skin graft rejections were reported unaffected, increased or decreased (Purchase et al., 1968; Dent et al., 1968).

In vitro PHA mitogenic response was reported to be depressed in LL virus infected chickens (Meyers et al., 1976; Smith and VanEldik, 1978). Meyers et al. (1976) used suboptimal doses of PHA to detect the depression in cellular immune fractions.

MATERIALS AND METHODS

Experimental Animals

Single Comb White Leghorn chickens were used and were a cross of inbred lines 15 I_5 males by 7_2 females maintained at the Regional Poultry Research Laboratory (RPRL). The chickens were susceptible to MD and LL and were free of the common poultry pathogens such as MD, LL, mycoplasmosis, and salmonellosis.

The turkey poults were obtained when 1 day old from Nicholas Turkey Breeding Farms, Inc., in California. These were Nicholas strain 02 from *Mycoplasma gallisepticum, Mycoplasma synoviae,* and *Mycoplasma meleagridis* free eggs. Chickens and turkeys were HVT antibody positive.

Isolation Units

Modified Horsfall-Bauer and plastic canopy isolators with automatic waterers and gravity flow feeders were used. The Horsfall-Bauer units (Labco Division, Part-Co, Inc., Columbus, OH) were stainless steel units under negative pressure with room air drawn into the isolator through 3 layers of 50-FG filter down (American Air Filter Co., Louisville, KY). The plastic units (Hazleton Systems, Inc., Aberdeen, MD) were under positive pressure with filtered air (Dri-Park and Astrocel filters, American Air Filter Co., Louisville, KY).

Tissue Culture

Chicken embryo fibroblasts (CEF) were prepared from 10- to 11-day embryos from inbred line 15_B specific pathogen-free (SPF) chicken flocks reared at the RPRL. Embryos used were from SPAFAS strain chickens (SPAFAS, Inc., Norwich, CT). Duck embryo fibroblasts (DEF) were prepared from 13-day-old embryos from Khaki Campbell ducks reared at the RPRL. Japanese quail embryo fibroblasts (QEF) were prepared from 7- to 9-day embryos obtained from the Poultry Science Department, Michigan State University.

Chicken embryo fibroblasts, DEF and QEF were grown and propagated in a mixture of medium 199 and Ham's F10 medium (F10-199 mixture, Microbiological Associates, Bethesda, MD). The mixture was supplemented with 4% calf serum, 100,000 units of penicillin, 100 mg of streptomycin/liter, and mycostatin (5 units/liter) or gentamycin (50 mg/liter) for propagation and maintenance of MDV and HVT. For the lymphoid leukosis viruses, the F10-199 mix medium was supplemented with 5% tryptose phosphate broth. Mycostatin and fungizone were added at the rate of 15,000 units and 1 mg/liter, respectively. Maintenance medium for normal and transformed cells was supplemented with 2% calf serum, 5% bovine amniotic fluid and 1% dimethylsulfoxide (DMSO). Rous minus quail cells (R-Q) were maintained in F10-199 growth medium supplemented with 1% DMSO and 1% chick serum. A medium with L-glutamine (RPMI 1640, Flow Laboratories, Rockville, MD) was used as the cell culture growth medium in the blastogenesis assays.

Cell Culture Preparations

Chicken embryo fibroblasts, DEF and QEF were prepared as described by Solomon (1975) and Solomon et al. (1971). Briefly, embryos were removed aseptically, decapitated and washed in phosphate buffered saline to remove blood. Whole embryos were then trypsinized in warm (37 C) trypsin solution (0.125%). Free cells were washed and then resuspended in growth medium. For primary cultures, 150 x 25 mm plastic plates (Falcon Plastics, Los Angeles, CA) were seeded with 2×10^7 cells in 25 ml of medium. For secondary cultures, 1.5×10^6 cells per plate were used. Plates used for secondary cultures were either 60 x 15 or 35 x 10 mm disposable plastic plates (Falcon, Los Angeles, CA).

Virus Inocula

MDV

Two MDV inocula were used: cell-culture propagated virus and tumor cell suspension. A cell-associated and cloned preparation of the Georgia (GA) isolate of MDV originally isolated from an ovarian tumor of a chicken (Eidson and Schmittle, 1968) was used. The virus stock consisted of infected DEF cells that were slowly frozen in F10-199 mix media containing 5% DMSO and stored at -196 C in the vapor phase of liquid nitrogen. This virus produced a high frequency of visceral tumors and nerve lesions in chickens.

An ovarian tumor was harvested from a 7-week-old chicken that had been infected with GA isolate of MDV at 1 day of age. The tumor was ground in a Tenbroek grinder and a 20% (w/v) homogenate was made in F10-199 mix medium and used fresh.

HVT

A cloned preparation of prototype strain FC 126 was used (Witter et al., 1970b; Okazaki et al., 1970). The virus stock consisted of HVT-infected CEF cells stored at -196 C.

LLV

Rous-associated virus-1 (subgroup A) was used as inoculum in Experiment IV and RAV-2 (subgroup B), RAV-49 (subgroup C) and RAV-50 (subgroup D) were used in the alternative phenotypic mixing tests. Inocula were obtained from P. K. Vogt, Department of Microbiology, University of Southern California School of Medicine. These viruses were propagated on CEF resistant to subgroup E virus (C/E) cells by the activation of nonproducer (NP) cells with cloned helper viruses.

Titration of Viruses

Assay of MDV and HVT

For *in vitro* plaque assays for MDV and HVT, serial 10-fold dilutions of stock virus or viable buffy coat cells were used. Blood was collected in heparin (20 units per ml) and viable buffy coat cells were obtained by flotation on bovine serum albumin (Parker, 1962). Diluted stock virus or 10^7 buffy coat cells in 0.2 ml medium were inoculated into secondary DEF cultures for assay of MDV and CEF or DEF for assay of HVT. Each sample was assayed in duplicate secondary cultures grown in 60 mm plastic plates. Uninoculated plates were kept as controls. The monolayers were maintained in liquid medium which was renewed every other day during the observation period. All cultures were incubated at 37 C in a humidified atmosphere containing 3 to 4% carbon dioxide. Turkey herpesvirus plaques were enumerated 6 to 8 days after inoculation, whereas MDV plaques were enumerated 11 to 12 days after inoculation. In dual infections the plaques induced by the 2 viruses could be identified on a morphological basis (Witter et al., 1976).

Alternative Phenotypic Mixing

To determine RAV-1 viremia in chickens and turkeys, a method of assay for exogenous leukosis viruses in which mixed cultures of transformed quail cells and C/E cells and assay of supernates for focus formation on C/E cells was used (Crittenden et al., 1979). In this assay, Japanese quail cells transformed by the envelope-defective BH titer strain of RSV (R-Q) were used as a source of RSV genome.

The test was set up by inoculating secondary SPAFAS cells at 0.5×10^6 and (R-Q) at 0.2×10^6/plate in 2 ml F10-199 medium with calf serum (4%) in 35 mm plastic plates. DEAE-dextran (Pharmacia, Upsala, Sweden) was incorporated at the rate of 2 μg/ml into the medium at the time of infection. When there was confluent growth, each plate was inoculated with 0.1 ml of the blood to be tested. Heparin was incorporated into the tissue culture media to a final dilution of 4 to 6 μg/ml during the first 24 hours of culture. The control plates were inoculated with RAV-1, RAV-2, RAV-49 and RAV-50. Ten-fold dilutions (10^{-1} to 10^{-4}) of each virus were made and 0.1 ml of each dilution was inoculated per plate. Maintenance medium was added on the second, fourth and seventh day after inoculation. On the ninth day the supernatant fluid was assayed on secondary SPAFAS cells (C/E) plated at 0.5×10^6 cells per 35 mm plate. The medium was supplemented with 4% calf serum and 2 μg/ml DEAE-dextran. Each plate was inoculated with 0.5 ml of the supernate. Controls were inoculated with 0.1 ml of RSV preparations (RAV-1, RAV-2, RAV-49 and RAV-50) per plate. Ten-fold (10^{-1} to 10^{-4}) dilutions were also used. After 24 hours, the plates were overlaid with hard agar base. The foci were counted 7 days after inoculation of the supernates.

Antibody Assay

Neutralization Test for RAV-1

The procedure described by Ishizaki and Vogt (1966) was followed. Plasma from heparinized blood samples was diluted 1:5 in F10-199 mix medium containing 4% calf serum. Complement and viruses that may have been present in the diluted plasma samples were inactivated by heating for 40 minutes at 56 C. The plasma samples were then mixed with equal volumes of a dilution of RSV (RAV-1) that gave approximately 100 foci per plate if no antibody were present in the sample. Known negative and known positive plasma samples were prepared similarly. All plasma-virus mixtures were further incubated at 37 C for 40 minutes to let the virus react with the plasma sample. Secondary CEF (C/E) cultures were grown in triplicate in 35 mm plastic petri dishes and inoculated with 0.2 ml of each sample. The following day the medium was discarded and the cultures were overlaid with agar media. Cultures were fed with 1 ml of F10-199 mix medium containing 2% calf serum and 0.5% DMSO every other day. Seven days after inoculation, foci were counted. Plasma samples that reduced the number of foci by 90% or more were considered positive, a reduction between 50 and 90% was considered suspicious, and a reduction of 50% or less was considered negative.

Antigenic Stimulation and
Antibody Titrations

Each chicken and turkey poult in groups allotted for antigenic stimulation trials was injected intravenously with 1 ml of phosphate buffered saline (PBS) containing 2.5 x 10^9 cells each of SE and formalin-killed *Brucella abortus*. A second similar injection was given 8 days later. Serum from birds was collected 7 days after the primary stimulation and 7 days after the secondary stimulation. All serums were heat inactivated at 56 C for 30 minutes.

The microagglutination tests for SE antibody were performed in microtiter U plates (Cooke Laboratory Products, Alexandria, VA) containing 8 x 12 wells. One drop of phosphate buffered saline (PBS) was placed in each well with a 0.025 ml plastic dispenser (Cooke Laboratory Products, Alexandria, VA). From each serum sample, 0.025 ml was taken with a microdilutor (Cooke Laboratory Products, Alexandria, VA) and 2-fold serial dilutions were made. To each serum dilution, 0.025 ml of 1% washed SE suspension in PBS was added. A PBS control was performed concurrently for each serum tested. A positive control was also performed. The plates were then shaken gently and incubated at 37 C for 1 hour, as for test serum. The end-point was the highest dilution of serum with complete agglutination.

For assay of the antibodies against *B. abortus*, a 1:8 dilution of the tube antigen of *B. abortus* was made in PBS. The sera were diluted as for the SE agglutination test. One drop (0.025 ml) of *B. abortus* antigen was dispensed in each well in the microplate containing the diluted serums. The test was allowed to proceed at room temperature after manual shaking and read the following day. For both SE and *B. abortus* tests, the titers were expressed as the $\log_2$ of the reciprocal of the highest dilution of serum causing complete agglutination of SE or *B. abortus*.

Microassay of Whole-Blood Lymphocyte Stimulation by Concanavalin A

The assay was performed according to the method described by Lee (1978). Blood was obtained from chickens and turkey poults by cardiac or venipuncture and was heparinized (20 units heparin/ml of blood). One hundred microliters of medium RPMI 1640 with L-glutamine (Flow Laboratories, Rockville, MD) without serum was placed in each of 5 wells in a 96-well flat-bottomed microtiter plate (Microtest II, Falcon Plastics, Los Angeles, CA). Ten microliters of whole blood from each bird were placed in each of the 5 wells containing the medium. A stock solution of Concanavalin A (Con A) (Cal Biochemical, Los Angeles, CA) was prepared in Dulbecco's PBS to a concentration of 10 mg/ml. Ten micrograms Con A per 0.1 ml medium were added in each of the 3 wells and each of the remaining 2 wells received 0.1 ml medium without Con A. The microtiter plates were incubated at 41 C in a 5% carbon dioxide atmosphere for 48 hours. Isotope 5-[^{125}I]iodo-2'-deoxyuridine (^{125}IUdR) (0.9 to 1.1 Ci/mM, Amersham Searle, Chicago, IL) was used at a concentration of 0.05 μCi in 50 μl medium/well in each of the 5 wells. The mixture was further incubated for 6 to 16 hours before counting the isotope incorporation. For assay of the radioactivity incorporated, cultures were harvested in an automatic 24-multiple culture cell harvester (Model M24V, Brandel, Rockville, MD). The sample disc filters (Reeves Angel, Clifton, NJ) with the labeled cells were then punched out and counted in a Beckman γ-counter (Beckman Instruments, Inc., Fullerton, CA). The stimulation index was calculated as counts per minute (cpm) of isotope incorporation in cells treated with Con A divided by cpm in cells not treated with Con A. Concanavalin A was preferred to PHA because preliminary experiments showed (unpublished) that turkey cells responded better to Con A than to PHA.

Body and Organ Weights

Chickens and turkey poults were weighed in an electronic balance (Model Autogram 1000, Ohaus Scale Corp., Union, NJ) or on a standard scale (Model 3180, Toledo Scale Co., Toledo, OH). The bursa of Fabricius, all lobes of the thymus, and the spleen were dissected out and weighed on an electronic balance (Mettler type H15, Mettler Scientific Products, Division American Hospital Supply Corp., Evanston, IL) or on an electronic balance (Mettler type P1200, Mettler Instrument Corp., Hightstown, NJ).

Pathologic Techniques

Autopsies were performed on all chickens and turkey poults in these experiments. All birds were examined for gross lesions. Vagus nerve, brachial plexus, sciatic plexus, liver, spleen, bursa of Fabricius, thymus, gonad, heart, skin, femur or humerus were fixed in 10% buffered neutral formalin solution. The bones were then placed in "decal" solution for decalcification. In Experiment III only gross lesions were recorded.

All tissues for histopathologic examination were embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin. Special stains included methyl green pyronine and reticulum stains. Histologic techniques were described by Siccardi and Burmester (1970) and Luna (1968). The system described by Witter et al. (1976) for scoring proliferative lesion response was followed. The lymphoproliferative lesions observed in each tissue were scored on a scale from 0 (none) to 4 (maximum). A very restricted lymphoproliferative lesion, often consisting of 10 to 20 cells, was scored as 1. Foci consisting of 20-40, 40-60, and 60-80 cells were scored as 2, 3, and 4, respectively. The mean scores of each group of chickens or poults were obtained by summing scores for all individual tissues examined and dividing by the number of tissues examined.

Experimental Design

Four experiments were conducted, each including chickens and turkeys but differing principally in the virus employed. The basic design of Experiments I, II, and IV is shown in Table 1. The design was slightly modified in all 3 experiments. A detailed description of individual experiments is given below. All birds were wing banded.

Generally, animals were inoculated with virus and the following observations were made. The body weights of chickens and turkeys were recorded weekly for the first 6 to 8 weeks. The weights of their lymphoid organs (bursa, thymus, spleen) were recorded at the time they were killed. The gross and microscopic examinations were done weekly as the birds were killed. Humoral immune responses were tested at 2 intervals during each experiment. Sheep erythrocytes, which carry a thymus dependent antigen, and *B. abortus*, a bursa dependent antigen, were used for stimulation of the humoral immune system. Concanavalin A stimulation of whole blood cells was done sequentially to monitor the kinetics of the cellular immune response. To assess viremic response, virus titrations were done each week. Demonstration of antiviral antibodies was done when appropriate.

Table 1. Basic experimental design for Experiments I, II and IV[†]

Host	Starting Number	Treatments	No. killed weekly[a] 1	2	3	4	5	6	No. for immunization with SE and *Brucella abortus*[b]	No. used[c] for Con A tests	No. Extra	Total No. Examined
Turkey	50	Inoculum I	4	4	4	4	4	4	6 x 2	8	12	
	50	" II	4	4	4	4	4	4	6 x 2	8	12	150
	50	none	4	4	4	4	4	4	6 x 2	8	12	
Chicken	50	Inoculum I	4	4	4	4	4	4	6 x 2	8	12	
	50	" II	4	4	4	4	4	4	6 x 2	8	12	150
	50	none	4	4	4	4	4	4	6 x 2	8	12	

[a]Used for sequential evaluation of body and organ weights, titration of virus present in the blood, and gross and histopathologic examinations.

[b]Used for evaluating humoral immune functions.

[c]Used for evaluating cellular immune functions.

[†]Variations from the basic design which appeared are described in the separate experiments. The design of Experiment III is different.

Experiment I

One hundred sixty-eight chickens and 159 turkey poults were divided into 9 groups each. Groups 1, 2 and 3 of the chickens contained 49, 47, and 36 chickens, respectively, and groups 1, 2, and 3 of the turkey poults contained 41 poults each. Groups 4, 5, 6, 7, 8, and 9 of both turkey poults and chickens contained 6 birds each. At day 1, each bird in groups 1, 4 and 7 was inoculated intraperitoneally (IP) with 4.53 x 10^6 PFU of GA-MDV. Groups 2, 5 and 8 were inoculated IP with 7.4 x 10^6 cells of MD tumor homogenate containing 8.2 x 10^4 PFU of MDV. The other groups of each species were left as uninoculated controls. Each group was housed in a separate isolator.

At weekly intervals, 4 birds were selected at random for killing from each of groups 1, 2 and 3. The observations of these birds included: live body weight, organ weights, titration of virus present in blood, and histopathologic examination. Chickens and turkey poults in groups 4, 5 and 6 were inoculated with SE and *B. abortus* at weeks 3 and 4. Chickens and turkeys in groups 7, 8 and 9 were inoculated with SE and *B. abortus* at weeks 7 and 8 and blood samples for microagglutination tests were taken 1 week after each inoculation. Blood samples from 8 birds of groups 1, 2 and 3 were obtained on days 24, 42 and 53 after infection for the Con A test. The experiment was terminated at day 60.

Experiment II

A total of 90 chickens and 90 turkey poults were divided into 8 groups, 4 groups of chickens and 4 of turkey poults. Both species were divided as follows: groups 1, 2, 3 and 4 contained 35, 35, 10 and 10 birds, respectively. Each bird in groups 1 and 3 was inoculated with 8.2 x 10^4 PFU of HVT IP at day 1. Groups 2 and 4 were left as uninoculated controls. Each of the 8 groups was kept in a separate isolator. All the investigations conducted in Experiment I were repeated in this experiment, except that inoculation with SE and *B. abortus* was done at 10 and 11 weeks after infection and blood for the blastogenesis assay was drawn at weeks 2 and 3. The experiment was terminated at day 42.

Experiment III

Twenty turkey poults were placed in 2 groups and 43 chickens were placed in 2 groups. One group of turkey poults and 1 group of chickens were vaccinated at day 1 with 8.2 x 10^4 PFU of HVT IP. After 10 days, each bird in the inoculated and uninoculated groups was given 8.3 x 10^4 PFU of GA-MDV IP. Each group was kept in a separate isolator until the experiment was terminated 81 days after vaccination. Six birds in each group were bled for titration of HVT and MDV present in the blood at 35 days of age. The experiment was terminated at day 81.

Experiment IV

One hundred thirty-five chickens and 135 turkey poults were divided into 9 groups each. Groups 1, 2 and 3 contained 33 birds each, and groups 4, 5, 6, 7, 8 and 9 contained 6 birds each. At 1 day of age, each bird in groups 1, 4, and 7 was inoculated with 10^5 infectious units of RAV-1 intravenously (IV) and each bird of groups 2, 5 and 8 was inoculated with 10^2 infectious units of RAV-1 IP. The remaining groups were left as uninoculated controls. Each group was kept in a separate isolator. Birds in groups 4, 5 and 6 were inoculated with SE and *B. abortus* at weeks 3 and 4. Birds in groups 7, 8 and 9 were inoculated with SE and *B. abortus* at weeks 10 and 11. Blood samples for the microagglutination test were obtained 1 week after each inoculation. Blood for Con A test was drawn on days 10, 20, 83 and 103 after infection. Body and organ weights, titration of virus present in the blood, and histopathologic examinations were done as in the previous experiments, but at 10-day intervals instead of weekly. The experiment was terminated at day 103.

Statistical Analysis

The data were analyzed as a one-way and two-way analysis of variance. Analyses were performed by an IBM 360 North Dakota State University computer using program statistical analysis. The analysis of variance was done to see if either week, treatment, or the week by treatment interactions were significantly different from one another.

Furthermore, calculation of statistical significance between means, as opposed to the overall significance, was performed according to Tukey's w-procedure for multiple comparisons among means. The Tukey test was performed at alpha levels of 0.05 and less. Thus, any statistical significance discussed is as a minimum $p \leq 0.05$.

RESULTS

Experiment I
Response of Chickens and Turkeys to MDV

Titration of MDV Present in the Blood

The virus associated with white blood cells was detected in chickens and turkeys inoculated with GA-MDV or with tumor homogenate (Table 2). Animals of both species remained persistently viremic through the observation period of 1 to 6 weeks. Generally, incidence of viremia and amounts of detectable virus were higher in chickens than in turkeys.

Pathology

The GA strain of MDV was highly pathogenic for chickens and turkeys (Table 3). Mortality during the 60-day observation period was high in both species, with the median day of death at about 40. The patterns of mortality in inoculated chickens and turkeys are shown in Figure 1. In both species a high percentage of birds had microscopic and macroscopic lesions (Table 3).

Gross lesions. Gross lesions were first observed in chickens 24 days after inoculation of GA-MDV and 32 days after tumor homogenate inoculation. In turkeys, lesions were seen 34 and 32 days after inoculation of GA-MDV and tumor homogenate, respectively.

In turkeys, gross lesions were seen in liver, spleen, kidneys, gonads, heart, thymus and peripheral nerves, with the liver and spleen

Table 2. Titrations of Marek's disease virus (MDV) in the blood from chickens and turkeys inoculated with Georgia (GA) isolate

	MDV Titers (mean PFU/10^7 WBC)[a]			
	Chickens		Turkeys	
Weeks Postinoculation	GA-MDV (tissue culture origin)[b]	Tumor Homogenate[c]	GA-MDV (tissue culture origin)	Tumor Homogenate
1	17 (4/4)[d]	133 (3/4)[d]	1 (2/3)[d]	1 (1/1)[d]
2	384 (4/4)	361 (4/4)	2 (3/4)	1 (2/3)
3	413 (4/4)	345 (4/4)	48 (4/4)	3 (2/4)
4	590 (4/4)	624 (4/4)	44 (2/4)	7 (4/4)
5	585 (4/4)	436 (4/4)	100 (2/3)	70 (4/4)
6	252 (2/2)	626 (4/4)	86 (4/4)	10 (4/4)

[a]Titers shown were calculated by averaging PFU per 10^7 white blood cells of all birds examined in each group. Four uninoculated controls per group examined at each interval lacked detectable virus.

[b]Inoculum consisted of 4.53×10^5 PFU/1-day-old bird and given intraperitoneally.

[c]Inoculum consisted of 7.4×10^6 cells with 8.2×10^4 PFU of MDV/1-day-old bird and given intraperitoneally.

[d]No. viremic/no. examined.

Table 3. Susceptibility of chickens and turkeys to GA isolate of MDV

			MD Response (%)[b]			
Animal	Inoculum	No. Examined[a]	Mortality (%)	Median Days to Death	Gross Lesions (%)	Total Microscopic and Gross Lesions (%)
Chickens	GA-MDV[c]	33	78.8	38	78.8	100.0
	tumor homogenate[d]	37	73.0	43	75.7	94.6
Turkeys	GA-MDV[c]	29	58.6	38	65.5	90.0
	tumor homogenate[d]	21	72.7	41	59.1	85.7

[a]Starting number less number removed during experiment and non-specific deaths.

[b]Experiment was terminated after 60 days.

[c]Inoculum consisted of 4.53×10^5 PFU/1-day-old bird and was given IP.

[d]Inoculum consisted of 7.4×10^6 cells with 8.25×10^4 PFU of MDV/1-day-old bird and was given IP.

Figure 1. Mortality patterns of chickens and turkeys inoculated with GA isolate of MDV. CV = chickens inoculated with GA-MDV, CT = chickens inoculated with tumor homogenate, TV = turkeys inoculated with GA-MDV, TT = turkeys inoculated with tumor homogenate. In CV and TV, each bird received 4.53×10^5 PFU of GA-MDV and in CT and TT 7.4×10^6 cells containing 8.25×10^4 PFU of MDV IP at day 1.

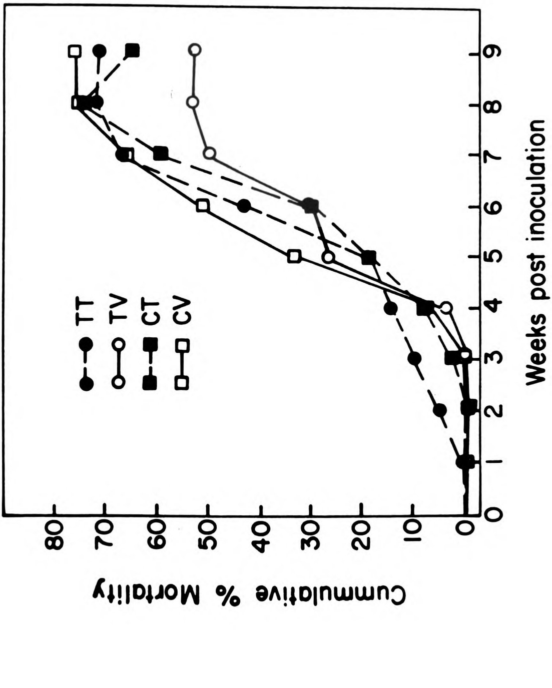

TT
TV
CT
CV
Cummulative % Mortality
0
10
20
30
40
50
60
70
80
Weeks post inoculation
0
1
2
3
4
5
6
7
8
9

Figure 1

being most frequently involved (Table 4). The same tissues in chickens had gross lesions, but gonads and nerves were more often affected in chickens inoculated with GA-MDV than in those inoculated with tumor homogenate.

Peripheral nerve lesions in both species were usually unilateral. The affected peripheral nerves were enlarged and yellowish and had poorly defined cross striations (Figure 2). The liver, spleen, gonad and kidneys were diffusely enlarged, sometimes up to 4 times their normal sizes. (Figures 3, 4, 5 and 6). Some affected livers and spleens had 1 mm to 1.5 cm white nodular lesions visible on the surface. The same type of foci were seen on the cut surface. Organs with focal lesions had moderate or no enlargement. In the hearts, diffuse and focal nodular lesions were seen.

Histopathology. Visceral and neural neoplastic lesions were similar in chickens and turkeys. A focal as well as a diffuse infiltration of lymphoid cells was seen in almost all organs examined in the turkeys. These infiltrating cells were heterogeneous populations of lymphocytes, including small and medium-sized lymphocytes, blast or basophilic cells (possibly neoplastic), and reticulum cells (Figure 7). The proliferating cells were sometimes seen invading the organ capsule and spreading into surrounding tissues and were also found in the lumens of vessels (Figures 8, 9 and 10). Degenerative and inflammatory lesions were observed in many turkeys, particularly in the lymphoid organs, liver, and skin (Figure 11). The initial lesion was perivascular cuffing of lymphoid cells around blood vessels (Figure 12). Accumulations of inflammatory cells included lymphocytes, heterophils, and some reticulum cells, accompanied by

Table 4. Distribution of macroscopic MD lesions in chickens and turkeys inoculated with GA isolate of MDV

Animal	Inoculum	No. Examined[a]	No. with MD Microscopic Lesions (%)[b]						
			Liver	Spleen	Kidneys	Gonads	Heart	Skin	Nerves
Chickens	GA-MDV[c]	33	11(33.3)	13(39.4)	8(24.2)	23(69.6)	5(15.2)	0(0)	23(69.6)
	Tumor homogenate[d]	37	7(18.9)	8(21.6)	11(29.7)	17(45.9)	14(37.8)	0(0)	14(37.8)
Turkeys	GA-MDV[c]	29	11(37.9)	13(44.8)	3(10.3)	3(10.3)	3(10.3)	0(0)	3(10.3)
	Tumor homogenate[d]	21	10(47.6)	13(61.9)	2(9.5)	1(4.75)	0(0)	0(0)	0(0)

[a]Starting number less number removed during experiment and non-specific deaths. Tumorous involvement and regression of the thymus and bursa were seen in both species but figures not presented.

[b]Experiment was terminated after 60 days.

[c]Inoculum consisted of 4.53×10^5 PFU/1-day-old bird and was given IP.

[d]Inoculum consisted of 7.4×10^6 cells with 8.25×10^4 PFU of MDV/1-day-old bird and was given IP.

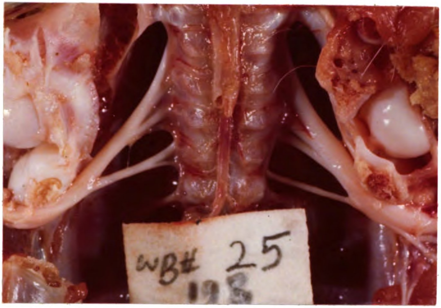


Figure 2. Enlarged sciatic nerve (right) in a turkey 51 days after inoculation with GA-MDV at day 1.

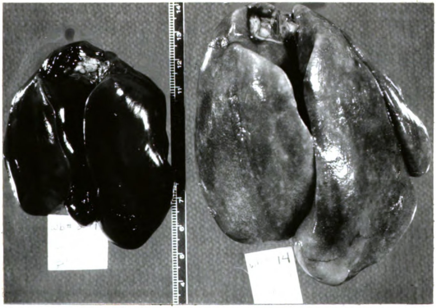


Figure 3. Diffuse enlargement with grayish discoloration and granular surface of the liver (right) of a turkey 44 days after inoculation with GA-MDV at day 1. Liver of uninoculated control of same age (left).

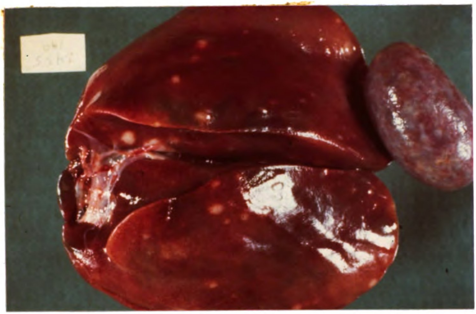


Figure 4. Focal discoloration and tumor nodules in the liver of a turkey 60 days after inoculation with tumor homogenate at day 1. Spleen (right) of same turkey had focal lesions and grayish discoloration but no enlargement.

Figure 5. Enlargement and focal discoloration (arrows) of the spleen (right) of a turkey 51 days after inoculation with GA-MDV at day 1. Spleen (left) from uninoculated control from turkey of the same age.

Figure 6. Diffuse enlargement of spleen (left) of turkey inoculated with tumor homogenate. Spleen from uninoculated control of the same age (right).

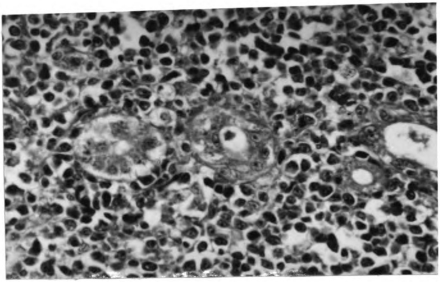


Figure 7. Heterogeneous lymphocytic and reticulum cell infiltration of the kidney of a turkey 60 days after inoculation with GA-MDV at day 1. H&E stain, X480.

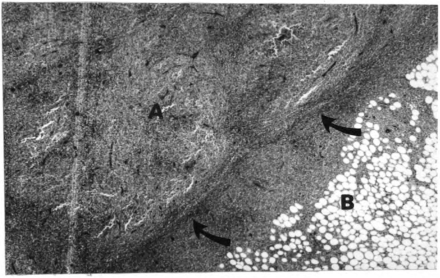


Figure 8. Lymphomatous infiltration of the thymus (A), capsule (arrow) and surrounding adipose tissue (B) in a turkey 60 days after inoculation with GA-MDV at day 1. H&E stain, X30.

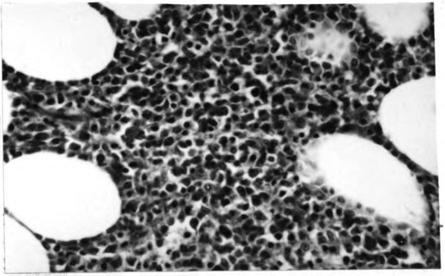


Figure 9. Higher magnification of adipose tissue seen in Figure 8. H&E stain, X480.

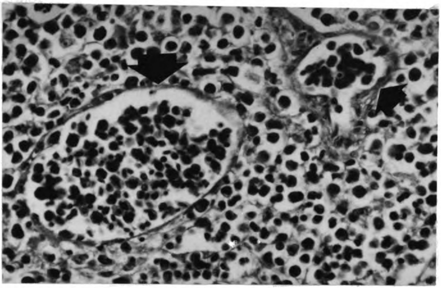


Figure 10. Neoplastic cells in vessels (arrows) in a turkey testis 60 days after inoculation with GA-MDV at day 1. H&E stain, X480.

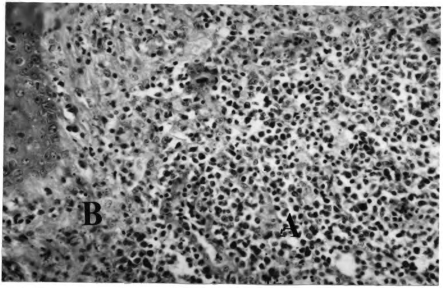


Figure 11. Lymphoproliferation (A) and degeneration (B) in the skin of a turkey 60 days after inoculation with GA-MDV at day 1. H&E stain, X48.

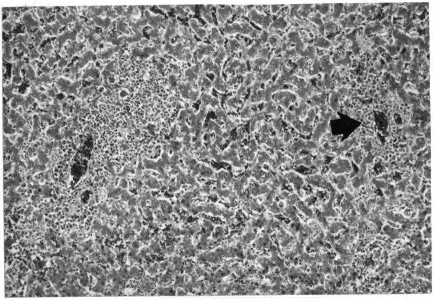


Figure 12. Perivascular cuffing (arrow) of blood vessels in the liver of a turkey 31 days after inoculation with GA-MDV at day 1. H&E stain, X48.

edema. Changes in the bursa also included lytic and inflammatory changes with proliferation of interfollicular connective tissue Figures 13, 14, 15 and 16). The bursal follicles, however, were depleted of lymphocytes. The most frequent lesion observed in the thymus was cortical regression (Figures 17 and 18). The spleen had lymphoproliferation (Figure 19) and cellular necrosis. The livers of many turkeys had inflammatory, degnerative and necrotic changes, including cellular vacuolation, focal necrosis, and perivascular cuffing with lymphocytes (Figure 20). The main types of lesions in peripheral nerves in turkeys were similar to those described for chickens. These included inflammatory lesions with edema (Figure 21) as well as invasion with neoplastic cells (Figures 22 and 23). Unlike turkeys, in which the spleen was the organ most frequently infiltrated with neoplastic cells, chickens had nerves, liver and gonads as organs most frequently involved by the neoplastic lymphocytic cell invasions.

Body Weights

The average body weights for the 4 treatment groups (i.e., 2 chicken and 2 turkey groups), inoculated with either GA-MDV or tumor homogenate, were plotted as fractions of the weights of uninoculated control groups, which was set at a value of one (Figure 24). Analysis of variance revealed a significant effect of time ($p<0.0001$) and infection (0.01), but not of the interaction between them in turkeys, and of time ($p<0.0001$), infection ($p<0.0001$) and their interaction in chickens ($p<0.0001$). The weights of the turkeys infected with GA-MDV were significantly depressed at week 4 ($p<0.05$), and those infected with tumor homogenate were significantly depressed at week 4

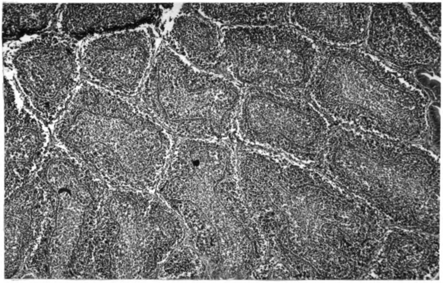


Figure 13. Normal bursa of uninoculated control turkey at day 60. H&E stain, X48.

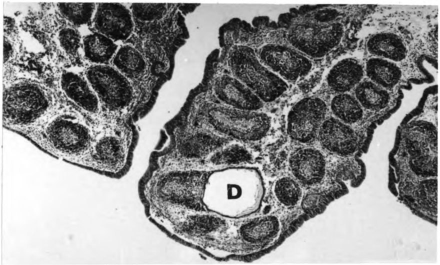


Figure 14. Proliferation of interfollicular connective tissue (C) and cystic degeneration (D) in the bursa of a turkey 60 days after inoculation with GA-MDV at day 1. Compare with Figure 13. H&E stain, X48.

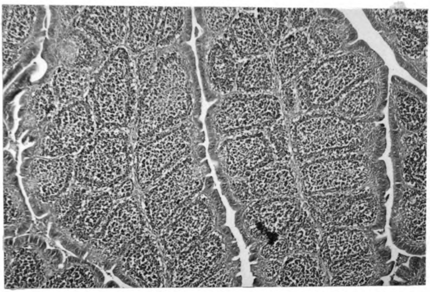


Figure 15. Normal bursa of uninoculated control turkey at day 10. H&E stain, X48.

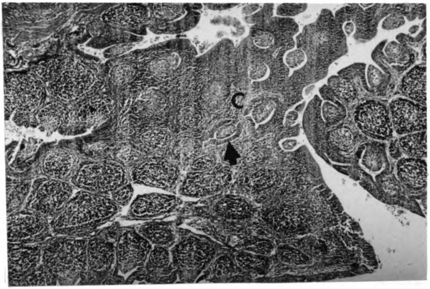


Figure 16. Atrophy and epithelial cell hyperplasia (arrow) in the bursal follicles and interfollicular connective tissue (C) proliferation in the bursa of a turkey (center) 10 days after inoculation with tumor homogenate at day 1. Compare with Figure 15. H&E stain, X48.

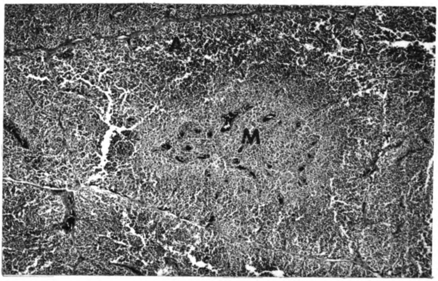


Figure 17. Normal thymus of uninoculated control turkey at day 60. Notice distinction between cortex (A) and medulla (M). H&E stain, X48.

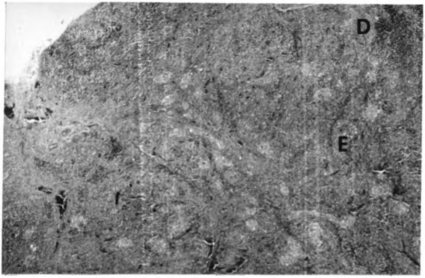


Figure 18. Cortical regression of the thymus of a turkey 60 days after inoculation with tumor homogenate at day 1. Note depletion of lymphocytes in cortex (D) and absence of distinction between cortex (D) and medulla (E). H&E stain, X48.

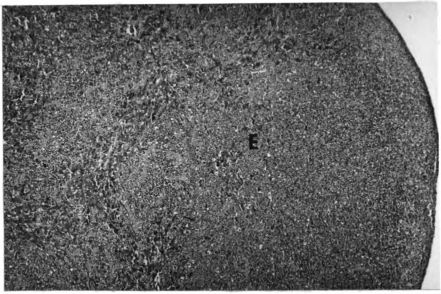


Figure 19. Lymphoproliferation (E) in the spleen of a turkey 60 days after inoculation with tumor homogenate at day 1. Note large focus (center and left). H&E stain, X480.

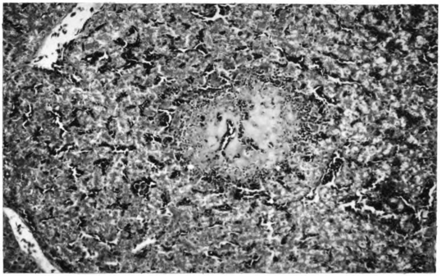


Figure 20. Focal necrosis (center) in the liver of a turkey 10 days after inoculation with tumor homogenate. H&E stain, X300.

Figure 21. Inflammatory lesion and edema in a nerve of a turkey 60 days after inoculation with GA-MDV at day 1. Notice interneural edema (F) and lymphoid cell infiltration (arrow). H&E stain, X120.

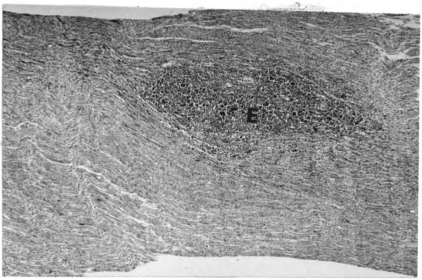


Figure 22. Lymphoproliferative infiltration (E) of nerve of a turkey 60 days after inoculation with GA-MDV at day 1. H&E stain, X48.

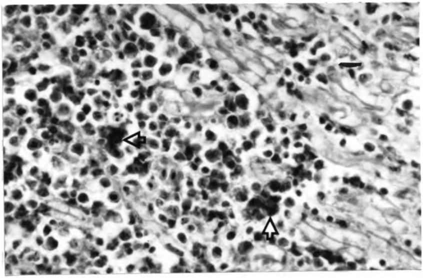


Figure 23. Neoplastic lymphoid cells in nerve of a turkey 41 days after inoculation with tumor homogenate at day 1. Notice many cells are in mitosis (arrows). H&E stain, X480.

Figure 24. The effect of GA isolate of MDV on body weights of chickens and turkeys. Each point represents an average of 4 birds. CV = chickens inoculated with GA-MDV, CT = chickens inoculated with tumor homogenate, TV = turkeys inoculated with GA-MDV, TT = turkeys inoculated with tumor homogenate. In CV and TV, each bird received 4.53×10^5 PFU of GA-MDV, and in CT and TT, 7.4×10^6 cells containing 8.25×10^4 PFU of MDV intraperitoneally at day 1. Weights of infected chickens and turkeys were significantly different from those of uninoculated controls at the following intervals: CV = weeks 2 and 5 ($p<0.01$) week 6 ($p<0.05$), CT = weeks 2, 4, 5 ($p<0.05$) and 6 ($p<0.01$), TV = week 4 ($p<0.05$), TT = week 4 ($p<0.01$).

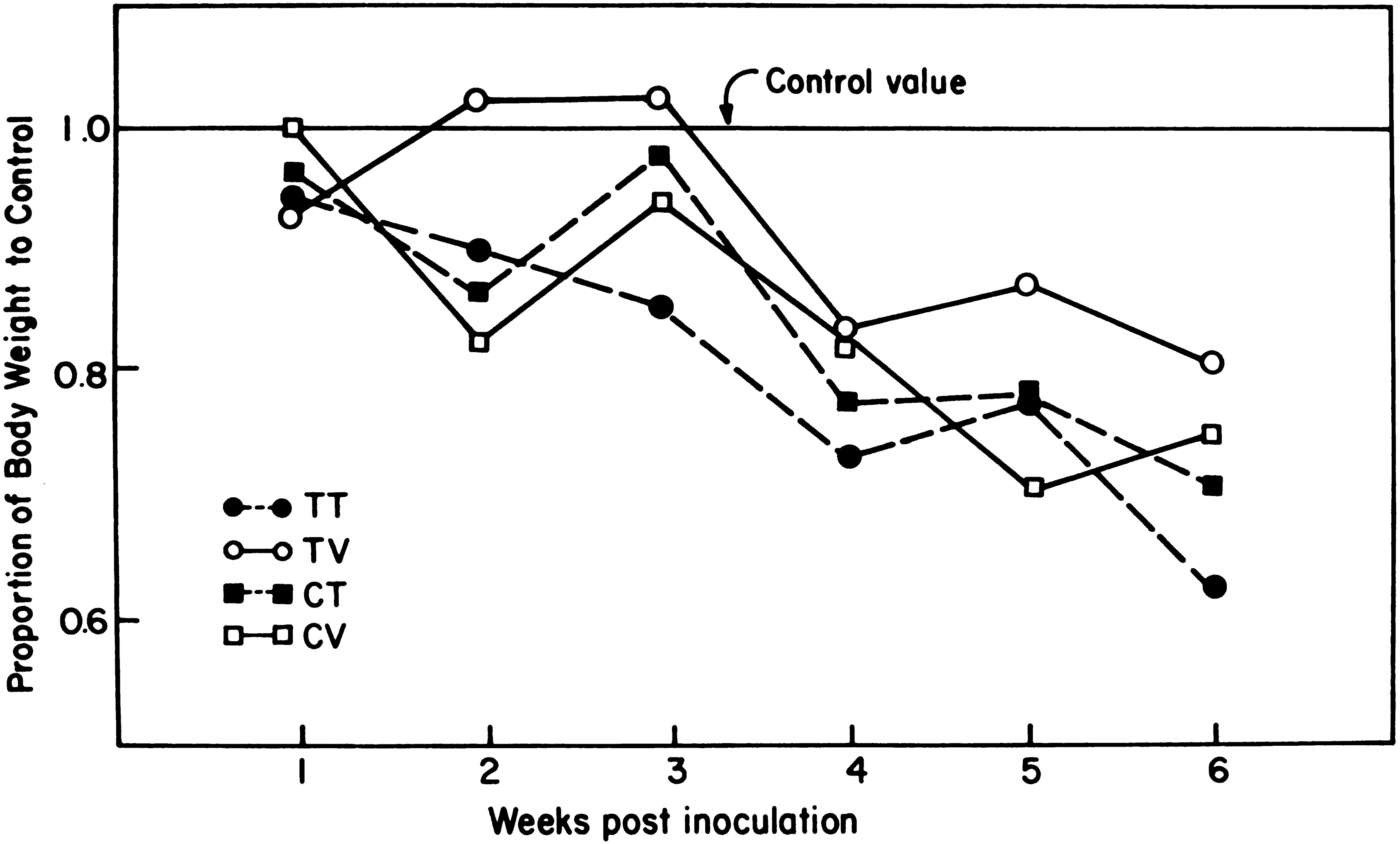

Control value
1.0
0.8
0.6
Proportion of Body Weight to Control
TT
TV
CT
CV
1
2
3
4
5
6
Weeks post inoculation

Figure 24

($p<0.01$) and week 5 ($p<0.01$). The weights of chickens infected with GA-MDV had significant depression at weeks 2, 5 ($p<0.01$) and 6 ($p<0.05$), while those infected with tumor homogenate had significant depression at weeks 2, 4 and 5 ($p<0.05$) and week 6 ($p<0.01$). The difference between the weights of the turkeys inoculated with GA-MDV and those inoculated with tumor homogenate was significant at week 2 ($p<0.05$). In both species, the decrease in weight due to the 2 inocula became more pronounced with age.

Organ Weights

Organ weights were calculated as mg/g of body weight and plotted in Figures 25, 26 and 27 as proportions of control values. The controls were set at a value of one.

Bursa. The bursas of chickens and turkeys 2 weeks after infection were smaller than those of controls of the same age. Analysis of variance of bursa to body weight ratios in turkeys revealed a significant effect of time ($p<0.05$) but of neither infection nor the interaction between time and infection. However, in chickens there was a significant effect of time ($p<0.05$) and infection ($p<0.0001$) but not of their interaction. The bursa to body weight ratios were significantly decreased compared to controls only at week 6 ($p<0.05$) in chickens infected with tumor homogenate. Moreover, there was a significant difference ($p<0.05$) between the bursa to body weight ratios of the 2 infected groups of chickens, the tumor homogenate inducing more depression than GA-MDV. The bursal weights were significantly decresaed at week 2 ($p<0.05$) and week 6 ($p<0.01$) in the group infected with GA-MDV and at weeks 2, 5 and 6 ($p<0.05$) and week 3 ($p<0.01$) in tumor homogenate-infected birds. The 2 inocula induced more depression

Figure 25. Effect of GA isolate of MDV on bursal weights of chickens and turkeys, shown as proportions of bursal weights to controls. Each point represents an average of 4 birds. CV = chickens inoculated with GA-MDV, CT = chickens inoculated with tumor homogenate, TV = turkeys inoculated with GA-MDV, TT = turkeys inoculated with tumor homogenate. In CV and TV, each bird received 4.53 x 105 of GA-MDV and in CT and TT 7.4 x 10^6 cells containing 8.25 x 10^4 PFU of MDV IP at day 1. Bursa to body weight ratios of infected chickens were significantly different from those of uninoculated controls at the following interval: CT = week 6 ($p<0.05$).

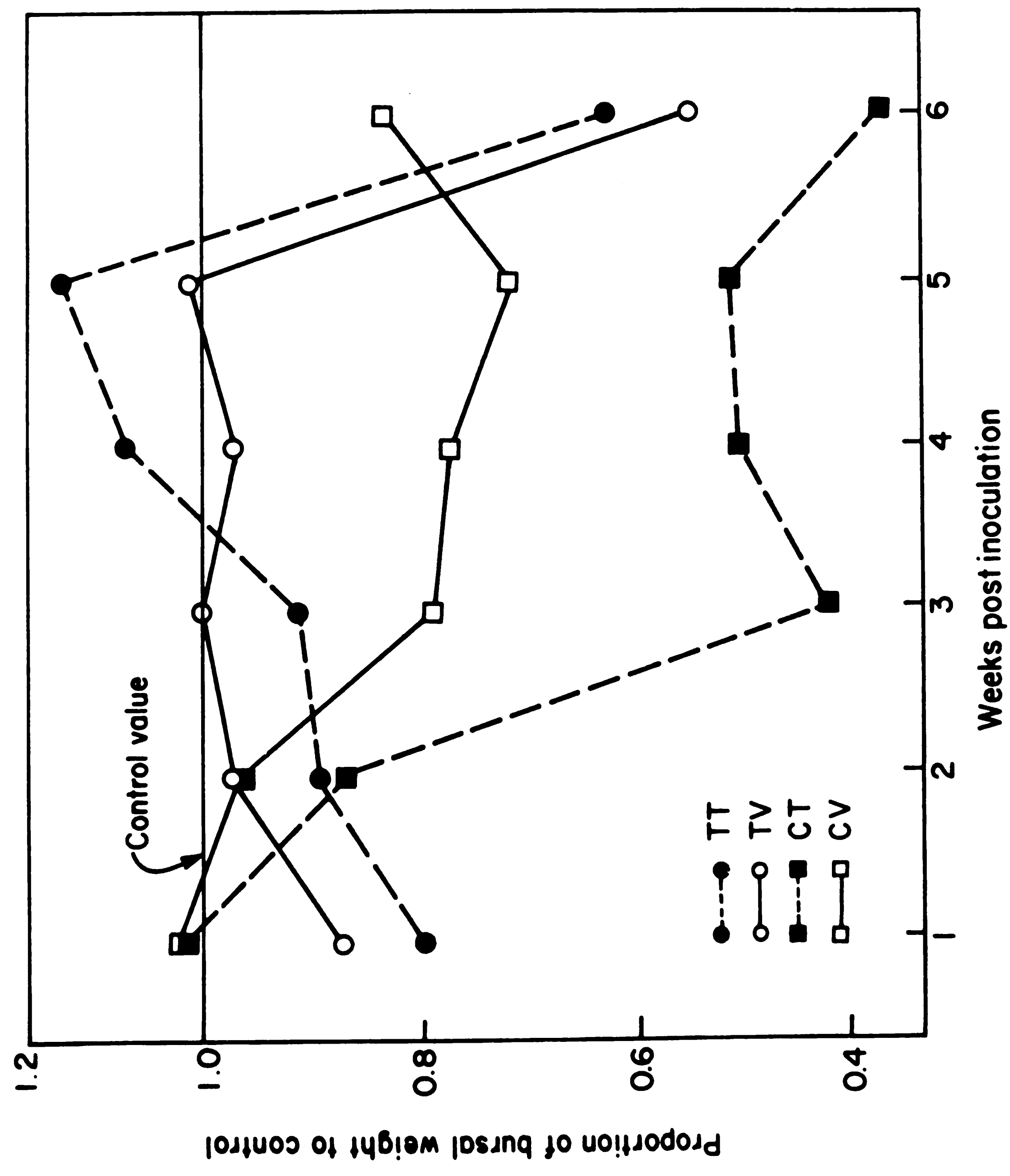

Proportion of bursal weight to control
1.2
1.0
0.8
0.6
0.4
Control value
TT
TV
CT
CV
1
2
3
4
5
6
Weeks post inoculation

Figure 25

in bursal weights in chickens than in turkeys during the 6-week observation period, but the difference was significant at weeks 3 and 6 only ($p<0.05$) (Figure 25).

Thymus. Analysis of variance showed a significant effect of time ($p<0.001$) but not of infection or interaction between infection and time in turkeys compared to controls. A significant effect of time ($p<0.0001$) and infection ($p<0.001$) but not their interaction was detected in chickens. No significant effect on thymus to body weight ratios was detected in the 2 infected groups of turkeys. Analysis of variance of thymus to body weight ratios in chickens revealed a significant effect at weeks 2 and 5 ($p<0.01$) in the group infected with GA-MDV and at weeks 5 and 6 ($p<0.05$) in the tumor homogenate-infected group. A significant difference was detected between the 2 infected groups at week 2 ($p<0.05$). The tumor homogenate induced a significant decrease in thymic weights of turkeys at week 2 ($p<0.05$) and week 4 ($p<0.01$). The difference in thymic weights between the 2 groups of infected turkeys was significant at week 2 ($p<0.05$) and week 4 ($p<0.01$). In the GA-MDV infected chickens, the significant difference in thymic weights was detected at weeks 2 and 5 ($p<0.01$). In the tumor homogenate-infected group, significant differences occurred at week 2 ($p<0.05$) and weeks 5 and 6 ($p<0.01$) (Figure 26).

Spleen. Spleens of all infected chickens and turkeys appeared larger than those of controls. Analysis of variance showed a significant effect of time ($p<0.0001$) and infection ($p<0.0001$) but not their interaction in chickens and turkeys. The mean spleen to body weight ratios were significantly increased at week 1 ($p<0.01$) and week 4

Figure 26. The effect of GA isolate of MDV on thymic weights of chickens and turkeys shown as proportions of thymic weights to controls. Each point represents an average of 4 birds. CV = chickens inoculated with GA-MDV, CT = chickens inoculated with tumor homogenate, TV = turkeys inoculated with GA-MDV, TT = turkeys inoculated with tumor homogenate. In CV and TV, each bird received 4.53×10^5 PFU of GA-MDV and in CT and TT 7.4×10^6 cells containing 8.25×10^4 PFU of MDV IP at day 1. Thymus to body weight ratios of infected chickens were significantly different from those of uninoculated controls at the following intervals: CV = weeks 2 and 5 ($p<0.01$), CT = weeks 5 and 6 ($p<0.05$).

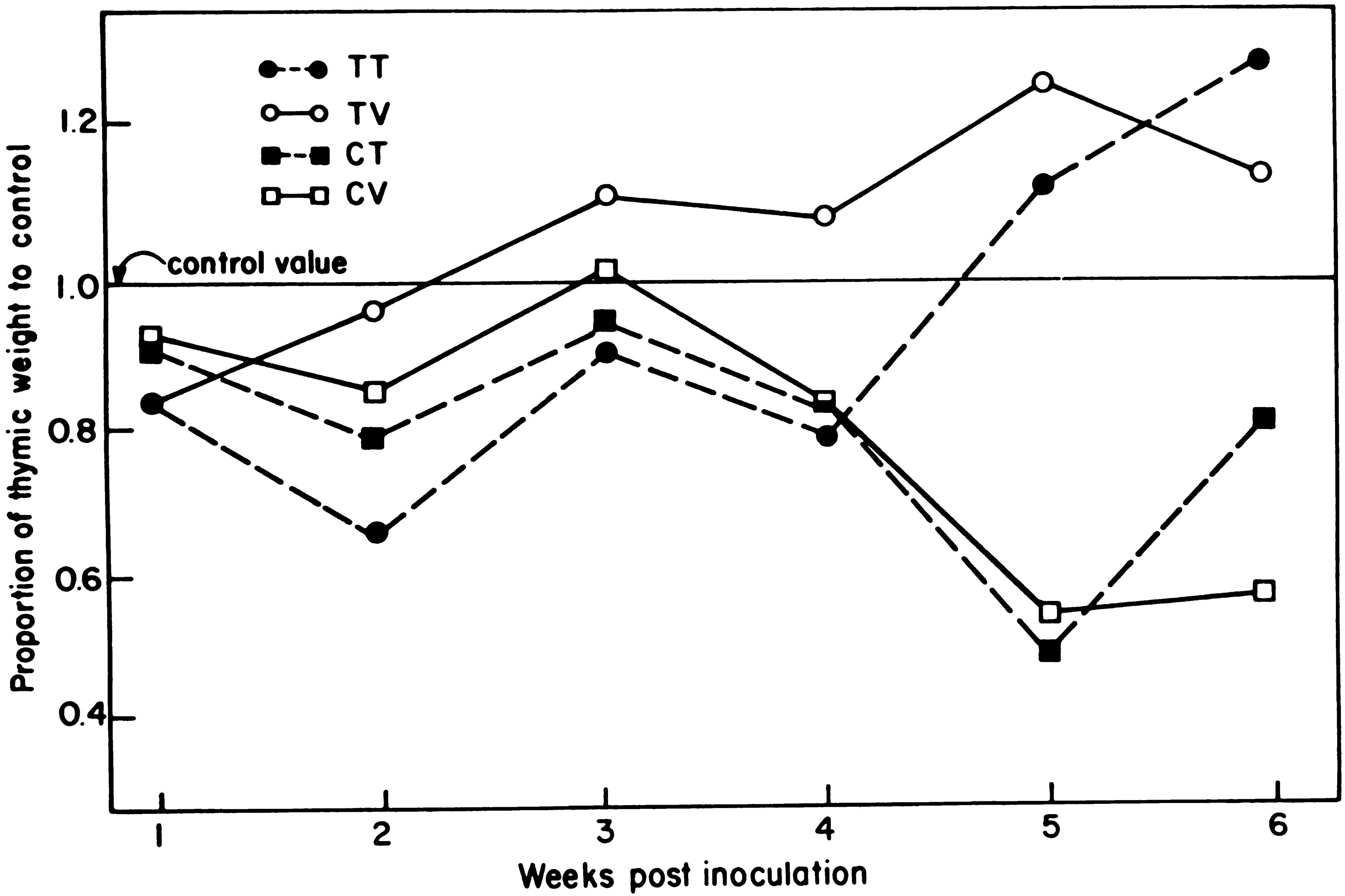

TT
TV
CT
CV
control value
1.2
1.0
0.8
0.6
0.4
Proportion of thymic weight to control
1
2
3
4
5
6
Weeks post inoculation

Figure 26

($p<0.05$) in turkeys infected with GA-MDV and at weeks 1, 2 and 5 ($p<0.05$) and week 3 ($p<0.01$) in tumor homogenate-inoculated birds. In chickens, a significant difference was detected at weeks 1, 2 and 4 ($p<0.01$) and weeks 3 and 5 ($p<0.05$) in the group infected with GA-MDV and at week 2 ($p<0.01$) and week 4 ($p<0.05$) in tumor homogenate-infected birds. The difference between the 2 infected groups of chickens was significant at week 1 ($p<0.01$) and week 2 ($p<0.05$). The weights were significantly increased only at week 1 ($p<0.01$) in turkeys infected with GA-MDV and at week 3 ($p<0.05$) in tumor homogenate-infected turkeys. In chickens, the significant reduction in the GA-MDV infected birds was found at weeks 1, 2 and 3 ($p<0.01$), and the differences between the 2 infected groups of chickens were significant at week 1 ($p<0.05$). The increase in the spleen to body weight ratios of the 2 infected groups of chickens was relatively constant throughout the observation period, while in turkeys these ratios decreased below that of chickens in weeks 2 and 3 and rapidly increased to more than that of chickens therafter. GA-MDV induced a greater increase in spleen to body weight ratios in chickens and a lesser increase in spleen to body weight ratios in turkeys after week 1 than did the tumor homogenate (Figure 27).

Antibody Response

Evaluations of humoral immune response are shown in Table 5. The primary response to SE was significantly decreased ($p<0.05$) in chickens injected with the 2 inocula at week 3. In turkeys, the primary response was significantly increased ($p<0.01$) in the group infected with GA-MDV, while the response was significantly decreased ($p<0.01$) in the group given injections of tumor homogenate. The

Figure 27. Effect of GA isolate of MDV on splenic weights of chickens and turkeys shown as proportions of splenic weights to controls. Each point represents an average of 4 birds. CV = chickens inoculated with GA-MDV, CT = chickens inoculated with tumor homogenate, TV = turkeys inoculated with GA-MDV, TT = turkeys inoculated with tumor homogenate. In CV and TV each bird received 4.53×10^5 PFU of GA-MDV and in CT and TT 7.4×10^6 cells containing 8.25×10^4 PFU of MD IP at day 1. Spleen to body weight ratios of infected chickens and turkeys were significantly heavier than uninoculated controls at the following intervals: CV = weeks 1, 2 and 4 ($p<0.01$) and weeks 3 and 5 ($p<0.05$), CT = week 2 ($p<0.01$) and week 4 ($p<0.05$), TV = week 1 ($p<0.01$) and week 4 ($p<0.05$), TT = weeks 1, 2 and 5 ($p<0.05$) and week 3 ($p<0.01$).

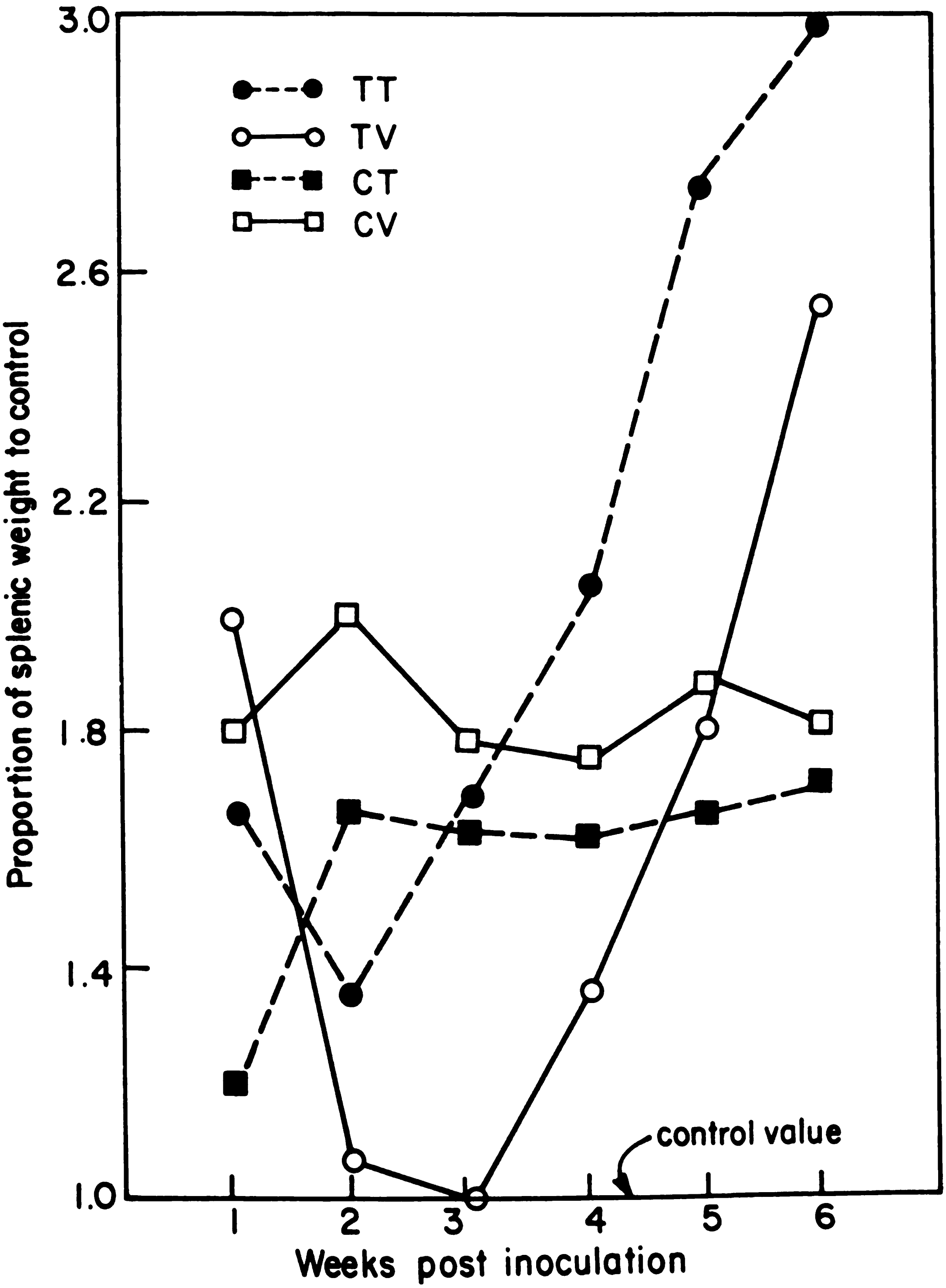

3.0
2.6
2.2
1.8
1.4
1.0
TT
TV
CT
CV
Proportion of splenic weight to control
control value
1
2
3
4
5
6
Weeks post inoculation

Figure 27

Table 5. Humoral antibody response of chickens and turkeys infected with GA isolate of MDV to sheep erythrocytes (SE) and *Brucella abortus* antigens

Test	Animal[a]	Inoculum	Week Post-inoculation	Antibody to SE[c] (hemagglutination test) (mean $\log_2$ titer ± SD) Primary Response	Secondary Response	Antibody to *B. abortus*[c] (plate agglutination test) (mean $\log_2$ titer ± SD) Primary Response	Secondary Response
1	Chickens	GA-MDV[d]	3	0.2 ± 0.4**	2.5 ± 0.5	2.0 ± 1.5*[b]	2.8 ± 0.5**
		tumor homogenate[e]		0.2 ± 0.4*	1.5 ± 1.0	1.5 ± 1.3	1.3 ± 1.0**
		none		1.7 ± 0.8	3.0 ± 0.6	3.2 ± 0.7	4.2 ± 0.8
	Turkeys	GA-MDV[d]		8.4 ± 1.1**	9.5 ± 0.6*	3.8 ± 2.4	6.4 ± 1.3**
		tumor homogenate[e]		3.0 ± 1.7**	4.5 ± 1.9*	2.0 ± 1.6	2.8 ± 1.9**
		none		6.2 ± 1.0	8.3 ± 0.8	3.3 ± 1.8	5.0 ± 1.1
2	Chickens	GA-MDV[d]	7	4.7 ± 1.5*†	4.5 ± 0.7†	2.0 ± 1.6	2.8 ± 1.9**
		tumor homogenate[e]		3.2 ± 2.7*	3.5 ± 2.1†	1.8 ± 1.7*	2.0 ± 0.0*†
		none		8.3 ± 3.0	4.5 ± 1.3	5.0 ± 0.8	4.5 ± 0.5
	Turkeys	GA-MDV[d]		5.3 ± 1.7*	5.5 ± 2.1†	4.3 ± 2.3*	3.5 ± 0.7†
		tumor homogenate[e]		5.0 ± 7.1*[b]	3.0 ± 0.0†	6.0 ± 2.8*†	2.0 ± 0.0†
		none		10.8 ± 2.0	7.0 ± 2.7	7.7 ± 1.0	4.8 ± 0.8

[a] Four to six animals examined in each group except "†" less than 4.
[b] Serum samples were tested for at week 4 (primary response) and at week 5 (secondary response).
[c] All birds were inoculated IV with SE and *B. abortus* (2.5×10^9 cells of each) at weeks 3 and 4 (test 1) and weeks 7 and 8 (test 2) after infection at day 1.
[d] Inoculum consisted of 4.53×10^5 PFU/1-day-old bird and given IP.
[e] Inoculum consisted of 7.4×10^6 cells with 8.2×10^4 PFU of MDV/1-day-old bird and given IP.
* ($p<0.05$)
** ($p<0.01$)

secondary response to SE was also significantly increased ($p<0.05$) and decreased ($p<0.05$), respectively, in turkeys given injections of GA-MDV or tumor homogenate. On the other hand, no significant difference in the secondary response to SE was detected between the groups of chickens. The primary response to *B. abortus* was significantly decreased ($p<0.05$) only in chickens inoculated with GA-MDV, while the secondary response was significantly decreased ($p<0.01$) in chickens after both treatments and significantly increased and decreased ($p<0.01$), respectively, in turkeys infected with GA-MDV and tumor homogenate.

At week 7, the primary responses to SE and *B. abortus* were significantly decreased ($p<0.05$) in both species after both treatments. The significant depression ($p<0.05$) in the secondary response to *B. abortus* was detected only in the 2 infected groups of chickens. Thus, the data suggest that the humoral antibody response was suppressed in chickens infected with GA-MDV or with tumor homogenate. In the turkeys inoculated with GA-MDV, the antibody response was also suppressed, although initially there was transient enhancement of the response.

Con A Stimulation of Whole-Blood Lymphocytes

The stimulation of blood T-cells by Con A was tested to measure the effect of the virus infection on cellular immune competence. The stimulation indices (counts per minute of ^{125}IUdR incorporation in cells with Con A divided by counts per minute in cells without Con A) were significantly decreased ($p<0.05$) at 52 days in chickens infected with tumor homogenate and in turkeys at 24 days after infection with GA-MDV or with tumor homogenate ($p<0.01$) (Table 6).

Table 6. Con A stimulation of whole-blood lymphocytes of chickens and turkeys inoculated with GA isolate of MDV

Animal[a]	Inoculum	Mean Stimulation Index ± SD[b] (days after inoculation) 24	42	52
Chickens	GA-MDV[c]	144.6 ± 145.4	70.5 ± 40.3	not done
	tumor homogenate[d]	234.7 ± 79.4	55.2 ± 25.6	74.7 ± 54.1*
	none	148.2 ± 79.4	45.8 ± 31.9	121.3 ± 29.0
Turkeys	GA-MDV[c]	29.6 ± 19.8**	12.0 ± 16.4	18.0 ± 14.4
	tumor homogenate[d]	26.4 ± 33.2**	13.0 ± 12.5	not done
	none	77.4 ± 19.8	6.3 ± 3.6	10.3 ± 10.0

[a]Eight animals per group were examined at each interval.

[b]Counts per minute of ^{125}IUdR incorporation in cells with Con A divided by counts per minute in cells without Con A.

[c]Inoculum consisted of 4.53×10^5 PFU/1-day-old bird and given IP.

[d]Inoculum consisted of 7.4×10^6 cells with 8.2×10^4 PFU of MDV/1-day-old bird and given IP.

*(p<0.05)

**(p<0.01)

Observations at 42 and 52 days after inoculation revealed no depression in Con A response.

Experiment II
Response of Chickens and Turkeys to HVT

Titration of Virus Present in the Blood

Chickens and turkeys infected with HVT (FC126) remained viremic throughout the entire observation period of 8 weeks (Table 7). At week 1 the virus titer was considerably higher in turkeys than in chickens. Subsequently, titers in both species dropped to comparable low levels. Uninoculated controls were not viremic.

Pathology

Gross lesions. Turkey herpesvirus (FC126) induced no detectable gross lesions or mortality in turkeys or chickens.

Histopathology. Microscopic examination of liver, kidney, gonad, heart, skin, bursa, thymus, and nerves revealed mild lymphoproliferative lesions in some chickens and turkeys. Lesions suggestive of neoplastic transformation were detected only in chickens. Lymphoproliferative lesions were first detected 1 week after inoculation and seemed to become somewhat more frequent over the next 5 to 6 weeks. However, the lesions diminished to negligible levels at week 8 (Table 8). The average number of organs with mild lymphoproliferative lesions throughout the examination period was slightly more in chickens than in turkeys, except at the third week (Table 8). The frequency of these lesions in different organs is shown in Table 9. The liver was most frequently involved in chickens, whereas the spleen was most frequently involved in turkeys. The bursa and kidneys were

Table 7. Titration of virus present in blood from chickens and turkeys inoculated with HVT (FC126)[a]

Weeks Post-inoculation	HVT Titer (mean PFU/10^7 WBC)[b]	
	Chickens	Turkeys
1	81 (4/4)[c]	182 (3/3)
2	33 (4/4)	6 (3/4)
3	17 (4/4)	28 (4/4)
4	15 (4/4)	12 (3/3)
5	57 (4/4)	9 (3/4)
6	11 (4/4)	12 (4/4)
7	13 (4/4)	5 (2/3)
8	25 (4/4)	14 (4/4)

[a]Inoculum consisted of 8.2 x 10^4 PFU/1-day-old bird and given IP.

[b]Three to four animals per group were examined at each interval. A similar number of uninoculated controls examined at each interval lacked detectable virus.

[c]Number viremic/total examined.

Table 8. Microscopic lymphoproliferative foci in chickens and turkeys inoculated with HVT (FC126)[a]

Weeks Post-inoculation	Average No. of Organs With Lesions[b]		Mean Lesion Score[c]	
	Chickens[d]	Turkeys[d]	Chickens	Turkeys
1	1.25	0.25	0.28	0.03
2	1.50	1.00	0.38	0.15
3	1.25	2.25	0.40	0.40
4	0.75	0.50	0.15	0.03
5	2.00	1.50	0.48	0.25
6	1.50	1.25	0.45	0.43
7	1.50	1.25	0.30	0.20
8	0.00	0.00	0.00	0.00

[a]Inoculum consisted of 8.2×10^4 PFU/1-day-old bird and given IP.

[b]Based on examination of liver, spleen, kidney, gonad, heart, skin, bursa, thymus and nerves (brachial and sciatic plexuses and vagus nerve).

[c]Scores are means (scale 0 to 4) of individual sections.

[d]Four birds were examined at each interval. A similar number of uninoculated controls examined at each interval lacked lesions.

Table 9. Frequency of microscopic lymphoproliferative foci in different organs of chickens and turkeys inoculated with HVT (FC126)[a]

Tissues Examined[b]	Animal	
	Chickens	Turkeys
Liver	22[c]	7[c]
Spleen	5	14
Kidneys	0	9
Gonads	7	7
Heart	2	3
Skin	7	5
Bursa	0	6
Thymus	1	1
Nerve	4	0

[a]Inoculum consisted of 8.2×10^4 PFU/1-day-old bird and given IP.

[b]Nine tissues from each of 32 birds were examined per group (4 birds per group each week for 8 weeks). A similar number of uninoculated controls examined lacked detectable lesions. Data pooled.

[c]Number of animals with lesions.

not involved in chickens. The nerves were not involved in turkeys but were involved in chickens. Uninoculated controls examined lacked detectable lesions.

The microscopic lesions in HVT-inoculated turkeys consisted of small focal lymphoid reactions in the liver, kidney, gonad, heart, and skin. Most of these contained mature lymphocytes, but a few were reactive foci with immature cells, none of which seemed to be neoplastically transformed. An increased number of bursa-dependent follicles was seen in the spleen at weeks 2 through 7. The changes in the bursa were detected during the first 3 weeks, with 50% of the turkeys having bursas with small follicles. A few bursas had proliferation of interfollicular tissue (Figures 28 and 29). Only one thymus had cortical regression (Figure 30).

In the HVT-inoculated chickens, the changes seen were similar to those in turkeys, but no bursal changes were detected. In addition, some of the lymphoid foci had immature cells suggestive of neoplastically transformed cells (Figures 31 and 32). Also, the nerves, which were not involved in turkeys, were infiltrated with lymphoid cells (Figure 33). The changes in the liver in the first 2 weeks included vacuolation around the lymphoid foci (Figure 34). The bursa-dependent follicles were seen in the spleen and, to a lesser extent, in the kidneys. In one instance, lymphoid cell proliferation accompanied by inflammation and necrosis was seen in the heart.

Body Weights

The average body weights of chickens and turkeys infected with HVT (FC126) are shown as a proportion of the uninoculated control

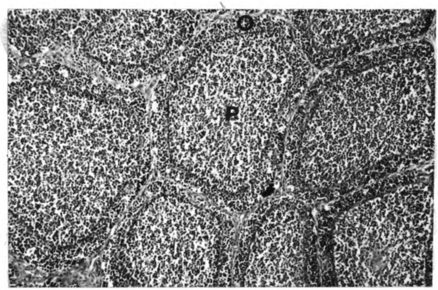


Figure 28. Normal bursa of uninoculated control turkey at day 14. Note distinct cortex (O) and medulla (P). H&E stain, X120.

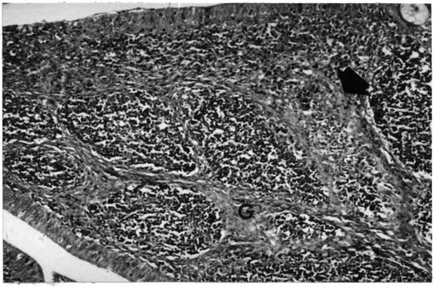


Figure 29. Necrosis and atrophy (arrow) in bursal follicles and proliferation of interfollicular connective tissue (G) in a turkey 14 days after inoculation with HVT (FC126) at day 1. H&E stain, X120.

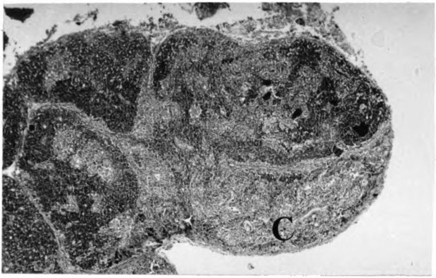


Figure 30. Depletion of cortical lymphocytes in the thymus of a turkey 21 days after inoculation with HVT (FC126) at day 1 (C). Notice less affected lobule with distinct cortex and medulla (left). H&E stain, X48.

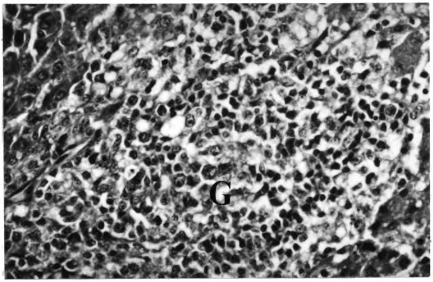


Figure 31. A focus of immature reticular-type cells (G) in the liver of a chicken 7 days after inoculation with HVT (FC126) at day 1. H&E stain, X300.

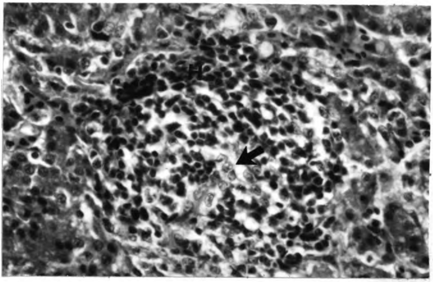


Figure 32. A regressing focus of immature reticular-type cells (arrow) surrounded by infiltration of small lymphocytes (H) in a chicken 21 days after inoculation with HVT (FC126). H&E stain, X480.

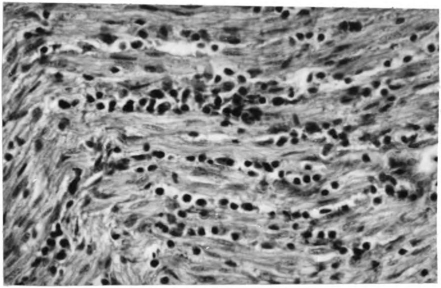


Figure 33. Infiltration of lymphoid cells in the nerve of a chicken 21 days after inoculation with HVT (FC126) at day 1. H&E stain, X300.

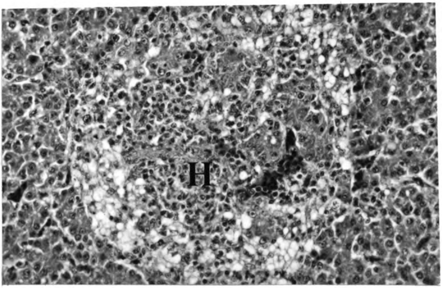


Figure 34. Vacuolation (arrow) around a focus of lymphoreticular cells (H) in the liver of a chicken 7 days after inoculation with HVT (FC126) at day 1. H&E stain, X480.

(Figure 35). The control was set at a value of one. Analysis of variance revealed a significant effect of time ($p<0.0001$) and infection ($p<0.05$) but not of the interaction between them in turkeys. In chickens time was significantly different ($p<0.0001$) but not infection or the interaction between them. The weights of the inoculated turkeys were significantly lower than the uninoculated controls at weeks 1 and 2 ($p<0.05$) and week 5 ($p<0.01$).

Organ Weights

Organ weights were calculated as mg/g body weight and then plotted in Figures 36 and 37 as proportions of controls. The controls were set at a value of one.

Bursa. Analysis of variance of turkey and chicken bursa to body weight ratios revealed a significant effect of time ($p<0.0001$) but not of infection or the interaction between them. The bursa to body weight ratios of inoculated turkeys were significantly larger than those of uninoculated controls at week 7 ($p<0.05$). No significant differences were detected between groups of chickens when the ratios calculated for each week were analyzed.

Thymus. Analysis of variance of turkey thymus to body weight ratios revealed a significant effect of time ($p<0.0001$) but not of infection or the interaction between them. Thymus to body weight ratios in chickens revealed no significant effect of time, but there was a significant effect of infection ($p<0.05$) and of the interaction between them ($p<0.01$). Weekly analyses of these ratios revealed no significant differences between inoculated and uninoculated turkeys. However, there was a significant decrease in the thymic weights of

Figure 35. Effect of HVT (FC126) infection on the body weight of chickens and turkeys. Each point represents an average of 4 birds. Each bird received 8.2 x 10^4 PFU/1-day-old bird (IP). Weights of infected turkeys were significantly lower than controls at weeks 1 and 2 ($p<0.05$) and week 5 ($0<0.01$).

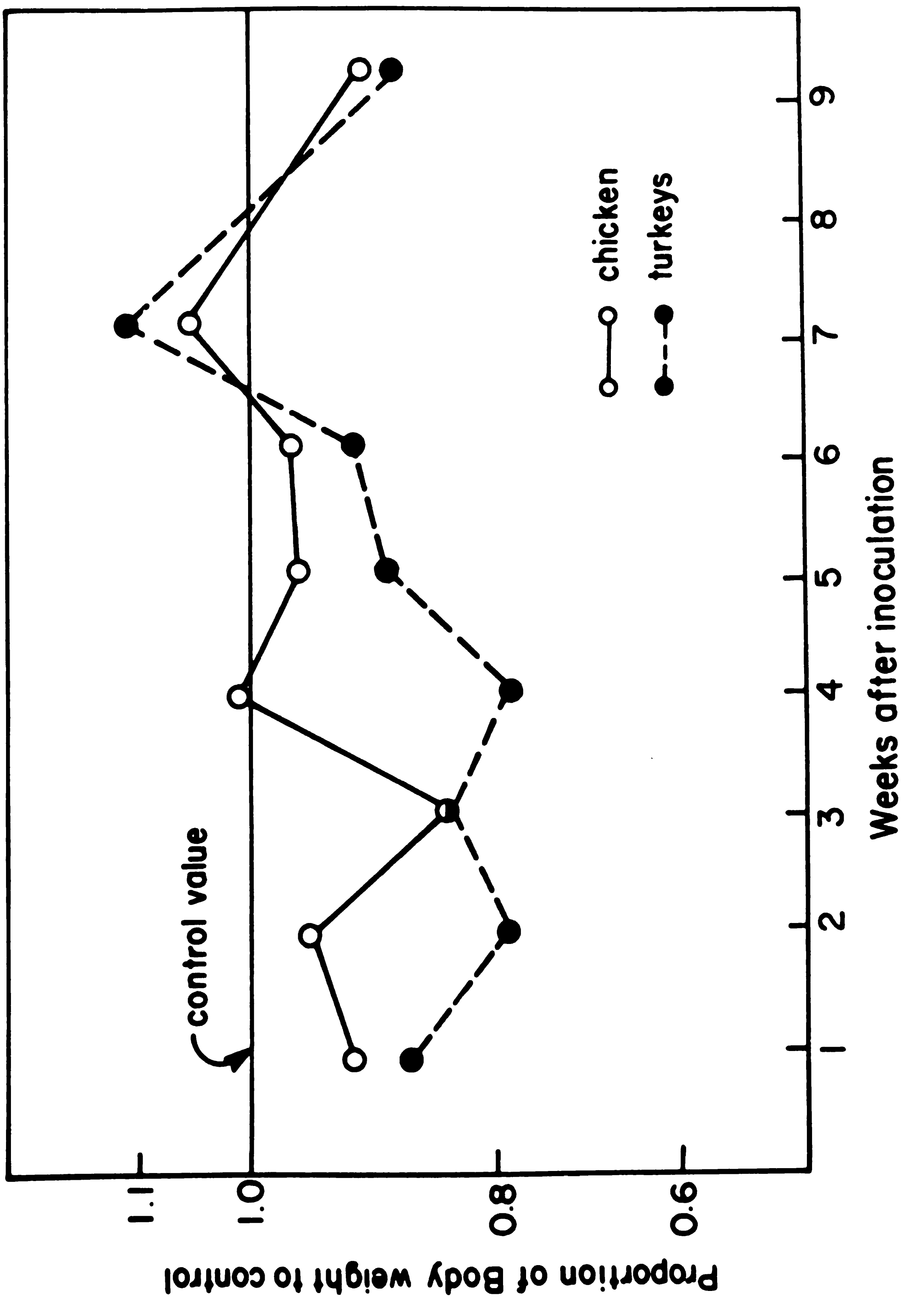

control value
chicken
turkeys
Weeks after inoculation
Proportion of Body weight to control
1.1
1.0
0.8
0.6
1
2
3
4
5
6
7
8
9

Figure 35

Figure 36. Effect of HVT (FC126) infection on bursal, thymic and splenic weights of turkeys, shown as proportions of organ weights to controls. Each point represents an average of 4 birds. One-day-old turkeys were injected IP with 8.2×10^4 PFU. Spleen to body weight ratios of infected turkeys were significantly heavier than those of controls at week 1 ($p<0.05$) and week 3 ($p<0.01$). Bursa to body weight ratios of infected turkeys were heavier than those of controls at week 7 ($p<0.05$).

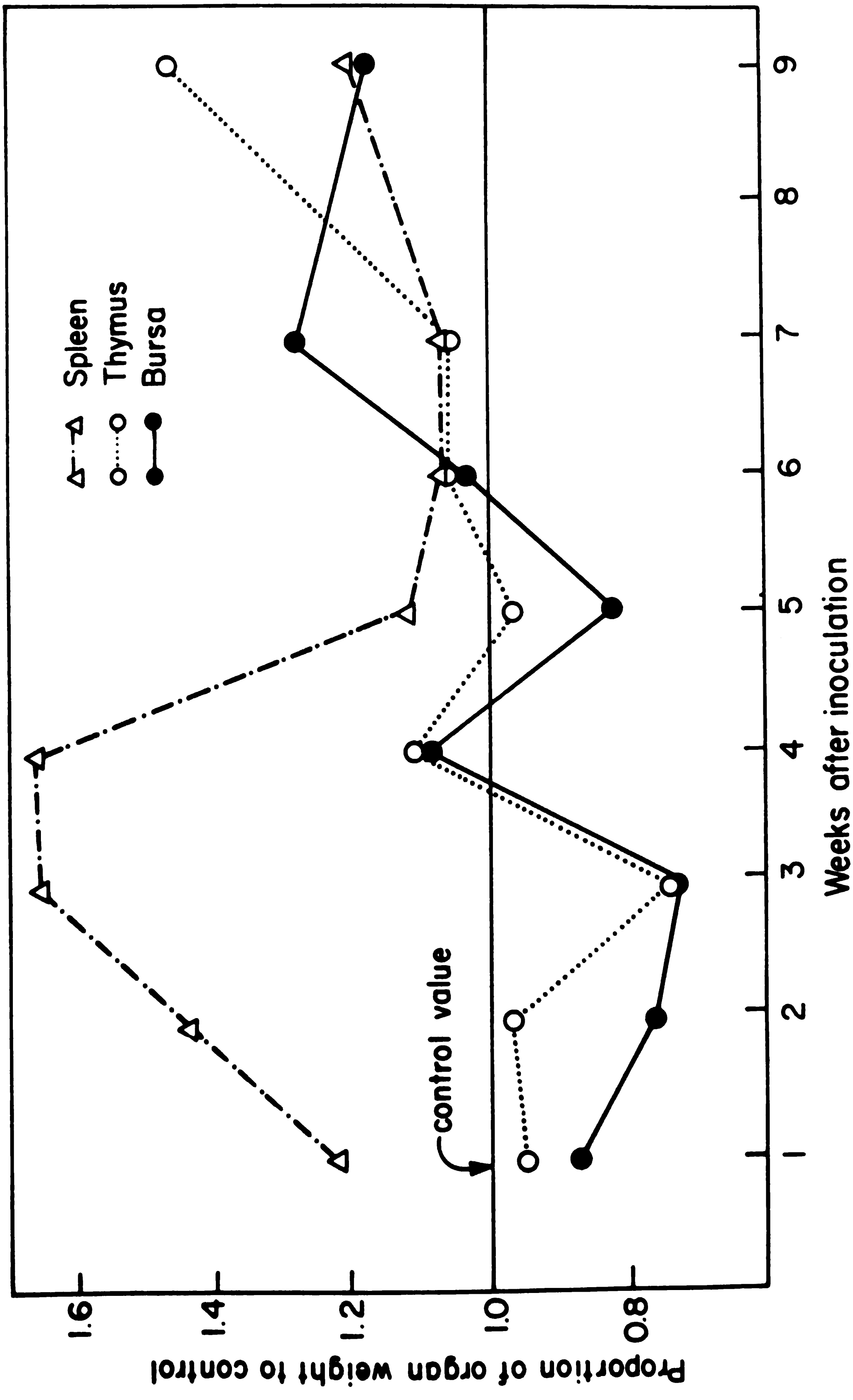

Spleen
Thymus
Bursa
control value
Proportion of organ weight to control
1.6
1.4
1.2
1.0
0.8
Weeks after inoculation
1
2
3
4
5
6
7
8
9

Figure 36

Figure 37. Effect of HVT (FC126) infection on bursal, thymic and splenic weights of chickens, shown as proportions of organ weights to controls. Each point represents an average of 4 birds. One-day-old chickens were injected IP with 8.2×10^4 PFU. Spleen to body weight ratios of infected chickens were significantly heavier than those of controls at week 1 ($p<0.01$) and weeks 6 and 7 ($p<0.05$). Thymic weight of infected chickens was lower than controls at week 1 ($p<0.05$) and heavier at week 6 ($p<0.05$) and week 7 ($p<0.01$).

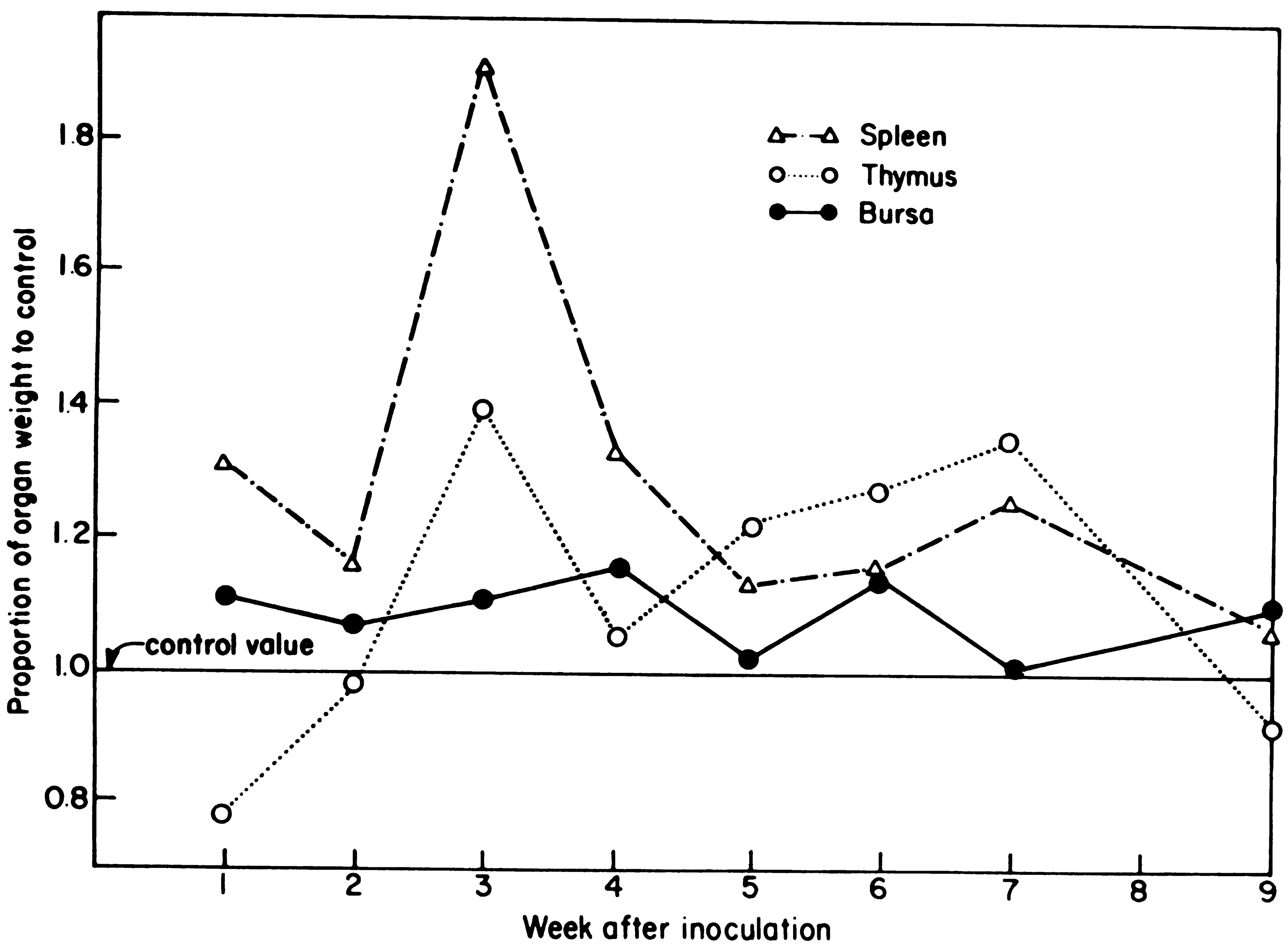

Spleen
Thymus
Bursa
Proportion of organ weight to control
1.8
1.6
1.4
1.2
1.0
0.8
control value
1
2
3
4
5
6
7
8
9
Week after inoculation

Figure 37

chickens at week 1 and a significant increase at week 6 ($p<0.05$) and week 7 ($p<0.01$).

Spleen. Analysis of variance of spleen to body weight ratios in turkeys revealed a significant effect of time ($p<0.0001$), of infection ($p<0.00001$), and of the interaction between them ($p<0.05$). In chickens there was a significant effect of time ($p<0.0001$) and of infection ($p<0.0001$) but not of the interaction between them. The spleen to body weight ratios of inoculated turkeys were significantly larger at week 1 ($p<0.05$) and week 3 ($p<0.01$) than those of uninoculated controls.

Antibody Response

The humoral response to SE and *B. abortus* was tested at week 10 after HVT inoculation. There were no appreciable differences in serum antibody concentrations in inoculated and control chickens or turkeys, except for a modest but significant reduction ($p<0.05$) in the primary antibody response to SE in inoculated chickens. Thus, HVT had no effect on the humoral antibody response of turkeys and a minimal effect in chickens (Table 10).

Con A Stimulation of Whole-Blood Lymphocytes

This *in vitro* test of cellular immunity was done at 2 and 3 weeks after HVT inoculation. There was no significant difference between the control and infected groups of chickens at both intervals. There was a significant decrease ($p<0.01$) in the stimulation index at 2 but not at 3 weeks after inoculation in turkeys. Thus, HVT had no detectable effect on this cellular immune response of chickens but caused a transient depression of this response in turkeys (Table 11).

Table 10. Humoral antibody response of chickens and turkeys infected with HVT (FC126) to SE and *Brucella abortus* antigens

		Antibody to SE[b] (hemagglutination test) (mean $\log_2$ titer ± SD)		Antibody to *B. abortus*[b] (plate agglutination test) (mean $\log_2$ titer ± SD)	
Animal[a]	Inoculum	Primary Response	Secondary Response	Primary Response	Secondary Response
Chickens	HVT[c]	3.6 ± 0.7*	4.3 ± 0.9	3.5 ± 0.8	3.2 ± 1.2
	none	4.5 ± 1.0	4.7 ± 0.5	3.3 ± 0.5	3.4 ± 0.5
Turkeys	HVT[c]	5.2 ± 1.0	6.0 ± 0.9	3.7 ± 1.9	6.0 ± 0.9
	none	5.9 ± 0.7	6.6 ± 0.5	4.1 ± 2.5	5.1 ± 1.2

[a]Six to ten animals were examined in each group.

[b]All birds were inoculated IV with SE and *B. abortus* (2.5×10^9 cells of each) at 10 and 11 weeks of age.

[c]Inoculum consisted of 8.2×10^4 PFU/1-day-old bird and given IP.

*($p<0.05$)

Table 11. Con A stimulation of whole-blood lymphocytes of chickens and turkeys infected with HVT (FC126)

Animal[a]	Inoculum[b]	Mean Stimulation Index ± SD[c] Days after Inoculation 15	21
Chickens	HVT (FC126)	96.7 ± 18.3	31.0 ± 3.1
	none	117.6 ± 14.7	32.6 ± 2.1
Turkeys	HVT (FC126)	1.9 ± 0.2**	3.7 ± 1.0
	none	11.1 ± 2.4	4.9 ± 1.2

[a]Eight animals per group were examined at each interval.

[b]Inoculum consisted of 8.2×10^4 PFU/1-day-old bird and given IP.

[c]Counts per minute of ^{125}IUdR incorporation in cells with Con A divided by counts per minute in cells without Con A.

**(p<0.01)

Experiment III
Response of HVT-Vaccinated Chickens and Turkeys to MDV

Since it was found that turkeys were susceptible to MDV infection, it was appropriate to investigate the possibility of vaccination against this infection. Vaccination with HVT was chosen because it is the most widely practiced method and because HVT is originally a virus of turkeys that is widely distributed through turkey populations.

The results of vaccination of chickens and turkey poults with HVT at day 1 and challenge with GA strain of MDV at day 10 are summarized in Table 11.

Titrations of Viruses Present in the Blood

At 35 days after inoculation, all chickens and turkeys were found to be viremic for HVT (Table 12). Virus titers were higher in chickens than in turkeys. Unvaccinated control chickens and turkeys lacked detectable viremia. Among vaccinated and MDV-challenged birds, 2 of 6 chickens and 0 of 5 turkeys had detectable MDV in blood. On the other hand, all the unvaccinated and MDV-challenged chickens that were tested had high MDV titers in their blood, while in the unvaccinated and MDV-challenged turkeys only 1 of 5 was viremic. Thus, vaccination with HVT decreased the incidence of MDV viremia in chickens. Because the viral titers and the incidence of viremia in unvaccinated turkeys were low, the effect of vaccination with HVT could not be assessed.

MD Response

Vaccination with HVT reduced MD mortality and gross lesion development in chickens but not in turkeys (Table 11). Therefore, it was concluded that HVT vaccination does not afford protection against challenge with virulent MDV in turkeys.

Table 12. Titrations of viruses present in blood, lesion response, and mortality in HVT-vaccinated and unvaccinated chickens and turkeys after challenge with virulent GA-MDV

Animals	Treatment		Virus Titers at 35 Days of Age (mean PFU/10^7 buffy coat cells)		MD Response (%)[d]		
	HVT (FC126)[a]	GA-MDV[b]	HVT	GA-MDV	No. of Animals[c]	Mortality (%)	Gross Lesions (%)
Chickens	+	+	26 (6/6)[e]	1 (2/6)	13	7.7	15.4
	-	+	0 (0/6)	184 (6/6)	7	23.5	70.6
Turkeys	+	+	2 (5/5)	0 (0/5)	17	29.4	29.4
	-	+	0 (0/5)	3 (1/5)	26	23.1	30.8

[a]Inoculum consisted of 8.2×10^4 PFU/1-day-old bird and given IP.

[b]Inoculum consisted of 8.3×10^4 PFU/10-day-old bird and given IP.

[c]Deaths unrelated to experimental infection were excluded.

[d]Experiment terminated at 81 days of age.

[e]No. positive/total examined.

Experiment IV
Response of Chickens and Turkeys to RAV-1

Titration of Virus Present in Blood and Antibody Response

The results of virus isolation and antibody evaluation from samples obtained from chickens and turkeys inoculated with RAV-1 are shown in Table 13. Control chickens and turkeys were free of detectable virus. All chickens and turkeys inoculated IV were viremic at week 2, and 7 out of 10 chickens inoculated IP were also viremic at week 10. The lower dose given IP did not produce 100% viremia in chickens or turkeys, but most chickens became viremic. However, no turkey was viremic at week 10, suggesting that immunologic tolerance may have been induced in some chickens but not in turkeys.

Serum samples from chickens and turkeys were examined for neutralizing antibodies to RAV-1 at week 10 after inoculation. Serum from 90% of the chickens tested in the 2 infected groups had antiviral antibodies. All turkeys given the higher dose IV and 8 of 9 turkeys given the lower dose IP had antiviral antibodies. However, 3 uninoculated control turkeys had antibodies but were not viremic.

Pathology

Table 12 shows the percentage of mortality after excluding deaths unrelated to RAV-1. In chickens and turkeys, the percentage of mortality was higher in the high-dose groups which were inoculated IV. Also, the 2 groups of infected turkeys had higher mortality than the 2 groups of infected chickens. The majority of deaths occurred during weeks 3 and 4 after inoculation in the 4 groups of infected turkeys and chickens. However, the deaths in chickens included 4

Table 13. Titers of virus present in blood, antibodies, mortality, and neoplastic lesions in chickens and turkeys inoculated with RAV-1

Animal	Inoculum	Titers[a] No. Positive/No. Examined 2 weeks	Titers[a] No. Positive/No. Examined 10 weeks	Antibodies[b] No. Positive/No. Examined 10 weeks	Mortality Dead/Total (%)	Neoplastic Lesions[d] No. Positive/No. Examined (%) microscopic	Neoplastic Lesions[d] No. Positive/No. Examined (%) gross
Chickens	high dose[e]	10/10	10/10	9/10	3/43 (9.3)	13/43 (30.2)	14/43 (32.6)
	low dose[e]	8/10	7/10	9/10	1/51 (2.0)	13/51 (25.3)	9/51 (17.6)
	none	0/10	0/10	0/10	0/43 (0.0)	0/43 (0.0)	0/43 (0.0)
Turkeys	high dose[e]	10/10	0/10	6/ 6	16/45 (37.5)	0/45 (0.0)	0/45 (0.0)
	low dose[e]	5/ 9	0/10	8/ 9	9/52 (18.4)	2/52 (3.8)	0/52 (0.0)
	none	0/10	0/10	3/10	0/51 (0.0)	0/50 (0.0)	0/51 (0.0)

[a]Ten uninoculated control animals per group examined at each interval lacked detectable viremia. Test used was alternative phenotypic mixing.

[b]Virus neutralization test was performed to detect the presence of neutralizing antibodies in samples of plasma.

[c]Deaths unrelated to experimental infection were excluded.

[d]A similar number of uninoculated controls lacked lesions.

[e]Inoculum consisted of 10^5 infectious units of RAV-1/1-day-old bird and given IV.

[f]Inoculum consisted of 10^2 infectious units of RAV-1/1-day-old bird and given IP.

cases of erythroblastosis in the high-dose group and 1 case in the low-dose group. Erythroblastosis did not occur in the infected turkeys.

Gross lesions. Macroscopic lesions were seen in some of the chickens and turkeys infected with each dose of RAV-1. All turkeys in the high-dose group had normal thymuses except one had severe thymic atrophy. In the high-dose group, the spleens were usually smaller than normal. However, some of the turkeys that died had enlarged spleens. Three of the spleens from those turkeys that died during the third week had dark focal lesions (Figure 38). Also, 5 of the spleens from the turkeys inoculated with the low dose that died during week 3 were grossly enlarged and had large dark focal lesions or small whitish focal lesions. The lesions ranged in size from 1 to 3 mm. Gross lesions associated with neoplastic cell proliferation were not detected in any of the infected turkeys.

On the other hand, gross lesions seen in RAV-1 infected chickens were neoplastic and could be divided into 2 types. The first type, seen in chickens that died between weeks 3 and 8, consisted of typical erythroblastosis. The 5 chickens involved (4 from the high-dose group and 1 from the low-dose group) had similar lesions confined to the liver and spleen. Both organs were enlarged, the spleen being 3 to 4 times the normal size. The enlargement was diffuse, the color was dark mahogany, and the consistency was soft and friable. The second type of gross lesions was seen after week 8 and consisted of typical lymphoid leukosis with bursal involvement. Lymphoid leukosis was more prominent in the high-dose group than in the low-dose group (Table 12). The bursa of Fabricius was the organ

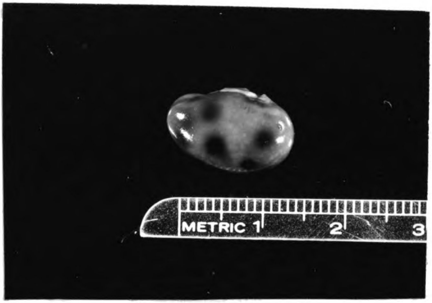


Figure 38. Dark focal lesions in an enlarged spleen of a turkey 10 days after inoculation with RAV-1 at day 1.

most often involved, and focal nodular lesions in bursal plicae were seen in almost every bursa examined after 3 months (Figures 39 and 40). Lymphoid tumors consisting of nodular, miliary or diffuse enlargement were also seen in gonad, liver, spleen, kidney and thymus. In the low-dose group, lesions were noted after 105 days and were usually confined to the bursa of Fabricius. One chicken also had severe thymic atrophy.

Histopathology. Data on microscopic lesions are given in Tables 12 and 13. Lesions caused by neoplastic cell proliferation and metastasis were observed in the 2 groups of infected chickens but only in the low-dose group of turkeys. Lesions in turkeys were detected in 2 bursas at 97 days after RAV-1 inoculation at day 1. In the turkey the affected bursal follicle was larger than the surrounding normal follicles and the distinction between cortex and medulla was lost (Figure 41), being composed entirely of large lymphoblastic cells with uniform size, some of them in mitosis (Figure 41). These cells had a poorly defined cytoplasmic membrane, abundant basophilic cytoplasm, and pyroninophilia was demonstrated by methyl green pyronine stain. The nuclei were vesicular with margination and clumping of chromatin and prominent nucleoli (Figure 42).

In the infected chickens of either group, the initial microscopic neoplastic lesion was a transformed bursal follicle. These were first seen at 64 days after inoculation of the high dose and at 40 days in the group inoculated with the low dose. The transformed follicles were very similar to those seen in turkeys, but in the chickens many bursal follicles became enlarged and some transformed follicles coalesced to form a large lesion that could be detected

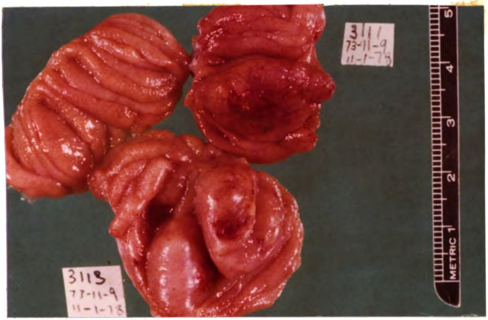


Figure 39. Tumorous involvement of the bursal plicae, inflammation and hemorrhage (right, above and below) of chickens 128 days after inoculation with RAV-1 at day 1. Bursa of uninoculated control chicken (left above) of the same age.

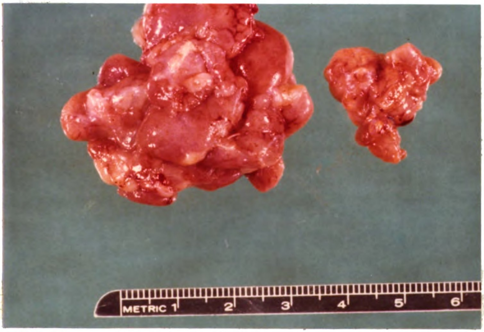


Figure 40. Tumorous involvement (left) and atrophy (right) of bursas in 2 chickens 128 days after inoculation with RAV-1 at day 1.

Table 14. Microscopic lesions in chickens and turkeys inoculated with RAV-1

Animals[a]	Inoculum	Average Number of Tissues with Lesions/10 Tissues Examined[b] Days after Inoculation								
		10	20	30	40	64	74	87	97	103
Chickens	high dose[c]	1.3	1.0	1.0	2.0	0.8[e]	2.5[e]	3.3[e]	3.8[e]	5.0[e]
	low dose[d]	0.0	0.5	0.8	3.3[e]	0.0	0.5	1.5[e]	1.8[e]	4.0[e]
Turkeys	high dose[c]	3.0	3.8	ND	ND	ND	ND	ND	0.5	ND
	low dose	2.0	3.8	1.8	2.8	1.8	1.0	0.8	0.8[e]	0.3

[a]Four animals per group were examined at each interval. A similar number of uninoculated controls examined at each interval lacked lesions, except for 1 turkey.

[b]Tissues examined were liver, spleen, kidney, gonad, heart, skin, bursa, thymus, nerves (vagus, brachial, sciatic), and bone marrow.

[c]Inoculum consisted of 10^5 infectious units of RAV-1/1-day-old bird and given IV.

[d]Inoculum consisted of 10^2 infectious units of RAV-1/1-day-old bird and given IP.

[e]Neoplastic lesions are included.

ND = not done.

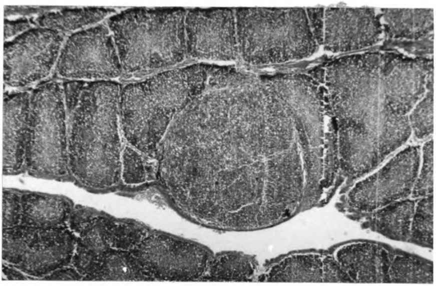


Figure 41. Lymphoblastic transformation of a bursal follicle in a turkey 97 days after inoculation with RAV-1 at day 1 (center). Affected follicle is larger than surrounding normal follicles with their distinctive medullary and cortical regions. H&E stain, X48.

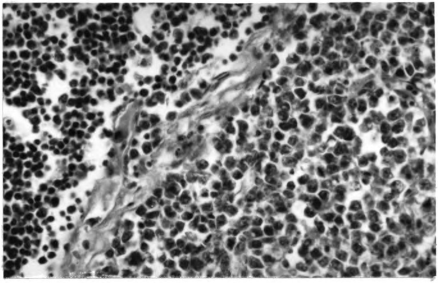


Figure 42. Higher magnification of above. Notice lymphoblastic cells in affected follicles (right below) are uniform and larger than normal small lymphocytes (left above) in unaffected follicle. H&E stain, X480.

grossly (Figures 39 and 43). In many cases the interfollicular tissue had been invaded by the transformed cells, adjacent normal follicles were displaced, and tumor cells accompanied by a few macrophages gave a "starry sky" appearance (Figure 44).

The metastatic tumors in chickens were detected in liver, spleen, kidney, gonad, heart, skin, bursa, thymus and bone marrow. The cells were similar to those seen in transformed bursal follicles. As lymphoid cells proliferated, they displaced and compressed surrounding normal cells and formed aggregates surrounded by fibroblast-like cells. Other lesions seen to a lesser extent included blood vessel distention and thrombosis (Figures 45 and 46), fibrous tissue proliferation (Figure 47) and erythroblastosis (Figure 48).

Somewhat different lesions were seen before the bursal transformation. These were lymphoproliferative and hyperplastic lesions and were more prominent during the first 6 weeks. They were seen in all 4 of the inoculated groups of chickens and turkeys. Lesions were more prominent in turkeys than in chickens and slightly more frequent in the high-dose groups. In turkeys, lesions were first seen at day 10 after inoculation, were most frequent at day 20, then gradually diminished. In chickens, they also appeared at day 10 in the high-dose group, but in the low-dose group they were first detected at day 20 after inoculation. In both groups of infected chickens, the lesions were most frequent at day 40 after inoculation (Table 13). These lesions in chickens were most frequent in the spleen, followed by heart, bone marrow, gonad, kidney, and liver. However, unlike these organs, cardiac lesions were not detected at day 10 after inoculation in any chickens or turkeys.

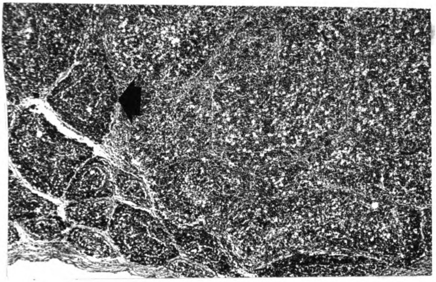


Figure 43. Neoplastic transformation in the bursal follicles of a chicken 105 days after inoculation with RAV-1 at day 1. Notice normal follicles with distinct medulla and cortex (arrow). H&E stain, X48.

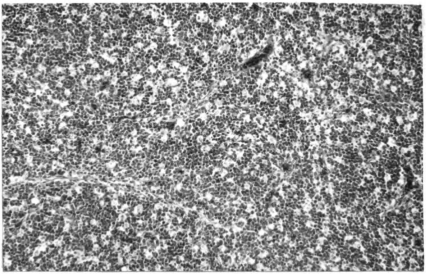


Figure 44. "Starry sky appearance" in a transformed bursal follicle of a chicken 97 days after inoculation with RAV-1 at day 1. H&E stain, X120.

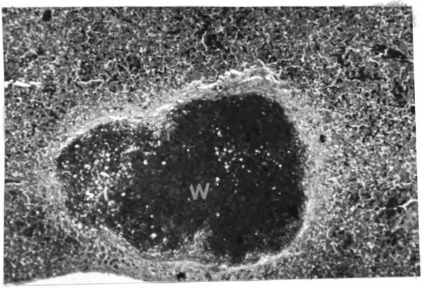


Figure 45. Blood vessel thrombosis (W) in the liver of a chicken 97 days after inoculation with RAV-1 at day 1. H&E stain, X48.

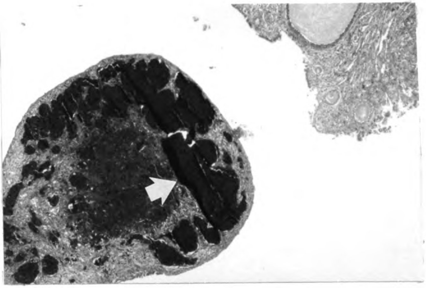


Figure 46. Blood vessel distention and thrombosis (arrow) on the surface of the ovary of a chicken 97 days after inoculation with RAV-1 at day 1. H&E stain, X48.

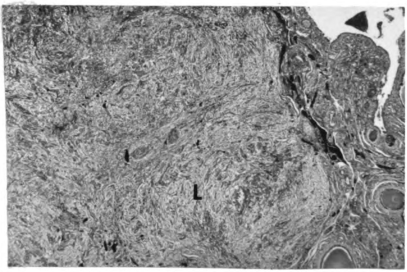


Figure 47. Fibromatous lesion (L) in the ovary of a chicken 97 days after inoculation with RAV-1 at day 1. H&E stain, X48.

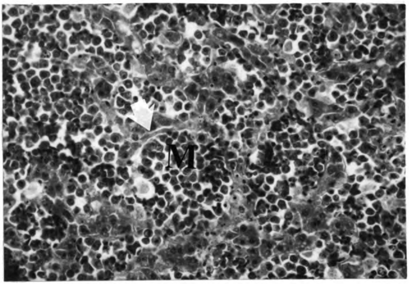


Figure 48. Erythroblasts (M) distending the sinusoids (arrow) in the liver of a chicken 54 days after inoculation with RAV-1 at day 1. H&E stain, X300.

Splenic lesions were present in all spleens from 4 turkeys examined in the high-dose group and in 3 out of 4 spleens from turkeys examined in the low-dose group at day 10. They consisted of a number of small discrete foci of variable sizes, disrupting the normal architectural pattern of the spleen (Figures 49 and 50). These lesions contained lymphoblasts, lymphocytes, reticular cells with a few immature heterophils, some stroma, and a proliferation of capillary vessels. Small mononuclear cells with pyknotic nuclei were seen, particularly near the center of many of these lesions (Figure 51). Many of these aggregates may have coalesced to form larger lesions. At day 20 after inoculation the spleens examined in both turkey groups had similar lesions. However, 1 spleen out of 4 in the high-dose group, and all spleens examined at day 30 after inoculation through day 103 after inoculation, had discrete circumscribed foci of large lymphocytes similar to the follicular lymphoid tissues described by Lucas et al. (1954b), which are referred to as bursa-dependent follicles (BF). The BF were especially numerous in spleens from turkeys that died during weeks 3, 4 and 5 after inoculation. The spleens that were grossly enlarged and dark in color consisted mainly of the BF (Figures 52 and 53) and a highly congested red pulp.

Splenic lesions in infected chickens were somewhat similar to those seen in the turkeys, except that the white pulp was increased. At day 10 after inoculation, splenic lesions were not seen in the low-dose group but were seen in all 4 chickens examined in the high-dose group. They were also seen at days 30 and 40 after inoculation in each of the groups of chickens, although only 1 to 2 out of 4 were involved. The BF were seen less frequently at days 40 to 105 after inoculation.

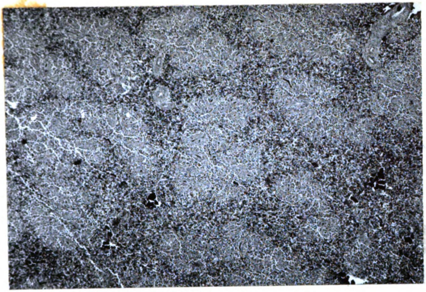


Figure 49. Normal spleen of uninoculated control turkey at day 10. H&E stain, X48.

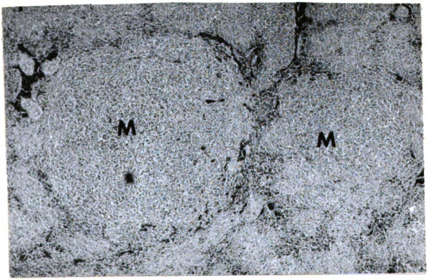


Figure 50. Focal lymphoreticular proliferation (M) in the spleen of a turkey 10 days after inoculation with RAV-1 at day 1. H&E stain, X48.

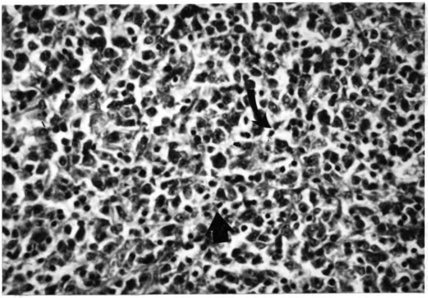


Figure 51. Higher magnification from Figure 50, showing cellular pleomorphism. Also notice presence of degenerative cells with pyknotic nuclei (arrows). H&E stain, X480.

Figure 52. Normal spleen of uninoculated control turkey at day 10. H&E stain, X30.

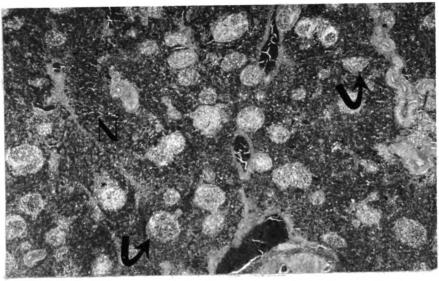


Figure 53. Proliferation of lymphoid follicles (arrow) in the highly congested red pulp (N) of the spleen of a turkey 10 days after inoculation with RAV-1 at day 1. H&E stain, X30.

Microscopic lesions in the heart were not seen in any of the chickens and turkeys examined at day 10 after infection. However, at day 20, 3 out of 4 chickens in the high-dose group, 4 out of 4 in the low-dose group, and 7 out of the 12 turkeys that died at weeks 3, 4 and 5 after inoculation had heart lesions. These lesions were detected less frequently at days 30 to 97 after infection. The main lesion was diffuse lymphoproliferation of varying amounts (Figures 54, 55 and 56). The lesions consisted of lymphoid cell accumulations amongst myocardial fibers. The predominant cell was an immature lymphocyte with a small nucleus but prominent nucleolus. Plasma cells were present, but to a lesser extent. There was congestion and some hemorrhage in some sections. There was much muscle fiber degeneration with loss of striation and, in a few cases, proliferation of myocardial cells (Figures 54 and 57) and accumulations of multinucleated giant cells (Figures 54 and 58). The frequency and extent of heart lesions in chickens were less than those in turkeys. Heart lesions in chickens were of diffuse and discrete types, which usually occurred separately (Figures 54, 55 and 59) but on rare occasions coexisted in the same sections. These were also seen in turkeys. The diffuse type was similar to that seen in turkeys, but no myocardial cell proliferation or multinucleated giant cell accumulations were seen. The discrete type was well circumscribed and similar to the BF in turkeys.

Affected livers of chickens and turkeys frequently had perivascular cuffing of lymphoid cells, small discrete lymphoid foci, vacuoles and, very rarely, BF. The changes in the gonad, kidney, and bone marrow, in chickens as well as in turkeys, included BF and discrete lymphoid foci formation (Figures 60 and 61). Atrophy of the

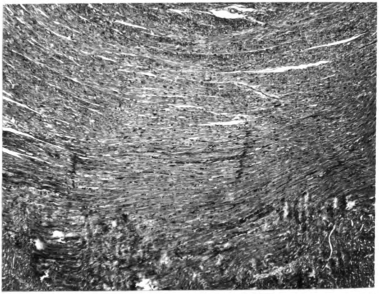


Figure 54. Normal heart of uninoculated turkey at day 20. H&E stain, X120.

Figure 55. Diffuse accumulations of lymphoid cells (1) among myocardial fibers (arrow) of the heart of a turkey 20 days after inoculation with RAV-1 at day 1. H&E stain, X120.

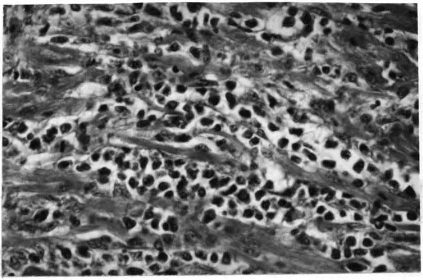


Figure 56. Higher magnification of Figure 55. H&E stain, X480.

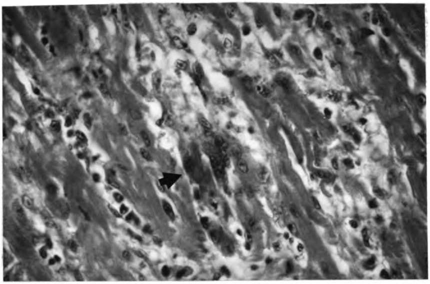


Figure 57. Regenerative attempts of cardiac muscle cells in a turkey 20 days after inoculation with RAV-1 at day 1. Notice enlargement of nuclei (arrow) of myocardial fibers and infiltration of cells. H&E stain, X480.

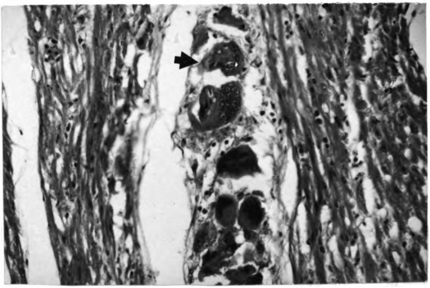


Figure 58. Multinucleated giant cell formation (arrow) in the heart of a turkey 29 days after inoculation with RAV-1 at day 1. H&E stain, X300.

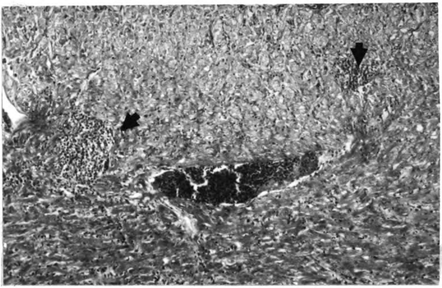


Figure 59. Focal infiltration of lymphoid cells in the heart of a turkey 20 days after inoculation with RAV-1 at day 1. Notice presence of 2 foci (arrows). H&E stain, X120.

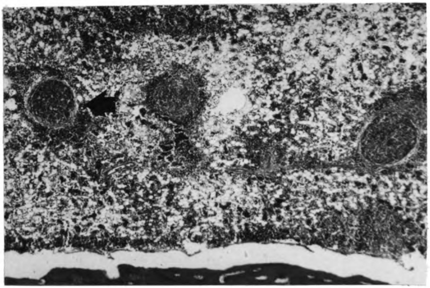


Figure 60. Lymphoid follicles (arrow) in the bone marrow of a turkey 40 days after inoculation with RAV-1 at day 1. H&E stain, X30.

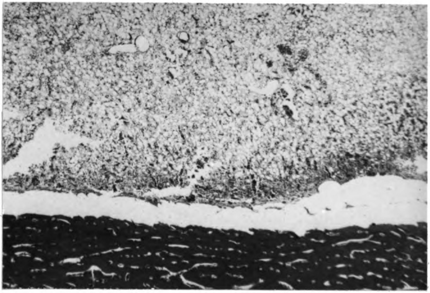


Figure 61. Normal bone marrow of uninoculated control turkey at day 40. H&E stain, X30.

cortex of the thymus was seen in 2 out of 4 turkeys examined at day 20 and in 7 of the turkeys that died during weeks 3, 4 and 5 after inoculation.

Body Weights

The average body weights of the 4 groups of treated chickens and turkeys were plotted as proportions of the uninoculated controls (Figure 62). The average weights of control birds are set at a value of one. Analysis of variance of chicken and turkey weights revealed a significant effect of time ($p<0.0001$) and infection ($p<0.0001$) but not of the interaction between them. The weights of turkeys and chickens infected with the high dose (IV) were significantly lower than those of uninoculated controls at day 20 after infection ($p<0.01$ and $p<0.05$, respectively). In turkeys that were inoculated with the low dose (IP), the decrease in body weight was significant at days 40, 87 and 97 after inoculation ($p<0.05$). In the low-dose group of chickens, the decrease in body weight was significant at days 20, 40 and 97 ($p<0.05$) and day 30 ($p<0.01$). The differences between the 2 treatments were significant at day 20 in turkeys and at days 30 and 97 in chickens ($p<0.05$).

Organ Weights

Organ weights were calculated as mg/g of body weight and then plotted in Figures 63, 64 and 65 as proportions of control. The average weight of control birds is set at a value of one.

Bursa. Analysis of bursa to body weight ratios revealed a significant effect of time ($p<0.0001$) but neither of infection nor the interaction between time and infection for turkeys and of time

Figure 62. Effect of RAV-1 infection on body weight of chickens and turkeys. Each point represents an average of 4 birds. CL = chickens inoculated with 10^2 infectious units of RAV-1 at day 1 IP; TL = turkeys inoculated with 10^2 infectious units of RAV-1 at day 1 IP; CH = chickens inoculated with 10^5 infectious units of RAV-1 at day 1 IV; TH = turkeys inoculated with 10^5 infectious units of RAV-1 at day 1 IV. *Not done after day 20 due to high mortality. Body weights of infected chickens and turkeys were significantly less than those of controls at the following intervals: CH = day 20 ($p<0.05$), CL = days 20, 40 and 97 ($p<0.05$), day 30 ($p<0.01$), TH = day 20 ($p<0.01$), TL = days 40, 87 and 97 ($p<0.05$).

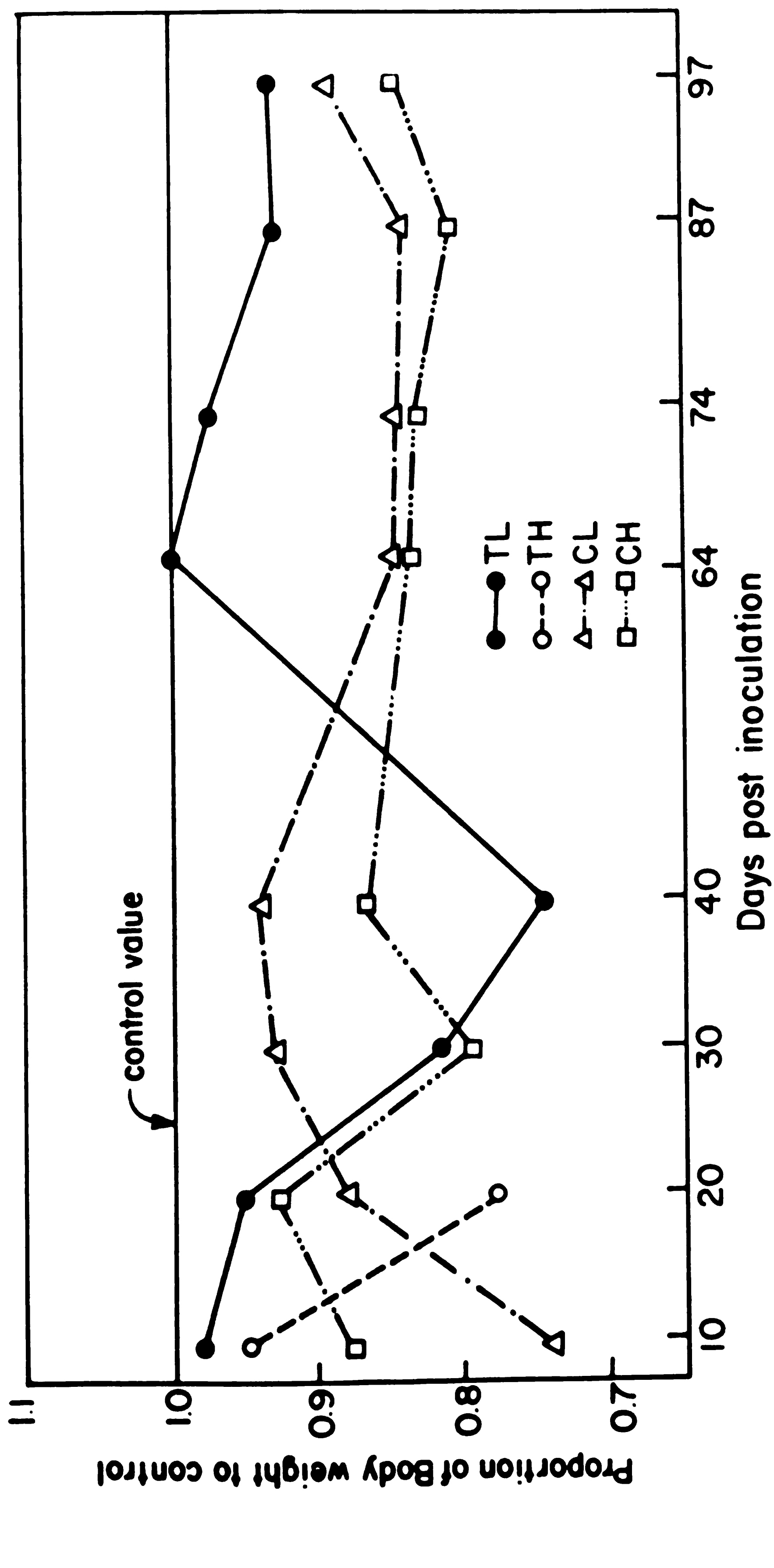
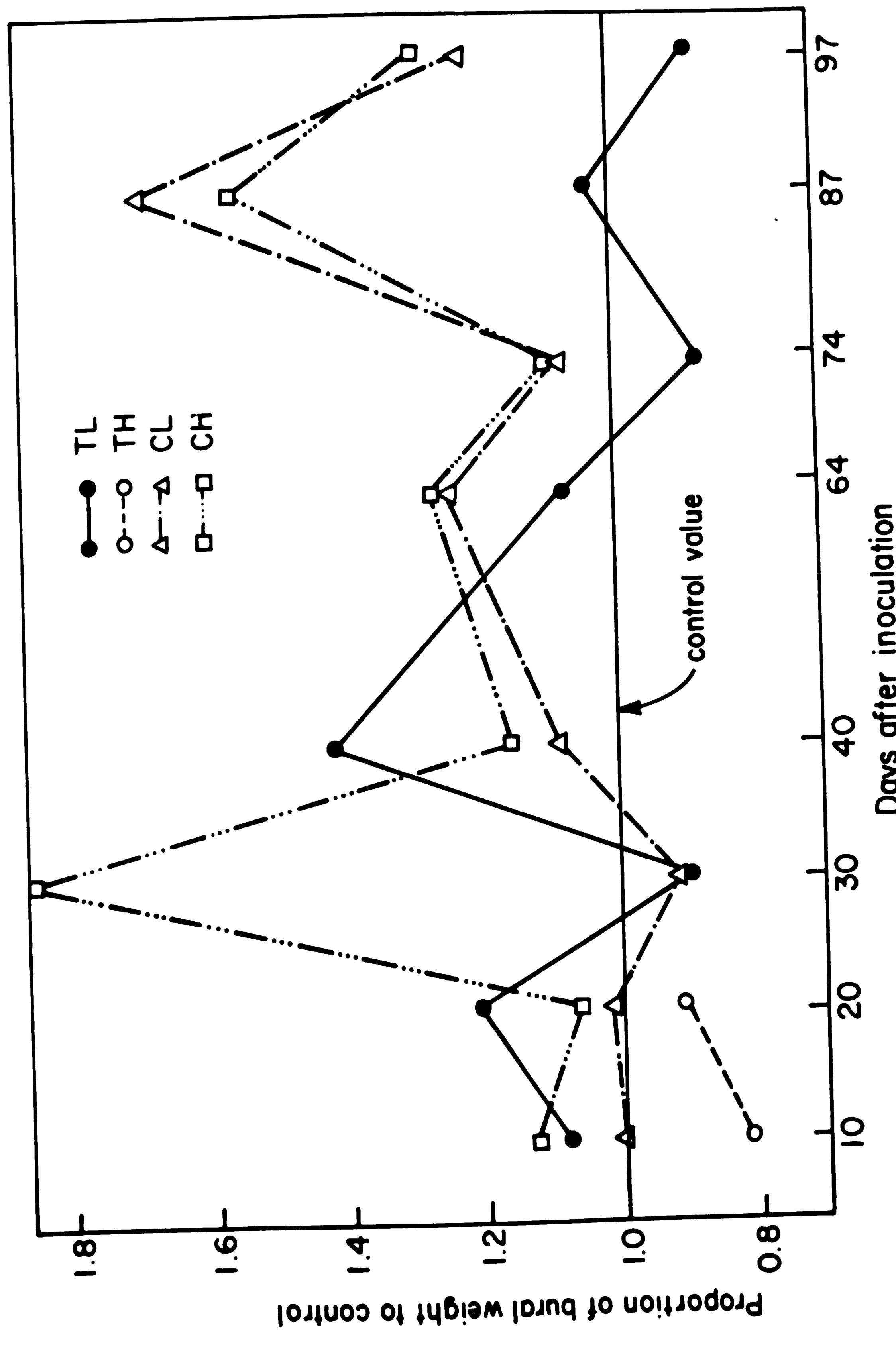

control value
Proportion of Body weight to control
1.1
1.0
0.9
0.8
0.7
10
20
30
40
64
74
87
97
Days post inoculation
TL
TH
CL
CH

Figure 62

Figure 63. Effect of RAV-1 on bursas of chickens and turkeys shown as proportions of bursal weights to controls. Each point represents an average of 4 birds. CL = chickens inoculated with 10^2 infectious units of RAV-1 at day 1 IP; TL = turkeys inoculated with 10^2 infectious units of RAV-1 at day 1 IP; CH = chickens inoculated with 10^5 infectious units of RAV-1 at day 1 IV; TL = turkeys inoculated with 10^5 infectious units of RAV-1 at day 1 IV. *Not done after day 20 due to high mortality. Bursa to body weight ratios of infected chickens were significantly different than those of controls at the following intervals: CH = day 40 ($p<0.05$), TL = day 40 ($p<0.01$).



TL
TH
CL
CH
control value
Proportion of bural weight to control
1.8
1.6
1.4
1.2
1.0
0.8
Days after inoculation
10
20
30
40
64
74
87
97

Figure 63

Figure 64. Effect of RAV-1 on the thymus of chickens and turkeys shown as proportions of thymic weights to control. Each point represents an average of 4 birds. CL = chickens inoculated with 10^2 infectious units of RAV-1 at day 1 IP; TL = turkeys inoculated with 10^2 infectious units of RAV-1 at day 1 IP; CH = chickens inoculated with 10^5 infectious units of RAV-1 at day 1 IV; TH = turkeys inoculated with 10^5 infectious units of RAV-1 at day 1 IV. *Not done after day 20 due to high mortality. Thymus to body weight ratios of infected chickens and turkeys were significantly different at the following intervals: CL = day 30 ($p<0.05$), TL = days 10, 30 and 97 ($p<0.05$).

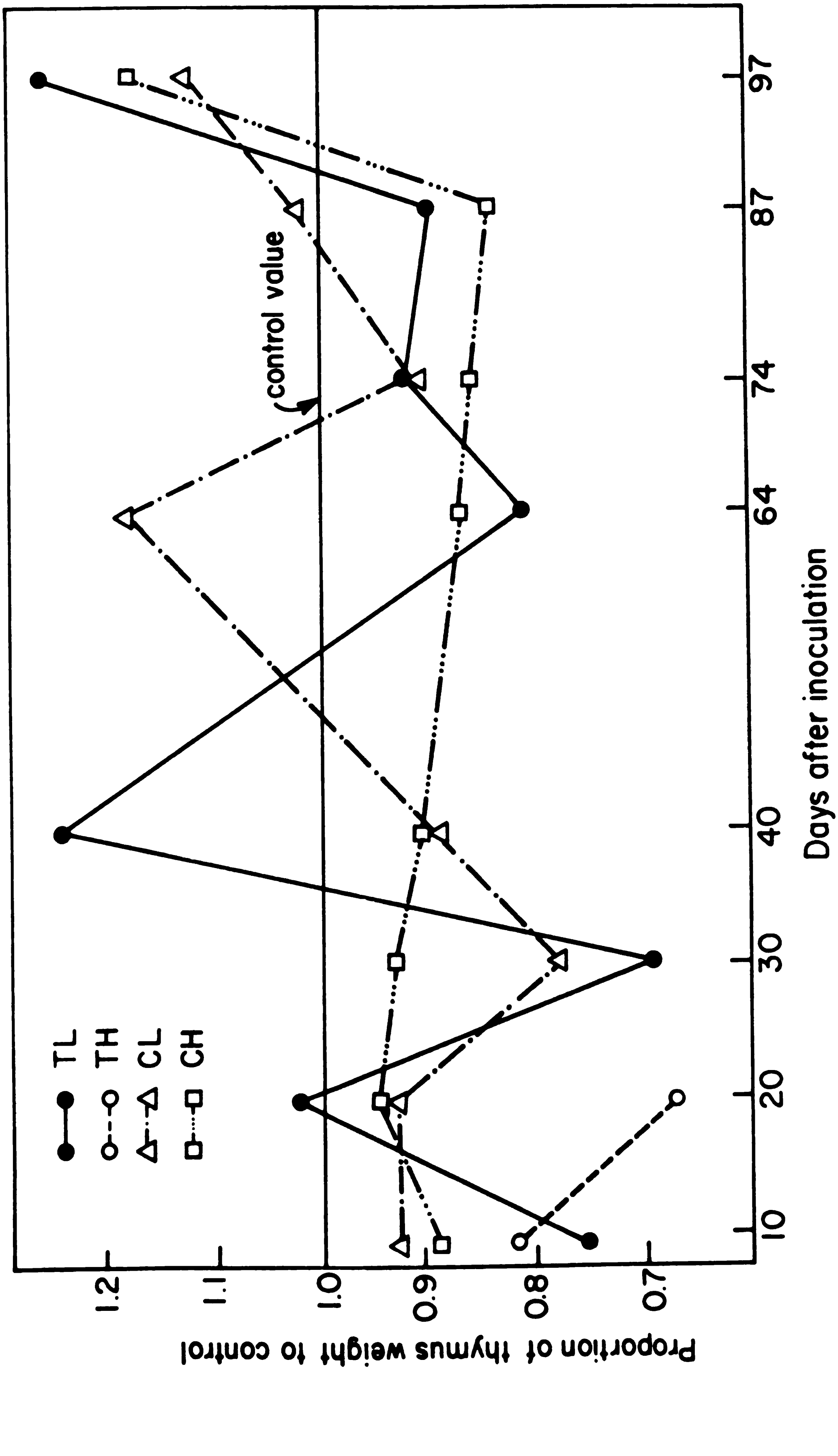
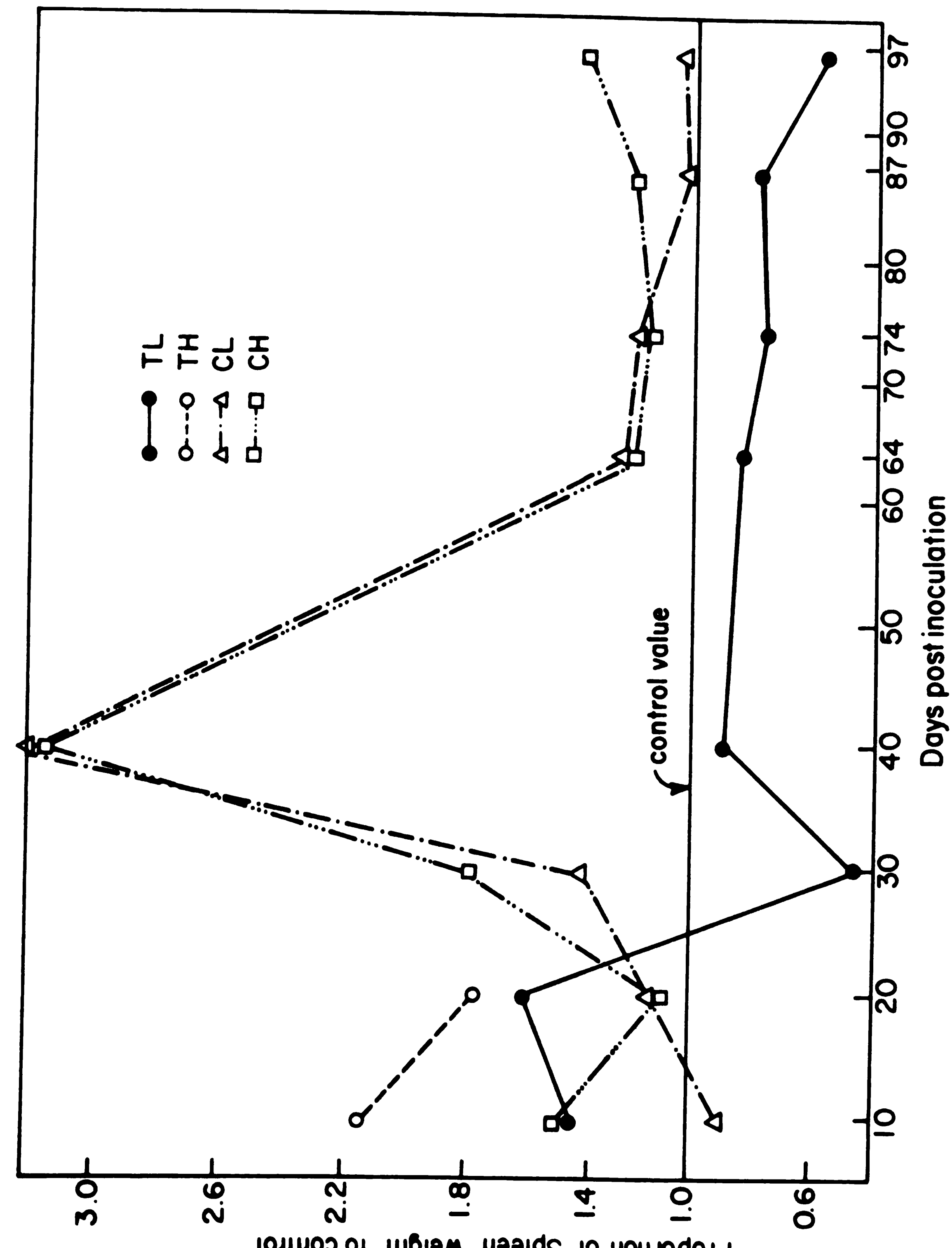

TL
TH
CL
CH
control value
Proportion of thymus weight to control
1.2
1.1
1.0
0.9
0.8
0.7
10
20
30
40
64
74
87
97
Days after inoculation

Figure 64

Figure 65. Effect of RAV-1 on the spleens of chickens and turkeys shown as proportions of splenic weights to controls. Each point represents an average of 4 birds. CL = chickens inoculated with 10^2 infectious units of RAV-1 at day 1 IP; TL = turkeys inoculated with 10^2 infectious units of RAV-1 at day 1 IP; CH = chickens inoculated with 10^5 infectious units of RAV-1 at day 1 IV. *Not done after day 20 due to high mortality. Spleen to body weight ratios of infected chickens and turkeys were significantly different than those of controls at the following intervals: CL = day 40 ($p<0.05$), CH = days 10 and 40 ($p<0.05$) and day 97 ($p<0.01$), TL = day 30 ($p<0.01$) and day 74 ($p<0.05$), TH = days 10 ($p<0.01$) and 20 ($p<0.05$).



TL
TH
CL
CH
control value
Proportion of Spleen weight to control
3.0
2.6
2.2
1.8
1.4
1.0
0.6
10
20
30
40
50
60 64
70 74
80
87 90
97
Days post inoculation

Figure 65

($p<0.0001$) and of infection ($p<0.05$) but not of the interaction between them for chickens. The bursa to body weight ratios were significantly higher at day 40 ($p<0.01$) in the turkeys inoculated with the low dose IP ($p<0.05$) and in chickens inoculated with the high dose IP, compared to the uninoculated controls. The differences between the 2 treated groups were significant at day 20 in turkeys and days 40 and 97 in chickens ($p<0.05$).

Thymus. Thymus to body weight ratios of low-dose inoculated turkeys and chickens were significantly lower than controls at day 30 ($p<0.05$) and at days 10 and 97 in turkeys ($p<0.05$). The differences between the 2 treated groups were significant at day 64 in chickens ($p<0.05$). Analysis of variance of the turkey thymus to body weight ratios revealed a significant effect of time ($p<0.0001$) and of the interaction between time and infection ($p<0.05$) but not of the infection itself. In chickens there was a significant effect of time ($p<0.0001$) but neither of infection nor of the interaction between time and infection.

Spleen. Analysis of variance of spleen to body weight ratios in turkeys revealed a significant effect of time ($p<0.0001$) and infection ($p<0.01$) and of the interaction between them ($p<0.01$), while in chickens there was a significant effect of time ($p<0.0001$) and of infection ($p<0.001$) but not of the interaction between them. Spleen to body weight ratios of infected turkeys were significantly larger than those of uninoculated control turkeys at day 10 ($p<0.01$) and day 20 ($p<0.05$) in the high-dose group and at days 30 ($p<0.05$) and 74 ($p<0.05$) in the low-dose group. In chickens, the spleen to body weight ratios were significantly larger at days 10 and 40 ($p<0.05$) in the high-dose group and at day 40 (p 0.05) in the low-dose groups. The differences between the 2 groups of treated chickens were significant at day 97 ($p<0.01$).

Antibody Response

The humoral immune response was evaluated at weeks 3 and 10 after inoculation of chickens and turkeys with RAV-1 (Table 15). At week 3 there was a significant decrease in the primary response to SE ($p<0.01$) and *B. abortus* ($p<0.05$) antigens in the infected chickens. The effect was more pronounced in the high-dose group. However, there was a trend to an increase in the secondary as well as in the primary response in each infected group of turkeys. In both groups of infected chickens at week 10, primary response was significantly increased for *B. abortus* ($p<0.05$) but not for SE. In turkeys, the response at week 10 tended to decrease in the infected groups compared to the control. Thus, there was no significant effect on humoral response in turkeys inoculated with RAV-1. The initial effect in chickens also tended to decrease or disappear later.

Con A Stimulation of Whole-Blood Lymphocytes

Concanavalin A stimulation of whole-blood lymphocytes from chickens and turkeys inoculated with RAV-1 at day 1 was evaluated at days 10, 20, 83 and 103 (Table 16). There was no significant effect in chickens. A significant decrease ($p<0.01$) in the stimulation indices was observed at day 103 in turkeys inoculated with low doses IP.

Table 15. **Humoral** response of chickens and turkeys infected with RAV-1 to SE and *Brucella abortus* antigens

Test	Animal[a]	Inoculum	Weeks Post-inoculation[b]	Antibody to SE[c] (hemagglutination test) (mean $\log_2$ titer ± SD)		Antibody to *B. abortus* (plate agglutination test) (mean $\log_2$ titer ± SD)	
				Primary Response	Secondary Response	Primary Response	Secondary Response
1	Chickens	high dose[d]	3	4.0 ± 0.0**	7.4 ± 2.3	3.8 ± 0.5*	4.6 ± 0.5
		low dose[e]		5.3 ± 0.5**	6.6 ± 1.1	4.5 ± 0.6*	5.2 ± 0.4
		none		7.2 ± 1.0	7.8 ± 0.8	5.0 ± 0.6	5.5 ± 0.8
	Turkeys	high dose[d]		10.0 ± 1.7	ND	6.0 ± 2.0	ND
		low dose[e]		9.2 ± 0.8	9.5 ± 1.0	7.8 ± 1.8	7.0 ± 1.4
		none		8.5 ± 1.3	9.0 ± 0.8	5.0 ± 3.5	6.5 ± 1.7
2	Chickens	high dose[d]	10	6.2 ± 0.8	6.5 ± 0.6	4.3 ± 0.5*	6.0 ± 0.3
		low dose[e]		5.8 ± 1.2	7.0 ± 1.1	4.8 ± 0.4*	5.3 ± 0.5
		none		6.0 ± 1.4	6.8 ± 1.0	3.7 ± 0.8	5.0 ± 0.6
	Turkeys	high dose[d]		ND	ND	ND	ND
		low dose[e]		5.2 ± 1.2	5.8 ± 0.9	4.7 ± 0.5	4.7 ± 0.8
		none		6.5 ± 1.1	6.5 ± 1.1	5.0 ± 0.6	5.3 ± 0.8

[a]Six animals in each group in test 1 and 3 to 6 animals in test 2.

[b]Serum samples were tested for antibody at 4 weeks (primary response) and at 5 weeks (secondary response).

[c]All birds were inoculated IV with SE and *B. abortus* (2.5×10^9 cells of each) at weeks 3 and 4 (test 1) and weeks 10 and 11 (test 2) postinoculation.

[d]Inoculum consisted of 10^5 infectious units of RAV-1/1-day-old bird and given IV.

[e]Inoculum consisted of 10^2 infectious units of RAV-1/1-day-old bird and given IP.

ND = not done.

*($p<0.05$)

**($p<0.01$)

Table 16. Con A stimulation of whole-blood lymphocytes of chickens and turkeys infected with RAV-1

Animals[a]	Inoculum	Mean Stimulation Index ± SD Days after Inoculation 10	20	83	103
Chickens	high dose[b]	85.6 ± 19.4	50.3 ± 11.4	41.8 ± 31.8	68.3 ± 43.8
	low dose[c]	81.1 ± 26.9	70.6 ± 16.1	46.7 ± 33.0	71.7 ± 28.9
	none	79.1 ± 17.2	51.9 ± 18.7	48.1 ± 14.9	26.4 ± 27.8
Turkeys	high dose[b]	37.7 ± 6.5	37.5 ± 8.3	33.5 ± 11.8	ND
	low dose[c]	30.8 ± 6.4	36.6 ± 8.1	29.7 ± 9.8	22.0 ± 9.3**
	none	27.7 ± 5.1	48.0 ± 7.6	30.3 ± 8.6	42.3 ± 8.6

[a]Eight animals per group were examined at 10, 20 and 83 days. At 103 days, 5 animals per group were examined.

[b]Inoculum consisted of 10^5 infectious dose of RAV-1/1-day-old bird and given IV.

[c]Inoculum consisted of 10^2 infectious dose of RAV-1/1-day-old bird and given IP.

[d]Counts per minute of ^{125}IUdR incorporation in cells with Con A divided by counts per minute in cells without Con A.

**($p<0.01$)

ND = not done

DISCUSSION

MDV Infection in Chickens and Turkeys

Although MDV does not infect turkeys under natural conditions, this study confirmed and extended earlier work (Witter et al., 1974; Divsheli, 1974; Paul et al., 1977) and established that turkeys are highly susceptible to tumor formation upon experimental exposure to MDV. The neoplastic lymphoproliferation found in turkeys was similar to that routinely detected in chickens affected with MD (Eidson and Schmittle, 1968; Witter et al., 1974; Paul et al., 1977). The inflammatory changes seen in the MD lesions may have occurred because the virus was lethal to some cells and the necrotic debris attracted the heterophils. Infection with MDV was also accompanied by significant loss of body weight in turkeys, as has been reported in chickens (Purchase and Biggs, 1967; Payne and Rennie, 1973; Darcel, 1973; Purchase, 1974). Weight loss probably was caused by reduced feed intake in the sick birds.

Susceptibility of turkeys to oncogenic manifestations of MDV may be of importance in experimental investigation, because turkeys may be used as a model for the study of MD. This susceptibility stressed the importance of avoiding direct contact between turkey and chicken flocks to minimize the danger of exposing turkeys to chicken MDV.

Marek's disease virus was isolated from naturally infected (Witter et al., 1974) and experimentally inoculated turkeys (Paul et al., 1977). However, this is the first sequential study and the first direct comparison of titers of this virus in the blood of turkeys and chickens. The results indicated that the GA isolate of MDV, which was originally isolated from chickens, replicated better in chickens than in turkeys. Chickens had higher virus titers in blood and more chickens were viremic when compared to turkeys. On the other hand, Witter et al. (1974) reported that strain TK809, which was originally isolated from turkeys, replicated better in turkey cells when compared with chicken cells. These findings may indicate that MDV propagates better in the homologous host than in the hterologous host. The gradual rise in viral titers in turkeys seemed to peak at week 5, while in chickens the peak was a week earlier. The fact that after the fifth week the titers tended to decrease in turkeys suggests that viremia in turkeys may not persist as long as it does in chickens. Further evidence for this possibility was obtained in Experiment III of this study, where chickens and turkeys were inoculated with MDV at day 10. In that experiment, when birds were tested at day 35, all chickens were viremic, the average titer being 184 PFU/10^7 buffy coat cells, whereas only 1 out of 5 turkeys was viremic, with a titer of 3 PFU/10^7 buffy coat cells. The differences in the titers of virus in blood between the 2 species could be attributed to a different phylogenetic makeup of the 2 species. Such differences were observed even in chickens of different breeds (Cho, 1975, 1977).

The depressive effect of MDV infection on relative weights of lymphoid organs was more pronounced in chickens than in turkeys. Generally, the percentage of the bursa, thymus and spleen weights to body weights in inoculated and control chickens was greater than in turkeys. The percentage of the weights of the bursa was constant for the 3 groups of turkeys but increased with time in the control group of chickens. The thymic weight ratios decreased with time in the 3 groups of turkeys and increased in the group of control chickens. The percentage of the spleen to body weight in chickens and turkeys increased with time in inoculated and in control groups.

The significant enlargement of the spleen in turkeys and chickens was due to the hyperplastic lymphoreticular tissue. The decrease in bursa and thymic weight of virus-infected chickens was expected, in spite of the fact that they were antibody positive, because of the high dose of MDV administered. This high dose may have overwhelmed the partial protective effect of antibodies so that the bursa and thymus could be adversely affected. Similar results were reported by Payne and Rennie (1973), Jakowski et al. (1969), and Payne (1967). However, Evans et al. (1971), who used susceptible chickens of undefined antibody status, reported an increase in spleen weights but found no significant differences between the bursas of inoculated and uninoculated groups. In addition, several workers (Purchase and Biggs, 1967; Jakowski et al., 1970; Purchase, 1970; Payne and Rennie, 1973; Evans et al., 1971; Payne et al., 1976) reported inflammatory-degenerative changes, and this work supports their findings. However, the degenerative changes were somewhat less in turkeys compared to chickens.

These previously unreported findings of MD in turkeys indicate that turkeys may respond to MDV in a manner similar to that of resistant chickens and chickens with maternal antibody. The effects of MDV infection on bursal and thymic weights in chickens were also more pronounced at a time coinciding with the time of maximum viral activity. This was also reported by others (Phillips and Biggs, 1972; Payne and Rennie, 1973).

In chickens, significant depression of the response of whole blood cells to Con A was seen during the overt development of the lymphomas (Lee et al., 1978b). The T-cell response to Con A or PHA was correlated with the immune competence and was found to be impaired in the presence of virus infection in many animals, including chickens (Dent, 1972). Lee et al. (1978b), using a whole blood microassay to sequentially monitor T-cell reactivity of peripheral blood lymphocytes to PHA, found an early depression in the response followed by varying degrees of recovery. However, they showed a second depression in PHA response at a later stage of infection, particularly in chickens with gross lesions. Similar correlations were reported by Burg et al. (1971), Alm et al. (1972) and Lu and Lapen (1974) using splenic T-cells and PHA. This study did not examine Con A response during the early stage of infection. The significant depression in response probably corresponded to that described during late infection in the study of Lee et al. (1978b). The finding in chickens is in agreement with Alm et al. (1972), Burg et al. (1971), Evans and Patterson (1971), Greaves et al. (1968), Kleven et al. (1972), Lee et al. (1978b), Payne (1970), Theis (1977), and Theis et al. (1975). The impairment of Con A response was detected at 24 days after infection in turkeys, although a depression later in the course of infection cannot be ruled out.

The result of MDV infection in chickens was clearly a suppression of the humoral response as indicated by the reaction to SE and *B. abortus*. These findings agree with previous work reported by Burg et al. (1971), Jakowski et al. (1973), Evans and Patterson (1971),

Kleven et al. (1972), and Payne (1970). These workers reported a reduced response to various bacterial agents. Purchase et al. (1968) reported the reduction of the response by using bovine serum albumin as the challenge antigen. The significant enhancement of antibody response at 3 weeks in turkeys infected with GA-MDV is of interest, because similar enhancement was not noted in chickens. This enhancement appeared to be transient and disappeared later and a depression of the response in turkeys occurred instead.

HVT (FC126) Infection in Chickens and Turkeys

Several reports indicated the inability of HVT to induce gross neoplastic lesions in chickens (Okazaki et al., 1970; Witter et al., 1970b; Witter et al., 1976). Mild lymphoproliferative lesions specifically caused by this virus were documented by Witter et al. (1976). The data in this study confirm the presence of microscopic lesions in chickens inoculated with HVT. Recently cells containing Marek's disease tumor associated surface antigen (MATSA) and immune cells which *in vitro* are cytotoxic to MATSA-bearing MD lymphoma target cells have also been noted in chickens vaccinated with HVT (Calnek et al., 1979; Sharma et al., 1978). Although chickens had clearly detectable lesions, in comparison turkeys inoculated with HVT had minimal lesions. Lymphoproliferative lesions in turkeys were much less frequent than those in chickens and lacked a detectable neoplastic component histologically. Witter (1972a) was unable to detect gross or microscopic lesions in turkeys at 10 weeks after inoculation with HVT. In chickens, viremia was detectable within 1 week after inoculation with HVT; virus titers peaked between 1 and 2 weeks and then gradually declined. Other workers have reported similar results (Churchill et al., 1973;

Zygraich and Huygelen, 1972; Witter et al., 1976). Viremia in turkeys had a similar pattern to that in chickens, except that turkeys had higher titers at peak levels and the titers declined more sharply. Unlike chickens, viremia could not be detected in some of the turkeys. A similar finding was previously reported by Witter and Solomon (1972). Reduced incidence of lesions and absence of detectable viremia in some turkeys indicate that this species is relatively resistant to experimental infection with HVT, an observation somewhat inconsistent with the fact that under natural conditions HVT occurs only in turkeys (Purchase et al., 1972; Witter et al., 1970b). Witter and Solomon (1972) also noted that turkeys appeared less susceptible than chickens to HVT-infected DEF cultures.

It was documented that vaccination of chickens with HVT had beneficial effects on their body weights (Honegger et al., 1972; Purchase et al., 1972; Biggs et al., 1972; Lee et al., 1978a). However, Colwell and Rose (1977) found that HVT significantly decreased the body weights of vaccinated chickens. In contrast to Colwell's finding, the present results showed that HVT has no significant adverse effect on body weight. There are no published data on the effect of HVT infection on body weight in turkeys, and the present study represents the first evidence that HVT infection significantly suppressed body weight gains of turkey poults soon after infection. Because HVT replication in turkeys was persistent, the decrease in weight might also be expected to be persistent. If so, it might have far-reaching practical implications to the turkey industry because HVT is ubiquitous in the field and turkeys acquire HVT infection early in life. However, the long-range effects of HVT infection on body weight are not really known. Clearly, further investigations are needed to confirm the presence of such a problem in the field and to delineate its dimensions. The desirability to control the natural infection is obvious if the lower incidence of MD in turkeys is not attributed to a protective effect of this ubiquitous infection.

The lack of a significant increase in the ratios of chicken bursal weights to body weights and the absence of detectable cytolytic activity confirm previous studies by Calnek et al. (1979), who reported reduced bursal weights in line P chickens inoculated with MDV isolates JM-10 or CU-2 but not with HVT-4 or SB-1 (Calnek et al., 1979; Smith and Calnek, 1974; Schat and Calnek, 1978; Bradley et al., cited by Payne et al., 1976). In the turkey, unlike the chicken, there was an initial reduction in bursal weight during the first 3 weeks, followed by a significant increase later. Thus, HVT was more lytic for bursal cells in turkeys than in chickens.

The effect of HVT infection on thymic weight has not previously been reported. The size of the chicken thymus was increased more by HVT infection than was the turkey thymus. Turkey herpesvirus infection caused a significant enlargement of the spleen in chickens and turkeys. Calnek et al. (1979) reported enlarged spleens in chickens infected with HVT.

A significant reduction in the primary antibody response to SE was noted in chickens after infection with HVT. This observation is in conflict with that of Schierman et al. (1976), who found no significant effect. This difference may reflect differences in experimental conditions, such as the time of testing or the strain of chickens used. In the turkey, antibody response was not significantly affected by infection with HVT. Thus, HVT may be immunosuppressive to the B-cell system in the chicken but not in the turkey.

The findings in this study also indicated that HVT infection in chickens does not have a significant effect on cellular immune function as measured by the mitogenic response of peripheral blood lymphocytes to Con A. This result is in agreement with Schierman et al. (1976), who found no difference in homograft rejection time between HVT-infected chickens and uninoculated controls. Lee et al. (1978b) found that in chickens, HVT depressed PHA response 2 weeks after infection, but in 1 of 2 replicates of HVT-inoculated chickens a significant increase in the mitogen response was detected after week 3 following inoculation. In the present study, there was no significant decrease in mitogenic responsiveness in infected chickens. In the turkey, HVT caused a transient but significant depression of the response of blood cells to Con A. This initial marked depression of cell-mediated immunity may be one factor helping this virus to establish itself in flocks of turkeys.

Effect of Challenge with GA-MDV on Vaccinated Chickens and Turkeys

This is the first study to report the ineffectiveness of HVT to protect its natural host, the turkey, against challenge with the pathogenic GA isolate of MDV. Divsheli (1974), in studying the effect of HVT against turkey MDV, found gross and microscopic lesions in 50% of his vaccinated turkeys, compared to 80% in the unvaccinated group. However, his inoculum had at least one log less PFU of MDV than in the present study and the period between vaccination and challenge was 3 weeks. Paul et al. (1977) speculated that MD was not a problem in turkeys in the field, because turkey flocks harbor HVT and might be protected against pathogenic MDV lymphoma. However, the results of this study strongly indicate that the presence of HVT may not be responsible for lack of MD outbreaks in turkeys. Because the mechanism of prevention of MD lymphoma formation by HVT is not yet fully understood, it is difficult to speculate on the failure of protection in turkeys at this time, and further work might offer an explanation.

The failure of isolation of MDV from the blood in most HVT-vaccinated and MDV-challenged chickens and the marked decrease in titer in those that remained viremic appears consistent with earlier reports (Purchase et al., 1972; Purchase and Okazaki, 1971; Witter et al., 1976; Spencer et al., 1974; Churchill et al., 1969b; Eidson et al., 1971).

Although HVT was isolated from all vaccinated and non-challenged chickens examined, the average titer was quite low. The fact that HVT titers decreased with time confirmed the reports of Purchase et al. (1972), Witter et al. (1976), Witter and Offenbecker (1978), and Cho (1977). Turkey herpesvirus and MDV in the blood of vaccinated and challenged turkeys was tested for at day 35. Marek's disease virus had disappeared from the blood of the vaccinated group, and in the unvaccinated group only 1 out of 5 remained viremic and had a low titer. Thus, MDV disappearance was faster in turkeys than in chickens. Viremic responses associated with HVT had a similar pattern to those of chickens, although the titers were lower.

The high degree of protection in vaccinated chickens agrees with the well documented protection offered by HVT against MD (Eidson et al., 1973; Okazaki et al., 1970; Purchase et al., 1972; Zanella et al., 1975; Witter et al., 1976; Witter and Burmester, 1979). Although a very high dose of the vaccine was administered, protection was not absolute. This might be because of the high dose of MDV used to challenge HVT's protective ability complicated by the short period between vaccination and challenge. It was previously established that there was no substantial difference in protection against MD when birds were vaccinated with as low as 500 PFU or as high as 10,000 PFU (Purchase et al., 1972; Okazaki et al., 1970; Eidson et al., 1973). However, in many published tests of potency, an interval of at least 2 weeks was allowed between vaccination and challenge to obtain a satisfactory protection (Okazaki et al., 1970; Patrascu et al., 1972; Purchase et al., 1972; Zygraich and Huygelen, 1973). When the interval was reduced, less satisfactory protection resulted. Okazaki et al. (1971) also found that when the challenge was done immediately after vaccination, the protection was less satisfactory than when it was done a week later. In this study the time and dose of challenge were chosen to emphasize any differences between the species if they occurred.

RAV-1 Infection in Chickens and Turkeys

In this study, neoplastic transformation of bursal cells was clearly detected in turkeys infected with RAV-1, thus indicating that this species is susceptible to the transforming effect of chicken LL viruses. This finding may have important practical implications as far as "turkey leukosis" is concerned. The etiology of turkey lesions is not clearly understood, and the diagnosis of this syndrome is generally made on the basis of pathology only (McKee et al., 1963; Simpson et al., 1957; Campbell, 1972). Reticuloendotheliosis virus has sometimes been associated with turkey leukosis (Paul et al., 1976). Recently Witter and Crittenden (1979) reported on the similarity of lesions of reticuloendotheliosis to those of lymphoid leukosis in chickens. Other neoplastic lymphoid lesions were associated with unclassified RNA viruses (McDougall et al., 1978a,b; Biggs et al., 1978). Thus, in the light of this study it becomes important to consider lymphoid leukosis virus as a potential etiologic agent of lymphoid neoplastic diseases in turkeys.

Although the earliest transformation due to LLV in chickens starts in the bursa of Fabricius by 4 to 8 weeks when infection occurs at hatching, metastasis and gross tumors in visceral organs only appear by 16 to 24 weeks. However, previous studies on the pathogenesis of RPL-12 virus-induced tumors in chickens indicated the presence of lymphoid abnormalities in the spleen, liver and pancreas as early as 23 days after infection (Lucas et al., 1954a,b). Calnek (1968) observed lymphoproliferative lesions in the spleen, heart, and testes and the dorsal root ganglion of chickens 4 to 10 weeks following inoculation at day 1 of age with RPL-12 virus. Early lesions similar to those reported previously were detected in chickens and turkeys in this study in the spleen, heart, liver, gonads, and kidney between 20 and 64 days after infection at 1 day of age. These lesions seemed to be dose dependent, as they were more intense in the high-dose group than in the low-dose group. Since lesions are transitory in nature, they may not have been observed by many workers. The increase in microscopic ectopic lymphoid foci in this study is in agreement with Lucas et al. (1954a,b), Witter (1964) and Calnek (1968). The turkey seemed more susceptible to the development of early lesions of RAV-1 infection than chickens. The lymphoreticular foci in the spleen were more conspicuous in turkeys and were characterized by hyperplastic and degenerative changes. Although immature cells were seen and their infiltrative and destructive nature was evident, the lesions lacked characteristics of neoplasia. The cellular reaction was greater and lesions were more frequent in turkeys than in chickens. Also, some mortality was associated with these lesions. There is no previously detailed study of such lesions in the turkey. Only one study reported the presence of lymphoid foci in the pancreas, spleen and liver of the turkey similar to those in the chicken (Lucas et al., 1954a,b). The significance of the early lesions due to LLV and their impact on the development of neoplastic lesions is not known.

The lesions in the heart, although more frequent in the turkey, did not persist, while those in the chicken were less frequent but seemed to persist after bursal transformation was evident. This persistence was also reported by Calnek (1968) and Witter (1964). Another previously unrecognized aspect of RAV-1 infection in chickens and turkeys was increased lymphoid follicle development in the spleen and bone marrow. In addition, degeneration was seen in many bursas and thymuses of infected turkeys, particularly in those that died of the infection, but not in chickens. Whether these degenerative changes were specific for the turkey is not known. Some of the early lymphoproliferative lesions caused by RAV-1 were somewhat similar in character to those caused by MDV, particularly in the chickens.

In this study, there were more cases of erythroblastosis in the group of chickens receiving the high dose (IV) than in those receiving the low dose (IP). This agrees with the findings of Burmester and Gentry (1956) and Burmester and Purchase (1970), who reported a higher incidence of erythroblastosis with an increased dose (IV) in line 15I chickens.

Almost all chickens examined later than 87 days after inoculation with RAV-1 had macroscopic tumors in the bursa and occasionally bursas were atrophied. These findings are in agreement with those of Dent et al. (1967), Cooper et al. (1968), and Burmester (1969). As noted previously, Purchase (1976a) and Purchase and Burmester (1978) reported the number of chickens with visceral tumorous involvement was less than those with bursal tumors. Purchase (1976a) attributed the arrest of growth of tumor cells in extra bursal sites to immunological intervention and indicated that the presence of normal lymphoid cells in and around tumors may arrest tumor progression. He also speculated that metastasis may be due to the lack of humoral or cellular immune response to the tumor antigen, or perhaps because the immune response is ineffectual possibly due to blocking antibodies or suppressor T cells.

In turkeys, in contrast to chickens, no gross tumors were seen in the bursa or visceral organs. All turkeys used in this experiment were males, while the chickens were males and females. Burmester (1945) noted that lymphomatosis was twice as common in females as in males. Cooper et al. (1968) found that males were more resistant to infection than females and that bursal regression occurred approximately 1 month earlier in males than in females. This was attributed to a direct or indirect hormonal effect on the bursa of Fabricius (Purchase and Burmester, 1978). Burmester and Nelson (1945) found that castration of males and females increased the incidence of lymphoid leukosis and testosterone increased the resistance of males and capons. It is likely that the above factors may have similar effects in turkeys, thus explaining in part the failure to develop tumors during the period of observation in this study. Also, RAV-1 may have a longer incubation period for transforming bursal follicles in turkeys.

This study presents the first reisolation of RAV-1 from experimentally infected turkeys. The natural host for this virus is the chicken, and it has not been isolated from other avian species except pheasants (Purchase and Burmester, 1978). It seems that immunologic tolerance might be more difficult to achieve in hatched turkeys compared to chickens, as indicated by the disappearance of detectable virus and development of antibodies in all turkeys tested in inoculated groups. The presence of antibodies in 3 turkeys in the control group might have been caused by exposure to virus due to failure to maintain isolation. The rapid disappearance of virus from the blood of turkeys might be the reason why lymphoid leukosis is not a serious problem in turkeys in the field. In other words, the innate resistance of turkeys may be great enough to overwhelm the oncogenic potential of LLV. Other unknown factors may also play a role.

The significant decrease of body weight in each of the infected groups of chickens agrees with the well established fact that leukosis causes emaciation and weakness of infected birds (Purchase and Burmester, 1978; Purchase, 1976a). Of interest is the finding that RAV-1 had a similar adverse effect on body weights of turkeys.

The significant increase in chicken bursal weights near 40 days after infection in both infected groups was most likely caused by the tumorous involvement in most of the infected chickens. Cooper et al. (1968) reported a similar finding. On the other hand, the bursal weight in turkeys was not significantly affected, presumably because turkeys did not develop gross bursal tumors. Decreased splenic weight in turkeys infected with RAV-1 may be due to lysis of splenic cells by the virus or movement of splenic cells from the spleen.

The significant depression of the primary responses to SE and *B. abortus* at 3 weeks after inoculation and before the appearance of transformed follicles in the bursa of Fabricius in RAV-1 infected chickens agrees with Peterson et al. (1966). They reported decreased primary responses to T2 bacteriophage and bovine serum albumin (BSA) in chickens 4 weeks after inoculation at hatching. Dent et al. (1968) also reported a depression of humoral responses at an early stage of infection. In the present study, the depression of humoral response was significant in both treatment groups, although the high dose that was administered intravenously produced greater depression of responses to both antigens than did the lower dose that was administered intraperitoneally. Thus, depression of humoral immunity may be a function of the amount of replicating virus that precedes bursal transformation. The significant elevation of the primary response to *B. abortus* in each of the infected groups of chickens was unexpected and is difficult to explain. The immunologic defect in LLV infection is not yet clearly defined, and the speculations center around antigenic competition and specific interference by the virus with the B cells' ability to produce antibody (Payne, 1970). More work is needed to sequentially characterize the responses at various stages of the disease in order to understand the mechanism of immunosuppression of LLV. Under the conditions of this experiment, the humoral immune system was not adversely affected in turkeys inoculated with RAV-1.

The results also indicated that cell-mediated immune responses in LLV-infected chickens were not significantly affected at 10, 20, 83, and 103 days after infection. Previous studies to assess cellular immunity in LLV-infected chickens have produced inconsistent results (Sharma, 1979; Purchase and Burmester, 1978). Graft versus host was reported to be unaffected or reduced depending on the strain of chicken used (Sharma, 1979). The same strain of chickens that showed depressed response in graft versus host tests did not show this depression when tested with another LLV strain (Dent et al., 1968). Skin graft rejection was either delayed, enhanced, or remained unaffected (Purchase et al., 1968). In one *in vitro* study using PHA, a depression of cellular immune response of LLV-infected chickens was reported (Meyer et al., 1976). However, the chickens used by Meyer et al. were congenitally infected with LLV of subgroup A and the depressed response was detected only when suboptimal doses of PHA were used. Unlike chickens, cellular immune response in turkeys was depressed by RAV-1 infection. The mechanism of this immune depression was not investigated.

SUMMARY AND CONCLUSIONS

Four experiments were conducted to compare in chickens and turkeys the pathogenesis of and immunosuppression by Georgia (GA) strain of Marek's disease virus (MDV), strain FC126 of turkey herpesvirus (HVT) and Rous-associated virus-1 (RAV-1) strain of lymphoid leukosis virus (LLV). One-day-old chickens and turkeys were each inoculated intravenously or intraperitoneally with varying doses of viruses. The observations included body and organ weights, gross and microscopic pathology, development of viremia and viral antibodies, primary and secondary antibody responses to sheep erythrocytes (SE) and *Brucella abortus*, and blastogenic response to Concanavalin A (Con A) of whole blood lymphocytes.

Marek's disease virus inocula were pathogenic for chickens and turkeys, resulting in high mortality (78.8% for chickens and 72.7% for turkeys). Lesions were induced in all infected chickens and in 90% of the turkeys during a 60-day observation period. Body weight gains were significantly depressed in both species. The depressive effect on relative weights of lymphoid organs was more pronounced in chickens than in turkeys, but the spleen was enlarged in both species. Viremia remained persistent in both species, but viral titers were higher in chickens than in turkeys. Although MDV infection resulted in decreased antibody responses to SE and *B. abortus* in both chickens and turkeys, turkeys showed significant enhancement of antibody production during the early stage of infection. The cellular immune response was also adversely affected in the 2 species.

Turkey herpesvirus did not induce gross lesions in chickens or turkeys. Histologically, lymphoproliferative lesions were found in both species but were more numerous in chickens. The body weight was adversely affected in turkeys but not in chickens. Spleens and bursas were significantly heavier than controls in turkeys. Turkey herpesvirus was immunosuppressive to the B-cell system in chickens but not in turkeys. Infection with HVT caused a significant transient depression of mitogenic response in turkeys but not in chickens.

Vaccination with HVT protected against pathogenic MDV in chickens, whereas similar vaccination was ineffective against MD in turkeys.

Infection with RAV-1 led to neoplastic transformation in the bursal follicles in chickens and turkeys. However, gross tumors were detected only in chickens during the 97-day observation period. Early lymphoreticular proliferative and infiltrative lesions accompanied by mortality occurred in turkeys as well as in chickens but were more pronounced in turkeys. Viremia was persistent in chickens but disappeared early after infection in turkeys.

Infection with RAV-1 caused a significant decrease in body weights of chickens and turkeys. The B-cell system was significantly depressed as early as 3 weeks after infection in chickens but not in turkeys. At week 10, antibody response in chickens was significantly elevated. The T-cell system was significantly depressed by RAV-1 infection in turkeys but not in chickens.

It was concluded that chickens and turkeys are susceptible to infection with MDV, HVT and RAV-1. There were differences in response between the 2 species, but the most notable difference was the inability of HVT to protect turkeys against MDV.

Turkeys may be a valuable tool for the study of avian oncogenic viruses.

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