Effect of lead on interferon production and antiviral effect of interferon on Marek's disease in chickens

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by

Vitolis Enrikas Vengris

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INTRODUCTION

The interferon system has become recognized as a major natural defense mechanism against viral diseases. It is believed to play an important role in the recovery from viral infections and serves to limit virus spread through the bloodstream (10, 110). There is good evidence that interferon and interferon inducers prevent some neoplastic diseases caused either by DNA (125, 160) or RNA (83, 111) viruses. The protective effect of interferon and interferon inducers against Marek's disease (MD) in chickens has not been extensively studied. Polyriboinosinic-polyriboctydlyc acid (polyI·polyC), a potent synthetic interferon inducer (54, 83), has been reported to inhibit or delay the appearance of a number of tumors in vivo (83, 108, 134) and in vitro (51, 141, 142). However, Nemes et al. (123) reported that polyI·polyC gave no protection against MD in baby chicks. Recently it was reported (16) that the administration of statolon parenterally to baby chicks within seven days after hatching has a prophylactic effect and is effective in improving the weight gains in chickens exposed to MD.

Interferon response may be altered by various factors. It has been reported that chemical carcinogens like urethan and polycyclic aromatic hydrocarbons depress interferon production in vivo (46) and in vitro (47). High concentrations of arsenicals inhibit both the synthesis and the action
of interferon (62).

Among the natural substances that man concentrates in his immediate environment, lead is one of the most ubiquitous (31). The most common sources of lead include lead-containing gasoline, used crankcase oil, machinery grease, lead-containing paints, pesticides and insecticide baits (31). Even with the increased awareness of lead toxicity today, it still continues to be a common toxicant of livestock, pets and man.

Lead interferes with numerous biological functions in the body (41). Subclinical doses of lead influence resistance to bacterial invasion and the immunologic reactivity of the host. Williams et al. (178) suggested that lead may inactivate antibodies. It was reported that subclinical intravenous (I.V.) doses of lead acetate increase sensitivity of the rat to endotoxin of various Gram negative bacteria by 100,000 times (147) and chicken sensitivity at least 1,000 times (161). Subclinical doses of lead nitrate suppressed mouse resistance to Salmonella typhimurium (81).

It is possible that lead has a suppressive effect on interferon synthesis or action and this could be one of the variety of mechanisms by which metals induce or activate the oncogenic viruses already present in the host.

The purpose of this study was to investigate the prophylactic and therapeutic effects of interferon and the interferon inducer statolon against MD in chickens. The
MD virus (MDV) can serve as a model for the study of DNA virus-induced neoplasms. Very little is known about the sensitivity of DNA viruses to interferon and the effect of interferon and interferon inducers on DNA virus oncogenesis.

An additional objective of this study was to determine the effect of long term lead exposure on interferon production in chickens.
LITERATURE REVIEW

Interferon

The role of a specific protein called interferon in the resistance of cells to viral infection was observed and described by Isaacs and Lindenmann (91) in 1957. They incubated cells from chorio-allantoic membranes with inactivated influenza virus and found an extracellular substance that interfered with the replication of live influenza virus. They concluded that the extracellular product had been produced in response to the inactivated influenza virus and that this substance could confer viral immunity on other cells.

Subsequent work by many investigators in virology, biochemistry and molecular biology has added much information about interferon. It is now known to be a protein of cellular origin which is capable of initiating non-specific intracellular inhibition of viral replication (91, 110).

Interferon inducers

Originally only viruses were thought to be capable of inducing the production of interferon (91). Subsequently various other agents have been shown to elicit the interferon response. Gram negative bacteria and bacterial endotoxins (83, 110), rickettsiae (85, 110), protozoa (110), mitogens (83, 110), synthetic materials (83, 85, 110), chlamydia (85), fungi and fungal extracts (85), anti-
biotics (85, 110), statolon (100) as well as different live and inactive (85, 110) viruses have been shown to be interferon inducers. With the exception of viruses and certain synthetic materials, most of these agents are not highly effective interferon inducers (85).

Statolon, a fermentation product of a mould, *Penicillium stoloniferum* (100) represents one of the most effective interferon inducers known. Its interferon-inducing activity has been shown to be associated with virus-like particles found in the mould (52).

Viruses of all the major groups can act as interferon inducers (110). The most effective inducers are members of the orthomyxovirus, paramyxovirus and togavirus groups (58). In general, enveloped RNA-containing viruses seem to be the best inducers of interferon (83), but DNA-containing viruses also induce interferon (83, 85). The ability to induce interferon varies greatly among strains of the same virus (156).

The synthetic double-stranded RNA copolymer polyI·polyC is one of the most potent inducers of interferon and has high antiviral activity *in vivo* and *in vitro* (54, 55, 83). Another complex, polyriboadenylic-polyribouridylic acid (polyA·polyU) is less active and double-stranded complexes of DNA or deoxyribohomopolymers do not induce interferon (55, 110).

Some common structural requirements of the synthetic
inducers must be kept in mind, namely, a sufficiently high molecular weight (42, 55), regular and dense sequences of negative charges on a long-chain backbone (42) and intactness of the double-stranded complex (29, 42). The integrity and molecular weight of polyl is much more important than that of polyC in determining the antiviral activity of the polyl−polyC complex (28). The potential utility of double-stranded RNA's as antiviral agents is constrained in part by a wide spectrum of toxicities (83). The toxicities appear to be related primarily to the presence of double-helical RNA (28, 29); the single-stranded compounds have little or no toxicity (131). Since polyC appears to have a "passive" role in induction, it is the candidate strand for further modifications to permit accelerated hydrolysis of the complex (28).

Other synthetic substances such as pyran copolymers (118) and polyacrylic acid (49) have recently been reported to induce interferon. The pyran copolymer appears to be of limited practical interest because of the large dose required to induce interferon and because of the lack of biodegradation (83).

Tilorone hydrochloride has been reported to be an orally active antiviral agent against different RNA and DNA viruses in mice (104). Recently it was reported that tilorone hydrochloride is neither safe nor effective in man (95).
Host response to interferon inducers

Interferon has been produced by and shown to be active as an antiviral system in the living host or cells of many species of vertebrates (110). Viral inhibitor substances resembling interferon have been described in plants too (137).

There is a marked difference in interferon-producing ability between species. Even individuals from the same species have been found to differ considerably in their capacity to produce interferon (58).

The spleen, bone-marrow, lung and to a lesser extent liver and peripheral lymphocytes are the most important sites of interferon formation in vivo following intravenous (I.V.) injection of inducers (58, 85). The relative importance of those tissues may differ for different inducers, animal species, age and routes of injection (26, 58, 110). Statolon administered intratracheally (I.T.) in chickens was practically ineffective as an interferon-inducer in the trachea or serum (56, 132). However, when administered I.V. or intramuscularly (I.M.) it induced high levels of circulating interferon (56, 132). On the other hand, polyI-polyC given I.V. did not produce circulating interferon, nor did it protect the chickens from challenge with the influenza virus (132). It was reported that although polyI-polyC is a relatively poor interferon-inducer in chickens when given I.M. or I.V., it can induce interferon and viral resistance in the trachea when given locally (56).
A number of different inducers stimulate production of interferon in chickens. Viruses like Newcastle disease virus (NDV) (11, 113), Marek's disease virus (MDV) (187), Chikungunya (11), Sindbis (11), influenza (11) as well as statolon (56, 132) are potent inducers. Tilorone hydrochloride (132) and polyI·polyC (56, 132) are poor inducers of circulating interferon in chickens.

The presence of a small amount of interferon or the absence of detectable interferon in serum is not an indication of protection or lack of protection (56). Interferon protection can be confined to a specific organ depending upon the interferon inducer used and its route of administration (56).

**Mechanism of interferon production and action**

The mechanism of interferon production is not yet understood and two hypotheses for the mechanism of induction have been proposed. One involves the de novo synthesis of interferon and the other the release of preformed interferon (110). Living agents such as viruses appear to induce synthesis of interferon de novo by derepression of the host genome to form a mRNA for synthesis of the corresponding interferon molecule (110, 112, 167). Biological extracts such as endotoxin and synthetic polyanions have been thought to release preformed interferon (110, 167). The distinction between the two patterns of induction has been based on
relatively indirect arguments such as a different sensitivity to inhibitors of RNA and protein synthesis and different kinetics of interferon production (42). A likely possibility is that each type of response requires de novo synthesis but that during viral induction additional steps occur such as uncoating of virions and formation of double-stranded RNA, which are more sensitive to metabolic inhibitors than interferon itself (43).

The observation of the phenomenon of superinduction in which inhibitors of protein synthesis actually enhanced interferon production (30) cast doubt on the de novo and preformed interferon induction hypotheses. To explain this enhancement it was speculated that metabolic inhibitors might prevent synthesis of a repressor substance which normally shuts off interferon production (167, 168). The superinduction phenomenon in vivo cannot be entirely explained by inhibition of synthesis of a repressor (30). It is possible that interferon is one of the several proteins which may be released into the circulation when cells are disrupted by cycloheximide or other compounds (30). Cycloheximide causes low level interferon production in vivo but not in vitro (30). It is possible that low levels of interferon produced by various stimuli (e.g. gut bacterial endotoxin or viral infections) in animals keep the clearance mechanism at least partially saturated with interferon which is released by the
cell-destructive effects of cycloheximide (30).

The derepression hypothesis of interferon production is widely accepted but it is not proven. None of the genetic elements of the interferon system have been isolated or characterized and likewise the molecule which triggers the depression of the host genome is not known for certain (112). The high interferon-inducing capacity of double-stranded RNA of both viral and synthetic origin in contrast to the inactivity of single-stranded RNA and double-stranded DNA was the basis for the hypothesis that the double-strandedness of RNA provides the final derepression for formation of mRNA and interferon (55). In 1969 Colby and Duesberg (39) demonstrated the presence of a virus-specific double-stranded RNA in chick cells infected with vaccinia virus which could explain the stimulation of interferon by DNA viruses. Bakay and Burke (8) think that neither viral DNA nor RNA are necessary for interferon formation with DNA viruses. They feel that some virus function, as yet unidentified, is necessary for interferon production. Lockart (112) stated that he could find no evidence indicating that double-stranded RNA or viral protein is responsible for induction of cells to produce interferon. He believes that the cell itself provides inducer molecules to a wide range of stimuli (112).

The mode of interferon action has been studied in viral infections and it was observed that interferon does
not act directly on virus (60, 84, 112). Interferon does not prevent virus attachment to the cell or penetration (60, 84, 93). Instead, it blocks the synthesis of new virus within the cell (60, 84, 93).

Experiments with protein and RNA synthesis inhibitors suggest that interferon might induce the synthesis of another protein by derepression of the host genome and transcription of the specific mRNA (60, 84, 93). This second protein, translation inhibitory protein (TIP), prevents the synthesis of new virus (60, 84, 93). Interferon or hypothetical TIP may act at the ribosomal level by interrupting the synthesis of viral-coded proteins which are required for virus multiplication (60, 84, 93). Ribosomes of interferon-treated cells have not been observed by some workers to form polypeptide complexes with the viral mRNA (84, 93). Others reported that ribosomes would combine with but not translate viral mRNA (116).

Interferon has been observed to have protective effect against phylogenetically higher organisms. Non-viral agents such as protozoa (140), bacteria (69), chlamydia (79) and rickettsiae (96) were reported to be inhibited by interferon and various interferon inducers. Interferon and interferon inducers suppress intracellular replication of Shigella flexneri in human and rabbit cell cultures (69). Since bacteria contain their own functional ribosomes, it now
seems unlikely that the ribosome is the only target of inter­feron action. Huang et al. (89) have proposed that interferon and interferon inducers suppress phylogenetically higher organisms by enhancement of phagocytosis.

Recent evidence implicates transcription of viral RNA as the basic defect in the virus replication mechanism in interferon-treated cells (14). It was also reported that interferon preparations have a growth-depressing effect on L-cells by inhibiting DNA synthesis (150). The authors hypothesize that viruses which are sensitive to interferon have as a requirement for their replication some mechanism which is modified by interferon and which is normally associated with cellular DNA synthesis (150).

It is apparent that an important factor which determines the susceptibility or resistance of a given virus to interferon is the host cell in which the virus infection occurs (78). Youngner et al. (180) reported that in the same cell a DNA virus can be refractory to interferon and a RNA virus can be susceptible. They raised the possibility that separate resistance factors may exist for RNA and DNA viruses (180).

Factors influencing the induction, production and action of interferon

The interferon response may be altered by various factors. Stress (154), hormones (60, 133), mycoplasma (152),
arsenicals (62) and chemical carcinogens (46, 47) have suppressive effect.

Corticosteroids given in amounts above the usual physiological level inhibited the interferon response to endotoxin (133) and virus (154).

Chemical carcinogens like urethan and polycyclic aromatic hydrocarbons depress interferon production in vivo (46) and in vitro (47). The noncarcinogenic counterpart benzo(e) pyrene did not affect the interferon response (47). This suggests a correlation between carcinogenicity and inhibition of interferon synthesis (47).

High concentration of arsenicals inhibited both the synthesis and the action of interferon (62), whereas low concentrations increased the antiviral activity of low levels of interferon (62).

Development of tolerance or refractoriness to repeated stimulation by interferon inducers is an important phenomenon in animals (85). Stimulators, be they virus, endotoxin or polyI·polyC, cause tolerance in animals which then remain tolerant to subsequent injection for 4 to 13 days after which they are again sensitive (85). Dextran has been reported (50) to eliminate the hyporeactive state. The mechanism of refractoriness is obscure. Some authors feel that interferon production somehow produces a negative feedback for its own production (85). A humoral factor has
also been suggested (42) while Vilcek (167) has proposed that an endogenous cellular inhibitor participates in refractoriness.

Properties of interferon

The physical and chemical properties of interferon have been determined by studying impure preparations and the extent to which impurities have contributed to the observed properties is as yet unknown (53).

The protein nature of interferon was first described by Isaacs and Lindenmann (91). Interferon is retained by dialysis membranes (91), it is inactivated by proteolytic enzymes (91), but not by nucleases or ether (53, 91) which suggests the absence of nucleic acids and lipids as essential components of interferon. Only little is known about the chemical composition and it is generally agreed that interferons are or at least contain protein (53) and a carbohydrate moiety (53).

Interferons are stable over a wide range of pH ranging from pH2 to 12 (53), they cannot be sedimented by centrifugation at 100,000g (53, 91) and they are relatively heat stable (91). The isoelectric point varies from pH 5.0 to 10.0 (53) depending on its origin, inducer and host system (53). Disulfide bonds are required for interferon activity (119). Some, but not all, interferons are inactivated by urea (119).
Interferons are heterogeneous with regard to molecular weight and the presence of multiple molecular species in a single interferon preparation has been reported (53). The range of molecular weight of interferon is very wide and varies from 28,000 to 160,000 (60). Carter and Pitha (27) think that human and mouse interferons are not a group of heterogeneous proteins. Rather they exist in multiple oligomeric forms and different inducers seem to trigger the release of different oligomeric species (27).

Several properties are shared by all interferons. Their mode of biological action is essentially species specific (58, 91). However, there are a few exceptions to the species specificity rule. Cross reactivity has been observed among species as different as mice and chickens (67). A clear exception to this general rule is the cross reactivity between interferons of man and rabbit (48); viral-induced interferon from human skin fibroblasts showed 20 times more antiviral activity in rabbit kidney cells than in the human cells (48).

It appears that interferon and antibody production are both highly specific functions of the lymphoid and reticuloendothelial (RE) systems and not merely consequences of non-specific stimulation of the RE system (43). Although interferons are proteins, they are poor antigens (127).

Interferons are active in a broad variety of viral
infections (42, 91, 110) and a large number of different viruses, both RNA and DNA are susceptible to interferon action.

Interferon has been observed to have protective effect against phylogenetically higher organisms (69, 79, 96, 140).

Interferon may act as a primer for its own production. Pretreatment of cells with interferon prior to viral induction results in earlier release and higher titers of interferon even by viruses which fail to induce without pretreatment (9). Interferon-containing preparations were found to enhance phagocytosis (89).

**Biological role**

It is widely assumed that interferon plays a major role in recovery from viral infections (10, 110). Measurable antibodies appear later and are more active in inhibiting the late spread of virus through the organism or in preventing reinfection (10, 110). Persons who suffer from agammaglobulinemia or hypogammaglobulinemia experience great difficulty in combating bacterial infections but recover from most of the viral diseases (110). Interferon limits virus spread through the blood stream (10). It appears that interferon and antibody complement each other in their function in the living organism (68). The action of interferon is limited by its rapid clearance from the circulation (110). In the serum of different mammals exogenous interferon I.V. injected has a
half life of 7 to 11 min. (86). The rapid loss from the circulation can be attributed to metabolism by cells and excretion (86). Interferon, while broad-spectrum in its action against different viruses, does require a close phylogenetic relationship between the host species used to produce the interferon and the species in which it is to be used (83, 110). All these limitations to the use of exogenous interferon in antiviral therapy suggest that endogenous stimulation may be another approach in practice (83). To be considered for application in clinical medicine the interferon inducers should be highly active, noninfectious, nontoxic and sufficiently nonantigenic to allow repeated use (83, 110).

A wide variation in the response of different viruses to inhibition by interferon has been observed (75). The togaviruses, vesicular stomatitis virus and vaccinia virus are considered the most sensitive viruses (75, 168). Herpesviruses, adenoviruses and the double-stranded RNA reovirus are relatively resistant (75, 168). Interferon susceptibility of viruses even of the same taxonomic group, varies depending on the virus strain, host system and inducer used (58, 168).

There is good evidence that interferon and interferon inducers can exert an antitumor effect in experimental animals inoculated with oncogenic viruses (73, 105, 108, 130, 134, 160, 175), with transplantable tumor cells (74) or with chemical carcinogens (111).
Repeated interferon administration significantly increased the survival time of mice infected with Rauscher virus (73) and markedly increased survival of tumor-inoculated mice (74). Exogenous interferon preparations inhibited cell transformation by mouse sarcoma (130) and SV 40 (160) viruses. Rous sarcoma and lymphoid leukemia viruses were found to be sensitive to the effect of exogenous interferon which inhibited their replication in tissue culture and the development of tumors in chickens (105). Wheelock and Larke (176) reported that Sendai virus, statolon or repeated injections of interferon, each significantly prolonged the life of mice when administered even 30 days after Friend virus inoculation, at the time when mice were beginning to die of leukemia.

PolyI·polyC was reported to inhibit or delay the appearance of experimental tumors in vivo (108, 134, 142) and in vitro (51, 141, 142). In addition carcinogenesis by dimethylbenzanthracene (DMBA) is strongly inhibited by suitable administration of polyI·polyC (111). However, polyI·polyC did not protect chickens against Marek's disease (123).

Statolon was reported (142, 175, 176, 177) to be very effective in inhibiting viral oncogenesis in mice. Statolon prolonged the life of mice even when it was administered 30 days after Friend leukemia virus (176). Recently it was reported (16) that the administration of statolon paren-
terally to chicks within seven days after hatching has a pro-
phylactic effect, improves the weight gains and reduces gross
lesions in chickens exposed to Marek's disease.

Gazdar and Ikawa (64) and Gazdar et al. (65, 66) re-
ported that polyI·polyC enhanced virus-induced sarcomas and
leukemias in mice and rats. Enhancement was dependent on
multiple factors including the mouse strains, age and time of
treatment. Pretreatment with polyI·polyC, polyA·polyU and
other polynucleotides enhanced tumor induction by Moloney
mouse sarcoma virus (MSV), but treatment with polyI, polyC,
and polyA·polyU·polyU had no effect. A correlation exists
between the MSV enhancement and the antiviral properties of
polynucleotides (64). Lack of correlation was observed be-
tween titers of circulating interferon and MSV tumor enhance-
ment (65, 66) and suppression (173). Tumor enhancement
could be due to alteration of immune response, increased
cell penetration or growth of tumor viruses (65, 65, 66).
Gazdar et al. (66) think that suppression of MSV oncogen-
esis by high doses or continuous treatment with polyI·polyC
may be mediated by a direct toxic or chemotherapeutic effect
of the polyI·polyC on the host or tumor cell.

The mode of antitumor effect of interferon and inter-
feron inducers is unknown. Does interferon inhibit the
replication of virus, multiplication of tumor cells directly
or does it in some manner enhance the host's ability to
reject the tumor? Some scientists feel that besides its
antiviral properties interferon increases the immune reactivity of the host (111). It was recently observed that interferon preparations enhance antibody formation and graft vs. host reaction (19). An apparent lack of correlation between serum interferon levels and antitumor activity has been observed (173). The mechanism of antitumor action of polyI•polyC and statolon is multifaceted (5, 111, 177). Since both are potent interferon inducers, it is likely that part of the antitumor action is through the interferon system (111). Synthetic polymucleotides have been shown to induce cellular resistance to VSV in the absence of detectable interferon in cell culture (169). Considerably higher concentrations of polyI•polyC are required to produce demonstrable interferon in cell culture (169). It has been assumed that cellular resistance produced by low polyI•polyC concentrations are mediated by endogenous cellular interferon (27, 169). PolyI•polyC was reported to enhance both humoral antibody formation and cell-mediated rejection (162). It also has direct chemotherapeutic action by inhibiting macromolecular synthesis in tumors in vivo, while having less inhibitory action on synthesis in normal organs (111). It is also suggested that rather than acting through interferon induction, low concentrations of polyI•polyC may directly interfere with virus replication by binding to the virus-specific RNA polymerase (98).

Badger et al. (5) believe that the polyI•polyC exerts
a cytotoxic effect by damaging the DNA as its primary antitumor activity. It was reported that cyclic AMP inhibits tumor cells in vitro and restores a normal phenotypic character in neoplastic cells exposed to cyclic AMP (88). Since interferon and interferon inducers increase activity of adenyl cyclase and cyclic AMP levels (19, 172) antitumor activity of interferon may also be attributed to cyclic AMP. Wheelock (177) indicated that the antitumor effect of statolon might be due to its capacity to restore immuno-competence to mice infected with Friend virus since this virus induces an immuno-depressive state.

Marek's Disease

Marek's disease (MD) first described by Marek (117) in 1907 is probably the most common lymphoproliferative disease of chickens. The etiological agent of MD was reported to be a cell-associated herpesvirus (36, 121, 155). Enveloped cell-free virus was extracted from the feather follicle epithelium (23, 122).

The MD virus can be propagated in cell cultures, in chickens or in embryonated eggs. Infection may result in cytopathic effect (CPE) as with chicken kidney or duck embryo fibroblast cells, or may be without observable effects (24). In vivo, the effects of infection vary from degeneration of lymphatic tissues to oncogenesis. The nature of the oncogenic response is not understood (24). Viral
plaques develop on the chorio-allantoic membranes (CAM) of chicken embryos following yolk-sac inoculation with MD virus (15). Blood or tumor suspensions inoculated into susceptible baby chicks are very often used for transmission of the virus (24).

Differences between isolates of MDV are apparent principally in their virulence and in tissue distribution of gross lesions (24). Serologically isolates appear to be indistinguishable (136) except for cell-culture-attenuated isolates which lack an antigen demonstrable in virulent isolates (37).

Feather follicle epithelium and perhaps feathers are thought to play an important role in the transmission of the disease by providing the enveloped virus, whether cell-free or within dead cells, which can survive in the environment or spread from bird to bird (12). Other potential means of virus spread include the darkling beetle (Alphitobius diaperinus) and possibly other vectors (24). The existence of carrier birds has been reported (149). Egg transmission is insignificant in spreading the disease among progeny (40).

The latent period is usually between three and four weeks in experimentally-induced MD in baby chicks (149). There is considerable variation in the latent period depending on virus strain, dosage, route of infection, genetic strain and sex of chickens (24). Clinical signs common to the disease are
those associated with asymmetric, progressive paresis and later, complete paralysis of one or more of the extremities. Since any one or several of the nerves in the body may be affected, signs vary from one bird to another (24). Depression, shrill cheeps, ataxia, gasping, dehydration and emaciation are very common (24, 102). Some birds die without extensive clinical disease (24). Blindness may result from involvement of the iris. Affected birds develop gross ocular lesions, show loss of normal pigmentation and paralysis of the iris (24, 102). Tumors are commonly found in the gonads, liver, heart, proventriculus, muscle and skin (102). Morbidity and mortality are related to the virus strain, dosage and route of exposure. Host sex, genetic constitution and age are very important factors (24). Organs associated with the immunological response, e.g. the bursa of Fabricius, the thymus and the bone marrow, are sites of early virus replication which can result in necrosis, atrophy and aplasia of the organs (22). The loss of lymphoid cells may have accounted for the decreased or delayed antibody response, lowered resistance to other disease and delayed homograft rejection all of which have been associated with MD (22, 128). According to Payne and Biggs (129) bursaectomy coupled with X-irradiations reduced the incidence but did not eliminate the disease in exposed birds. This is in contrast to the situation with lymphoid leukosis where the bursa of Fabricius is required for the development of the
disease (24).

Antibody to MD acquired naturally from the dam, or injected prior to, or at the time of virus inoculation, failed to prevent infection but early destructive lesions of hematopoietic tissue were largely prevented (22). The presence of passive antibody at the time of virus entry can reduce the subsequent incidence of neoplastic lesions (35). Maternal antibodies (if present) are depleted by about three weeks after hatching (24). Since chickens are most susceptible to MDV when very young, hyperimmunization of the dams in commercial flocks can reduce losses due to the disease. Witter et al. (179) described a nonpathogenic herpesvirus from turkey (HVT) which is used for vaccine production.

Biological Effect of Lead

**History**

Lead was one of the first metals discovered by man and has been widely used for domestic, industrial and medical purposes during the last two thousand years. Some of the clinical signs of lead poisoning were known to the ancients long before they were ascribed to the action of lead. Hippocrates (370 B.C.) was probably the first to recognize lead poisoning (4). He reported severe attacks of colic in a man who extracted metals. The Greek poet-physician Nicander first described the disease more than two thousand
years ago (4). Several reports about the development of lead colic appeared in the literature in the seventeenth century. Citois reported that wine contaminated with lead was the cause of colic (4). An experimental study of lead poisoning was conducted by Orfila in the nineteenth century. He administered lead orally as well as intravenously and reported that lead was more toxic orally (4). Gombault (70) reported a segmental demyelination in chronically poisoned guinea pigs. The soldering, painting and pottery industries constituted the largest industrial hazard during the nineteenth century leading to chronic lead poisoning in workers. Teleky (158), Oliver (124) and others did extensive work on the public and industrial hygiene aspects of lead poisoning.

The decline of lead poisoning cases in the last two or three decades is likely a result of improved hygiene and medical supervision of workers in the lead industry (76). Lead poisoning is still very common in ship and car building, storage battery and pottery industries (76). There is much evidence that lead wastes have been accumulating during the past century, particularly in congested urban areas (31). The most common sources are related to the burning of lead-containing gasoline, used crankcase oil, machinery grease, lead-containing paints, pesticides and insecticide baits. Lead is one of the most common causes of child poisoning (32).

The history of lead poisoning in animals is more
recent. Lead poisoning has been recognized as a factor in waterfowl mortality since the turn of the century (7). Morgan (120) in 1924 reported chronic lead poisoning in sheep and ponies and observed that animals might acquire a taste for lead. Gardner (63) reported development of ricketts in lambs when kept in lead-mining areas. Since then many reports have appeared in the literature concerning incidents of lead poisoning in many species of mammals (3, 33, 181) and birds (7, 38, 40, 159).

Very little is known about lead poisoning in chickens. Signs of poisoning ascribed to the feeding of lead ars enate were described in 1932 (159). Salisbury et al. (144) reported lead poisoning in chickens caused by feeding a grit which contained 32 per cent lead oxide. Simpson et al. (151) described abnormalities of erythrocytes and renal tubules of chickens poisoned with lead.

**Biological effects on the animal organism**

Although no population group is apparently as yet being subjected to levels of lead high enough to cause symptoms, it is clear that a continued rise in the pollution of the human environment with lead could eventually produce levels of exposure that could have adverse effect on human, plant and animal health (31). The scientific literature is replete with reports on the toxic manifestations, diagnosis and treatment of lead poisoning,
As far as is known, lead is not a trace element essential to nutrition but this particular question has not been adequately studied (31). The usual daily dietary intake of lead in adult man averages about 0.3 mg. Of this, about 90 per cent passes through the intestinal tract and is not absorbed. The small amount absorbed is also excreted and under normal conditions there is no net retention of lead in the body (97). Lead enters the body also through the respiratory tract and skin (97). When the rate of lead absorption exceeds the rate of excretion, higher levels of lead result in the blood and metabolic, functional and clinical responses follow. At the level of cellular metabolism the best known adverse effect of lead is its inhibition of the activity of enzymes that are dependent on the presence of free sulfhydryl (SH) groups for their activity (171). Lead interacts with SH groups in such a way that they are not available to certain enzymes that require them. In the living organism, under most conditions this inhibition is apparently partial (171). The clearest manifestation of the inhibitory effect on the SH enzymes is the disturbance lead causes in the biosynthesis of heme (31, 171). Lead is implicated specifically in the metabolism of delta-amino-levulinic acid (ALA) and in the final formation of heme from iron and protoporphyrin. Both of these steps are mediated by mitochondrial enzymes. Almost all the information which exists on the effects of lead on the
synthesis of heme comes from observations of red blood cells (RBC). Yet all cells synthesize their own heme-containing products, notably the cytochromes (31). In the blood the functional effect of lead is anemia (31, 171). The decrease in heme synthesis leads at first to a decrease in the lifespan of RBC's and later to a decrease in the number of cells and in the amount of hemoglobin per cell (171). Lead in ionic form has a great affinity for RBC's, especially for their membranes and this interaction at excessive levels of lead brings on alterations of the RBC's and shortens their lifespan (41). In compensation, the blood-forming tissue steps up its production of RBC's and immature red cells, reticulocytes and cells with basophilic stippling appear in the circulation (31, 41, 171). The stippling represents remnants of the cytoplasmic constituents of red cell precursors such as clumps of ribosomes and mitochondria (92). Basophilic stippling was not seen in birds with lead toxicity (38, 151). However, dumbbell, bottle, oat, sickle and teardrop forms (38) and various stages of mitosis were observed in RBC's of birds exposed to lead (151).

A significant positive correlation between coproporphyrinuria and the occurrence of lead in the urine and the blood was reported (157). An increase in protoporphyrin 9 in blood is one of the best indicators of lead poisoning (41).

The activities of enzymes other than those associated
directly with hematopoiesis are also influenced by lead. Increases in the activity of serum aldolase, serum glutamic oxaloacetic and pyruvic transaminases have been observed (170). Alkaline phosphatase activity diminishes significantly in experimental animals exposed to toxic doses of lead (101). Enzymatic alterations associated with toxic effects exerted upon cardiac and skeletal muscle, small blood vessels and the cells of the liver, kidney, intestine and salivary gland have been reported (41). Lead is capable of suppressing cellular oxidation-reduction processes and of inhibiting the active transport of high-energy organic phosphates in muscle (41). Marked inhibition of succinate oxidation and ADP-stimulated respiration in vitro was reported (25). It is assumed that lead interacts with the mitochondrial membrane (41).

In acute lead poisoning observable changes in the kidney and kidney functions were reported (31). Much of the excess lead is concentrated in the form of dense acid-fast inclusions in the nuclei of certain cells, including those lining the proximal renal tubules (72). These inclusions consist of a complex of protein and lead (72). Apparently inclusions serve as a protective device to keep lead in the nucleus, away from vulnerable mitochondria (72). Kidney dysfunction is expressed in what is called the Fanconi Syndrome, characterized by loss of amino acids, glucose and phosphate in urine because the damaged tubular
cells fail to reabsorb these substances (72). Long term plumbism was implicated as a cause of a chronic nephritis (107).

The toxic effect of lead on the central nervous system is little understood (31). Chronic overexposure to lead causes peripheral nerve disease, affecting primarily the motor nerves of the extremities. Mitochondria of the Schwann cells are damaged resulting in demyelination (31).

Striking changes were observed in the amount of various amino acids in the blood and liver of experimental animals exposed to lead (17). Decrease of alanine, cysteine, glutamic acid, leucine and methionine was very significant. Other amino acids, with the exception of lysine, were affected as well (17).

Caccuri (21) reported a distinct relationship between lead poisoning and the metabolism of nicotinic acid. Animals poisoned by lead exhibit a profound decrease of blood nicotinic acid content with the consequent reduction of NAD and NADP. Lead was reported to inhibit the biosynthesis of the DNA and RNA of E. coli (103). It is suggested that lead exerts serious effects upon the pyridine nucleotides, either by blocking their synthesis or by enhancing the degradation of nicotinic acid (41).

It was reported that lead poisoning interferes with lipid metabolism leading to morphologic changes in arterial
walls similar to those seen in atherosclerosis in rats (146).

Consistent body weight losses and emaciation were observed in birds which were given high doses of lead for prolonged periods (38, 151).

Metals rank among the most prominent industrial and environmental carcinogens. Arsenicals, chromium and nickel compounds are accepted as carcinogens for humans. Iron, as hematite and iron-dextran, is strongly suspected (13). So far lead has not been shown to be a carcinogen in man but lead compounds obviously induce tumors in animals. Cerebral gliomas (126) and renal tumors in rats (18) and mice (163) were induced with dietary lead.

Influence on immunologic responses

There is considerable evidence that toxic substances exert adverse effects upon the resistance of the body to disease by influencing immunologic mechanisms. Fonzi et al. (59) have reported that lead-treated animals, subsequently subjected to procedures of active immunization, developed lesser quantities of gamma globulin, complement underwent progressive diminution and anti-typhoid antibody titers were lower. The activity of lysozyme is reduced in the spleen and serum of dogs poisoned by the prolonged administration of lead (1). Interference with phagocytic activity of polymorphonuclear leukocytes has been reported in cases of lead toxicity in man (138). Williams et al. (178) suggested
that lead may inactivate antibodies. Lead has also been reported to bind antibodies \textit{in vitro} and could potentially do so under \textit{in vivo} conditions (178). This ability to bind proteins could also interfere with the functional activity of the properdin and complement systems (81). It was reported that the subclinical I.V. administration of lead acetate increases sensitivity of the rat to the endotoxin of various Gram-negative bacteria by 100,000 times (147) and chicken sensitivity at least 1,000 times (161). Mice treated with subclinical doses of lead nitrate for thirty days showed greater susceptibility to challenge with \textit{Salmonella typhimurium} than controls which received no lead (81).
CELL CULTURES

MDBK cells

The Madin-Darby bovine-kidney cell line (MDBK) was used for interferon assay and for pseudorabies virus (PVR) propagation.

The cells were propagated in 250 ml plastic tissue-culture flasks in Eagle's basal medium (BME) with Earle's salts, supplemented with 10 per cent newborn calf serum and 0.11 per cent sodium bicarbonate. After trypsinization with a 0.2 per cent trypsin- versene solution (see later), the cells were diluted 1:3 in growth medium and 2 ml were inoculated per well into 35 x 10 mm wells of plastic tissue-culture plates and 20 ml into 250 ml plastic tissue-culture flasks. Monolayers usually developed in 24 to 48 hours.

CEF cells

The primary chicken embryo fibroblast (CEF) cells were used for vesicular stomatitis virus (VSV) propagation, titration and interferon assay.

CEF cells were prepared from ten and eleven-day-old

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^aFalcon Plastics, Los Angeles, California.

^bGrand Island Biological Company, Grand Island, New York.

^cLinbro Chemical Co., New Haven, Connecticut.
embryos using a modification of a previously described technique (145). Eggs were obtained from the specific-pathogen-free flock maintained at the Veterinary Medical Research Institute, Iowa State University. Egg shells were disinfected over the air sac with 70 per cent alcohol and embryos were aseptically transferred into petri dishes containing saline G (see later). They were then decapitated, eviscerated and their feet and legs were removed. Embryos were then transferred to a 35 ml syringe and forced with the plunger directly into a trypsinization flask containing 0.2 per cent Ca-free trypsin\(^a\). Trypsinization was performed on a magnetic stirrer for 5 - 15 min. at room temperature until cells were dispersed. The trypsinated cell suspension was then centrifuged twice at 800 rpm for 10 min. and resuspended in growth medium at a concentration of 0.5 ml of packed cells per 100 ml medium. Growth medium consisted of BME with Earle's salts, supplemented with 5 per cent fetal calf serum\(^b\), 10 per cent tryptose phosphate broth\(^c\) (TPB) and 0.11 per cent sodium bicarbonate. One ml of cells suspended in growth medium was used to inoculate each disposable glass tissue-culture tube\(^d\) and 2 ml per well

\(^a\text{Difco Laboratories, Detroit, Michigan.}\)
\(^b\text{Grand Island Biological Company, Grand Island, New York.}\)
\(^c\text{Grand Island Biological Company, Grand Island, New York.}\)
\(^d\text{Kimble Glass Company, Toledo, Ohio.}\)
in the 35 x 10 mm wells of plastic tissue-culture plates. Monolayers usually developed in 48 to 72 hours. Primary CEF cells were passed up to five times.

Buffers and Diluents

**Saline G**

Sterile Saline G (135) was used as a virus diluent and during preparation of CEF cells.

**Phosphate buffer**

A 0.01M phosphate buffer (PBS) pH 7.5 was used as a dialysis solution for the serum and as the diluent in the hemagglutination (HA) and hemagglutination-inhibition (HI) tests.

To prepare the phosphate buffer 8.5 ml of 1M \( \text{Na}_2\text{HPO}_4 \) and 1.5 ml of 1M \( \text{NaH}_2\text{PO}_4 \) were mixed and made up to one liter with physiological saline solution.

**Buffer pH 2**

A 0.1M KCl-HCl buffer (pH 2) was used as a dialysis solution for chicken serum prior to interferon assay. This buffer was prepared by mixing 0.1M KCl and 0.1M HCl until the desired pH was obtained.

**Physiological saline**

Physiological saline solution was used to wash cell cultures during interferon assay by the dye-uptake method (57) and for interferon characterization experiments.
Mcllvaine-Lillie buffer

This buffer (pH 4.2) was used for dye extraction in interferon assays by the dye-uptake method. Stock solutions of 0.1M citric acid and 0.2M disodium phosphate (Na$_2$HPO$_4$·7H$_2$O) were made in 25 per cent methanol. Stock solutions were diluted 1:25 in water before use and mixed until the desired pH 4.2 was obtained.

A mixture of equal volumes of this buffer and absolute ethanol was used as the extraction fluid.

Water

The water used in all procedures and solutions was glass distilled and deionized. It routinely contained less than 0.05 μg/ml of sodium chloride equivalents as measured with a conductivity meter$^a$.

Maintenance Medium

Eagle's basal medium with Earle's salts supplemented with 2 per cent newborn calf serum and 0.11 per cent sodium bicarbonate was used for the maintenance of cell monolayers, for the cell washing and as a diluent in the interferon titrations. The medium was filter sterilized.

Trypsin-Versene Solution

A trypsin-versene solution was used to remove cells from the surface of the flasks after propagation of the MDBK

$^a$Barnstead Company, Boston, Massachusetts.
and CEF cells. The formulation was as follows:

- Trypsin\(^a\) 2.0 g
- NaCl 7.0 g
- KH\(_2\)PO\(_4\) 0.2 g
- Na\(_2\)HPO\(_4\) 1.15 g
- H\(_2\)O 1 liter
- Ethylenediamine
- Tetraacetic acid, disodium 1.0 g

**Agar-Overlay Medium**

A double concentration of Eagle's basal medium with Earle's salts and supplemented with 10 per cent newborn calf serum and 0.22 per cent sodium bicarbonate was combined with an equal volume of 1.8 per cent Noble Agar\(^b\). This was used as an agar-overlay medium giving a final concentration of 0.9 per cent agar, 5 per cent serum and 0.11 per cent sodium bicarbonate. Ten thousand units of penicillin and 10,000 micrograms of streptomycin were used per 100 ml agar-overlay medium.

**Viruses**

**Vesicular stomatitis virus**

The **Vesicular stomatitis virus, Indiana strain (VSV-In)**

\(^a\)Difco Laboratories, Inc., Detroit, Michigan.

\(^b\)Difco Laboratories, Inc., Detroit, Michigan.
at the unknown passage level was used. The third passage level on BHK cells was propagated on CEF cells in 250 ml tissue-culture flasks. When the cytopathic effect (CPE) was nearly complete, the growth medium containing virus and cells was frozen, thawed and centrifuged at 300g in order to remove the cell debris and then stored at -90 \(^\circ\)C. VSV-In at the third and fourth passage levels on CEF cells was used for the interferon assay.

**Pseudorabies virus**

The Pseudorabies virus strain DR (PRV-DR) was isolated\(^a\) on MDBK cells from the brain of a calf which died of pseudorabies. PRV-DR was cloned three times on MDBK cells and was used as inoculum at the twelfth passage level on MDBK cells.

**Swine influenza virus**

The Shope strain of the swine influenza virus\(^b\) (SIV) of undetermined passage level was used. The virus was propagated by allantoic-cavity inoculation of eleven-day-old chicken embryos. The virus was titrated by the HA test and used for interferon induction experiments.

\(^a\)Dr. C. J. Mare, College of Veterinary Medicine, Iowa State University.

\(^b\)Obtained from Dr. W. P. Switzer, Veterinary Medical Research Institute, Iowa State University.
Newcastle disease virus

The B₁ strain of Newcastle disease virus (NDV-B₁)² of undetermined passage level was used. The virus was propagated by allantoic-cavity inoculation of eleven-day-old chicken embryos. The virus was titrated by the HA test and was used for interferon induction in chickens and chicken embryos.

Marek's disease virus

Sevoian's (148) virulent Marek's disease virus, the JM-V strain (MD-JMV), which causes lymphoblastic leukemia and death at five to ten days after inoculation into baby chicks, was used at an undetermined passage level in chicks.

The virus was propagated by intraperitoneal (I.P.) administration of 0.2 ml MD-JMV to four-day-old chicks. When the first chicks started to die all chicks were bled by cardiac puncture. Freshly-collected blood was pooled, stabilized with 5 per cent dimethylsulfoxide, put into 2 ml ampoules, slowly frozen and stored in liquid nitrogen. The virus was titrated in four-day-old chicks and the titer was calculated using the Reed and Muench method (139). The MD-JMV virus was used in Experiment I for chicken inoculation.

Plaque Staining

Crystal violet was used (174) for plaque staining.

²Obtained from Dr. M. S. Hofstad, Veterinary Medical Research Institute, Iowa State University.
When visible plaques appeared under agar, 2 ml of a 10 per cent formalin solution was added to fix the cells in the 35 x 10 mm wells of plastic tissue-culture plates. After 15 min. the agar was removed, cell sheets were washed under tap water and 1 per cent crystal violet in 20 per cent ethanol was added for one minute. Stain was washed off under tap water and plaques were counted.

Virus Titration

Tenfold serial dilutions of VSV and PRV virus were made in saline G. One ml of each dilution of virus was inoculated into each of two 35 x 10 mm wells of the plastic tissue-culture plates containing CEF monolayers. After an adsorption period of one hour at 37°C the virus was aspirated and the monolayers were overlayed with the agar-overlay medium, 2 ml per well. The plates were then incubated until plaques were seen. The crystal violet staining technique was then used. The titers of the viruses were expressed in plaque forming units (pfu) per ml. Marek's disease virus was titrated in chickens.

Virus Propagation in Chicken Embryos

Fertilized eggs were obtained from the specific-pathogen-free flock maintained at the Veterinary Medical Research Institute, Iowa State University. The eleven-day-old chicken embryos were inoculated into the allantoic cavity with 0.1 ml
of NDV or SIV. Inoculated eggs were incubated in a humidified 37 °C incubator. As soon as death occurred, allantoic fluid was harvested, pooled and the virus was stored at -90 °C.

Purification and Concentration of Virus

Purification and concentration of NDV and PRV were performed by centrifugation (164). When CPE was nearly complete, the growth medium containing virus and cells was partially purified by centrifugation at 300g for 10 min. to remove cellular debris. The virus was then pelleted by centrifugation at 30,000g for 90 min. The pellet was suspended in saline G and stored at -90 °C until needed.

Polyriboinosinic-Polyribocytidylic Acid

PolyI·polyC² (serial number 76645) was used as the interferon inducer in chickens at the doses indicated later. The polyI·polyC was stored at 4 °C in vials until used.

Statolon

Statolon² (lot 354-1080B220) was stored in a desiccator at 4 °C until used. Immediately before use, the powder was weighed and suspended in distilled water at the concentrations

²Microbiological Associates, Bethesda, Maryland.

²Obtained from Dr. W. J. Kleinschmidt, Eli Lilly and Co., Indianapolis, Indiana.
indicated later. The statolon was used as an interferon inducer in chickens as described later.

**Tube Hemagglutination Test**

Serial twofold dilutions of the allantoic fluid containing NDV or SIV were made in PBS (pH 7.5). Equal volumes of 0.5 per cent chicken erythrocytes in PBS (pH 7.5) were added to the virus dilutions and thoroughly mixed. The test was held for 60 min. at room temperature. A positive hemagglutination pattern consisted of a uniform thin layer of erythrocytes covering the bottom of the tube. The negative pattern consisted of a round "button" of sedimented cells. The reciprocal of the highest dilution of allantoic fluid which gave positive agglutination was considered to represent the titer of the virus.

**Tube Hemagglutination-Inhibition Test**

The HI test was used to detect NDV antibody titers in chickens. The constant-virus and decreasing-serum method was used.

Serial two-fold dilutions of the serum were made in PBS (pH 7.5). Equal volumes (0.25 ml) of serum and NDV containing 80 HA units/ml were mixed and incubated for 60 min. at room temperature. An equal volume (0.5 ml) of 0.5 per cent chicken erythrocytes in PBS was added, the tubes were shaken and results were read after 60 min. at room
temperature. The endpoint of the inhibitory activity of the serum was the highest dilution of serum in which hemagglutination was completely inhibited. The reciprocal of that dilution was considered to represent the antibody titer.

Agar Gel Precipitation Test

The agar gel precipitation (AGP) test was used to determine antibody titer to Marek's disease (34) in baby chickens.

The plastic 60 x 15 mm dishes containing agar\(^a\) which was prepared utilizing the method of Churchill et al. (37) were stored at 4 \(^\circ\)C in plastic bags until used.

A central hole cut in the gel was filled with feather follicle antigen\(^a\) (77) and the surrounding six holes filled with test sera and positive control serum\(^a\). The plates were then sealed and incubated at room temperature. Results were recorded after five days when one or more clear precipitation lines were visible with control positive serum.

Preparation of Exogenous Interferon

The exogenous chicken interferon was used in Experiment 1.

Eleven-day-old chicken embryos were inoculated by the allantoic cavity route with 0.1 ml of NDV containing

\(^a\)Obtained from Dr. P. D. Beard, USDA, APHIS, Veterinary Services, Ames, Iowa
1280 HA/ml. Allantoic fluid was harvested at 60 hours postinoculation, pooled and dialyzed at 4°C against 100 volumes of 0.1M KCl-HCl buffer (pH 2) for 72 hours and then back dialyzed against PBS (pH 7.5) for an additional 72 hours. Precipitants were removed by centrifugation at 30,000g for 90 min. at 4°C and allantoic fluid was then concentrated ten times by pressure filtration at 4°C. Pressure filtration was carried out in dialysis tubing suspended in a flask maintained at a negative pressure of 15 pounds per square inch. Concentrated allantoic fluid was then sterilized by filtration through 300 nm Millipore filters and stored at -20°C until assayed for interferon activity. Bovine serum albumin (0.5 ug/ml) was added to stabilize the interferon during storage (106).

The viral inhibitor produced in chicken embryos was considered to be an interferon since it was acid-resistant, non-dialyzable, trypsin sensitive and possessed species specificity.

Serum Preparation for Interferon Assay

Blood samples were collected from baby chicks by cardiac

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*b Millipore Filter Corporation, Bedford, Massachusetts.

*c Pentex Bovine Albumin, Research Product Division, Miles Laboratories, Inc., Kankakee, Illinois.
puncture. Jugular and wing vein bleeding was used in older chickens. After firm clots were formed at room temperature, the blood was incubated for 1 - 1.5 hours at 37 °C and serum was harvested after centrifugation for 15 min. at 2,000 rpm. The sera were dialyzed in cellulose tubing at 4 °C against 0.1M KCl-HCl buffer (pH 2) for 24 hours and then against PBS (pH 7.5) for 24 additional hours. Precipitation developed in most specimens after dialysis and was removed by centrifugation at 30,000g for 60 min. at 4 °C. The supernatant fluids were collected, filtered through 300 nm Millipore filters and stored at -20 °C until assayed. Bovine serum albumin (0.5 µg/ml) was again added.

Neutral Red

Neutral red solution (1:20,000) was used in interferon assay.

One and a half grams of neutral red was ground and mixed with absolute alcohol to give a 10 per cent (w/v) solution, diluted in water to 1 per cent, filtered through filter paper and autoclaved at ten pounds per square inch for 20 min. and used as a stock solution.

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National Aniline Division, Allied Chemical Corporation, Rochester, New York.
Interferon Assay

**Dye uptake method**

Exogenous chicken interferon prepared in chicken embryos was assayed by the dye-uptake method (57).

Serial twofold dilutions of the dialyzed and centrifuged fluids were made in maintenance medium supplemented with 2 per cent heat-inactivated bovine serum. One ml of each dilution was inoculated into three 12.5 x 1.5 cm rubber stoppered glass tubes containing CEF monolayers and were incubated at 37°C for 16 to 18 hours. The cultures were then drained, washed twice with serum-free maintenance medium and infected with VSV containing $2 \times 10^2$ pfu/0.2 ml. Tubes were incubated at 37°C in a humidified atmosphere containing 5 per cent CO$_2$ until marked cell destruction was seen in virus control tubes. Half ml of neutral red (1:20,000) was added to the medium in each tube. After two hours incubation at room temperature the medium was decanted and the cell sheets were drained by inversion and washed twice with 2 ml physiological saline. The dye from each tube was extracted into 2 ml of ethanol, buffered with an equal volume of Mollvaine-Lillie buffer, pH 4.2. Dye solutions from replicate tubes were pooled and measured colorimetrically at 540 nm using a Beusch and Lomb Spectronic 20 Colorimeter. Interferon titer in 50 per cent dye-uptake units was the reciprocal of the dilution
giving dye uptake midway between the virus and cell culture controls.

**Plaque reduction method**

This technique was used in Experiments II and III.

Serial twofold dilutions of the dialyzed and centrifuged serum were made in maintenance medium supplemented with 2 per cent bovine serum. One ml of each dilution was inoculated into each of two 35 × 10 mm wells containing CEF monolayers and incubated at 37°C for 16 to 18 hours. The control wells were inoculated with 1 ml of the maintenance medium and were incubated for the same period of time. At the end of the incubation period the fluids were aspirated, the plates were washed twice with the maintenance medium and 1 ml of VSV (calculated to contain 50 to 100 pfu) was added to each well. After adsorption of the virus at 37°C for one hour the excess viral fluids were aspirated and the cultures were covered with an agar-overlay medium. After incubation until the visible plaques were seen, crystal violet staining was used. The interferon titers were expressed as the reciprocals of the dilutions which produced 50 per cent reduction in the number of viral plaques when compared with the number in the control plates.
Characterization of Interferon

**Acid stability and dialyzability**

All samples were dialyzed for 24 hours against 50 to 100 volumes of 0.1M KCl-HCl buffer (pH 2) at 4°C in cellulose dialyzer tubing. This was followed by a similar dialysis against PBS (pH 7.5) for 24 additional hours. The final samples were assayed for interferon activity as described previously.

The representative pooled interferon-containing samples were tested for species specificity, heat stability, trypsin sensitivity and the effects of ultracentrifugation (165). Suitable controls were included with each test.

**Host species specificity**

The interferon-containing samples produced in chickens were tested for their activity on MDBK cells by the plaque reduction assay.

**Heat stability**

Heating of sera was performed in a water bath at 56°C for 60 min. After the required length of time, sera were immediately diluted in a maintenance medium and assayed for interferon.

**Trypsin sensitivity**

Equal volumes of interferon-containing serum and a
solution of trypsin\textsuperscript{a} containing 2 mg/ml in physiological saline were mixed. This mixture was incubated for two hours at 37 C. After the incubation, serial twofold dilutions of the interferon were made in BME supplemented with 15 per cent heat-inactivated newborn calf serum and the interferon was assayed for antiviral activity against VSV on CEF cells. The number of plaques was compared with the controls which consisted of equal volumes of the interferon and physiological saline solution incubated for two hours at 37 C.

**Ultracentrifugation**

The samples were ultracentrifuged in an International B 60 ultracentrifuge\textsuperscript{b} at 100,000g for 60 min. and the supernatant fluid was assayed for antiviral activity.

**Experimental Chickens**

White Leghorn chickens were obtained from the specific-pathogen-free flock maintained at the Veterinary Medical Research Institute, Iowa State University. All the chickens for a given experiment were hatched together and subsequently held in the same wire-floored battery brooders in an isolation unit (Experiment I) or in wire cages (Experiments II and III). Sera of the chickens used in Experiment I

\textsuperscript{a}Trypsin 1:250, Difco Laboratories, Inc., Detroit, Michigan.

\textsuperscript{b}International Equipment Co., Boston, Massachusetts.
were negative for Marek's disease (MD) antibody in the AGP test.

Weights

All birds used in Experiment II were individually weighed before and after lead treatment.

Lead Acetate

Lead acetate\(^a\) dissolved in water was used as a lead source for chickens at the doses indicated below.

Determination of Lead in Blood

Blood was collected from chickens into 100 x 16 mm Vacutainer tubes containing sodium heparin\(^b\) as an anticoagulant and was frozen at -20 C until tested. The method described by Hessel (82) for lead determination was used\(^c\). The blood was hemolyzed by using 5 per cent TX-100 solution\(^d\) and was chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted by methyl isobutyl ketone. The organic supernatant solution was analyzed by atomic adsorption

\(^a\)Mallinckrodt Chemical Works, St. Louis, Missouri.
\(^b\)Becton, Dickinson and Co., Columbus, Nebraska and Rutherford, New Jersey.
\(^c\)Analyses for lead in blood and tissues were performed by J. Hurd, Veterinary Diagnostic Laboratory, Iowa State University.
\(^d\)TX-100 is alkyl phenoxy polyethoxyethanol. Rohm and Haas, Philadelphia, Pennsylvania.
spectroscopy. Standards were made using the blood collected from chickens which were not exposed to lead.

Standards having concentrations of 1, 2, 3, 4 and 5 parts per million (ppm) of lead respectively, were made using Pb(NO\textsubscript{3})\textsubscript{2}. Thorough shaking of the standards was found to be a very important factor.

Five ml of unclotted test blood collected from chickens were pipetted into 150 x 20 mm test tubes and 1 ml of the 5 per cent TX solution was added to each. One ml of the 2 per cent APDC solution was then added to each test tube followed by mixing. Five ml of water-saturated methyl isobutyl ketone was then added, test tubes were sealed with screw caps and were shaken for at least one minute by hand, followed by centrifugation for ten min. at 2,000 rpm. The organic supernatant was aspirated into an atomic absorption spectrophotometer\textsuperscript{a} at a wave length of 2835Å (range selector UV) and the burner was kept under normal flow condition of air and acetylene as described by Hessel (82).

**Determination of Lead in Tissues**

Tissues were weighed into a porcelain crucible and one ml aqueous magnesium acetate solution (20 mg of magnesium acetate per ml of water) was added per gram of tissue (71). The crucible content was well dried on a steam bath and then

\textsuperscript{a}Perkin-Elmer Model 303, Perkin-Elmer Corp., Walthum, Massachusetts.
was ashed in a furnace at 450-475 °C for four to five hours. The ash was dissolved in a 2 N hydrochloric acid and then diluted with water to an appropriate volume. The Perkin-Elmer atomic absorption spectrophotometer, Model 303, was used for all determinations. Instrument settings were those recommended in the Analytical Methods for Atomic Absorption Spectrophotometry (2).

Hematological Procedures

Disposable tuberculin syringes were used to collect blood from the wing veins of chickens. Blood was immediately transferred into 50 x 12 mm Vacutainer glass tubes with EDTA as anticoagulant.

Blood from each sample was used for leukocyte, erythrocyte and differential counts and hemoglobin and packed cell volume (PCV) determinations.

Total leukocyte counts were estimated using the Rees-Ecker method as reported by Lucas and Jamroz (114). The total number of cells counted from the four corner squares of a hemocytometer counting chamber was multiplied by 50 to give the total leukocyte count per cubic mm.

Slide smears were air-dried and stained with standard Wright's staina for differential leukocyte counts.

aHarleco, Philadelphia, Pennsylvania.
Erythrocyte counts were made using a Coulter counter\(^a\) (80).

Packed cell volume was determined by the micro-hematocrit method using capillary tubes. The tubes were heat-sealed and centrifuged at 15,000 rpm for three minutes. The percentage of packed erythrocytes was determined. Hemoglobin values were determined using the cyanmethemoglobin technique (80).

**Necropsy Examination**

Birds that died during the course of the experiments were necropsied.

All surviving birds in Experiment I were necropsied six weeks after administration of virus.

Four chickens selected at random from each of the treatment groups in Experiment II were necropsied at the time of death or 35 days after the start of lead administration. Gross lesions were observed and liver, kidney and lung samples were fixed in 10 per cent buffered formalin and processed for histopathologic examination. The lung, liver, kidney, brain, bone and muscle were collected for the chemical lead analysis. These tissues were stored at -20 C until tested.

\(^a\)Coulter Electronics, Inc., Hialeah, Florida.
Histopathological Procedures

The tissues collected at necropsy were fixed in 10 per cent buffered formalin. All tissues were dehydrated in graded ethanol solutions, cleared in xylene and embedded in paraffin. Sections were cut at 6 μm and mounted with Permount\textsuperscript{a} mounting medium on glass slides. All sections were stained with Harris's hematoxylin and eosin (H and E). Some selected tissues were also stained by Ziehl-Neelson acid fast stain. These procedures were carried out as described in the Armed Forces Institute of Pathology's Manual of Histologic Staining Methods (115).

\textsuperscript{a}Fisher Scientific Company, Fair Lawn, New Jersey.
EXPERIMENTAL PROCEDURES AND RESULTS

Experiment I: The Effect of Interferon and Statolon on the Survival of Chickens Inoculated with Marek's Disease Virus (MD-JMV)

Kinetics of interferon production in chickens by statolon

Four-day-old chickens were inoculated intraperitoneally (I.P.) with statolon at the dosage level of 500 mg/kg. Six chickens were exsanguinated by cardiac puncture at each of the following times: 0, 2, 6, 12, 24, 48, 72 and 96 hours postinoculation. The sera of the chickens bled at each time interval were pooled and stored at -20 C until assayed for interferon by the plaque-reduction method.

MD-JMV virus titration

Tenfold serial dilutions of virus were made in BME medium with Earle's salts. Two tenths of one ml of each dilution of the virus was inoculated I.P. into six four-day-old chicks free of MDV antibodies. The LD$_{50}$ titer of the virus was calculated using the Reed and Muench method (139).

Effect of exogenous interferon

Four-day-old chicks were divided into four groups containing 27 or more chicks per group. Each group was inoculated I.P. with 0.2 ml of MD-JMV virus containing 39 LD$_{50}$. Three of the above groups received 0.5 ml of concentrated crude interferon preparation I.P. for 14
consecutive days as indicated below. The interferon titer was 2048, 50 per cent dye-uptake units per ml (DU<sub>50/ml</sub>). Interferon administration was initiated simultaneously with virus in the first group 72 hours after virus inoculation in the second group and 168 hours following virus inoculation in the third group. The control group received allantoic fluid from 14-day-old uninfected chicken embryos.

**Effect of statolon**

The experiment described below was performed twice. All the four-day-old chicks in this trial were inoculated with 39 LD<sub>50</sub> MD-JMV I.P. In the first trial seven groups of 36 or more chicks per group received a single dose of 500 mg/kg statolon I.P. at different times. Chicks of the first group received statolon six hours before virus inoculation and other groups at 0, 6, 24, 48 and 120 hours after virus inoculation.

In the second trial eight groups of 32 or more chicks per group received a single dose of 500 mg/kg statolon at different times. Chicks of the first group were inoculated at 24 hours before virus and the other groups at 0, 6, 24, 48, 72 and 96 hours postinoculation.

Control chicks received 0.25 ml of BME media with Earle's salts intraperitoneally.

Chicks that died during the course of the experiments were necropsied. The experiment was terminated six weeks
after virus administration at which time surviving chickens were killed and necropsied.

Results

Kinetics of interferon induction

Low levels of circulating interferon were detected in chicks bled at six hours postinduction. The maximum titer of 128 was detected in serum 24 hours after induction and decreased thereafter. The results are presented in Table 1.

The viral inhibitor, produced in the chicks by statolon, was characterized as being acid-resistant non-dialyzable and species specific.

Table 1. Serum interferon titers in chicks given intra-peritoneal injections of statolon

<table>
<thead>
<tr>
<th>Interferon titers(^a) per ml (hours postinoculation)</th>
<th>Inducer</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statolon 500 mg/kg</td>
<td></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>128</td>
<td>64</td>
<td>16</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\)Interferon titers are expressed as the reciprocal of the dilution which produces a 50 per cent reduction in the number of viral plaques.
**MD-JMV virus titration**

The inoculation of MD-JMV at lower dilutions into chicks was characterized by death occurring as early as six days postinoculation and as late as 12 days. The experiment was terminated six weeks after virus inoculation.

<table>
<thead>
<tr>
<th>Virus dilution</th>
<th>Mortality ratio</th>
<th>Total deaths</th>
<th>Total survival</th>
<th>Per cent death</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-1}$</td>
<td>6/6</td>
<td>25</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>$10^{-2}$</td>
<td>6/6</td>
<td>19</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>6/6</td>
<td>13</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>6/6</td>
<td>7</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>1/6</td>
<td>1</td>
<td>5</td>
<td>17</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>0/6</td>
<td>0</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>0/6</td>
<td>0</td>
<td>17</td>
<td>0</td>
</tr>
</tbody>
</table>

The MD-JMV virus titer calculated by the Reed and Muench method (139) was 39810 LD$_{50}$ per 0.2 ml.

**The effect of exogenous interferon**

The I.P. inoculation of 39 LD$_{50}$ MD-JMV into four-day-old control chicks resulted in 96.7 per cent mortality.

A survival rate of 17.3 per cent was observed when I.P. interferon administration (1024 DU$_{50}$ units per dose) was
initiated simultaneously with virus. When exogenous interferon administration was started 72 hours after virus inoculation, only 7.4 per cent of chicks survived. Interferon treatment at 168 hours after virus inoculation, at the time when the first chicks started to show clinical signs and die, neither protected nor prolonged the survival of the chicks. The results are summarized in Table 3.

A statistical analysis by the chi-square test (153) indicated no significant difference (P<0.05) between chicken survival in the interferon-treated and the control groups.

Death in chicks in interferon-treated and control groups occurred at 7 to 13 days after viral inoculation. Depression and weakness were observed only for four to five hours before death.

No macroscopic lesions typical of Marek's disease were observed during the necropsy.

The effect of statolon

The effect of statolon on the survival of chicks inoculated with MD-JMV was marked. The time of statolon administration was critical.

In the first trial statolon administered six hours prior to virus inoculation protected 21.1 per cent of the chicks as compared to 5 per cent survival in the control group. Simultaneous I.P. injection of statolon and virus
protected 26.3 per cent. Fifty per cent protection was observed when statolon was given six hours after viral inoculation. The maximum protection rate of 56.8 per cent was achieved when statolon was administered 24 hours after the virus. When statolon was given 48 hours postinoculation, the rate of survival in chicks decreased to 32.4 per cent. No protection was observed when statolon was administered 120 hours after the virus.

The preceding experiment was repeated and similar findings were observed. The best protection was again achieved when statolon was administered 24 hours after virus. The protective effect of statolon diminished when it was given later. The results of both trials are summarized in Table 3.

A statistical analysis by the chi-square test indicated significant differences ($P<0.05$) between chicken survival in statolon-treated and control groups except when statolon was administered 96 hours or later after the virus.

Death in chickens in statolon-inoculated and control groups occurred at 7 to 14 days after viral inoculation. Depression and weakness were observed only four to five hours before death.

Macroscopic lesions typical of Marek's disease were not observed in the necropsied chickens.
Table 3. The effect of interferon and statolon on survival of chicks inoculated with MD-JMV<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. survived/No. inoculated</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td>Interferon</td>
<td>Simultaneously with virus</td>
<td>5/29</td>
</tr>
<tr>
<td></td>
<td>72 hours after virus</td>
<td>2/27</td>
</tr>
<tr>
<td></td>
<td>168 hours after virus</td>
<td>0/28</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>1/30</td>
</tr>
<tr>
<td>Statolon&lt;sup&gt;d&lt;/sup&gt;</td>
<td>24 hours before virus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>6 hours before virus</td>
<td>8/38</td>
</tr>
<tr>
<td></td>
<td>Simultaneously with virus</td>
<td>10/38</td>
</tr>
<tr>
<td></td>
<td>6 hours after virus</td>
<td>18/36</td>
</tr>
<tr>
<td></td>
<td>24 hours after virus</td>
<td>21/37</td>
</tr>
<tr>
<td></td>
<td>48 hours after virus</td>
<td>12/37</td>
</tr>
<tr>
<td></td>
<td>72 hours after virus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>96 hours after virus</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>120 hours after virus</td>
<td>1/40</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2/40</td>
</tr>
</tbody>
</table>

<sup>a</sup>39 LD<sub>50</sub> intraperitoneally.

<sup>b</sup>Daily for 14 days 1028 DU<sub>50</sub> units intraperitoneally.

<sup>c</sup>Normal allantoic fluid 0.5 ml.

<sup>d</sup>Single dose 500 mg/kg I.P.
Experiment II: Lead Poisoning in Chickens

Six-week-old chickens of both sexes were divided at random into groups containing twelve chickens each. Chickens were given lead acetate dissolved in water individually per os daily for 35 consecutive days. Two separate trials were conducted. In trial one the control group received no lead and the remaining three groups received 20 mg/kg, 40 mg/kg and 80 mg/kg respectively. In the second trial the control group received no lead and the remaining three groups received 160 mg/kg, 320 mg/kg and 640 mg/kg of lead respectively.

All chickens were individually weighed prior to and after the treatment. Group mean weight was used in determining the quantity of lead used for treatment. Equal quantities of blood were obtained from all chickens within each group and the blood was pooled according to group. The chickens were bled on the 10th, 21st and 30th day after treatment initiation for the determination of lead levels in blood. The blood of four chickens selected at random from each of the treatment groups was used for hematological studies. White-and red-blood-cell counts, packed cell volume, hemoglobin determinations and differential leukocyte counts were determined on the 15th and 30th days after lead treatment began.

Four chickens selected at random from each of the treatment groups were necropsied at 35 days after lead
administration and lung, liver, kidney, brain, femur and gastrocnemius muscles were collected for the chemical analysis. Liver, kidney and lung samples were used for histopathological studies.

All chickens were necropsied at the time of death.

Results

Chickens tolerated levels of lead (Table 4) as high as 160 mg/kg for 30 days administered per os without exhibiting clinical signs or hematological changes (Tables 5 and 6). There were no significant weight differences between control and lead-treated groups nor were gross lesions observed.

Chickens treated at a level of 320 mg/kg of lead exhibited early signs of lethargy, tiring, weakness and depression. Anorexia, anemia and apparent loss of weight followed and marked paralysis of both legs and wings was observed in most birds a day or two prior to death. One chicken in the 320 mg/kg treatment group died on the 11th day and five others at 21-30 days following initiation of lead treatment. Six chickens of this group survived and all but one showed depression, anemia and loss of weight. The total erythrocyte counts and the leukocyte differential counts remained within normal limits after 30 days of treatment. A fall in hemoglobin concentration was noted in chickens after 15 days of treatment and all chickens had decreased levels of hemoglobin after 30 days of treatment (Table 6). The PCV
remained within normal limits in all tested chickens except chicken number 27 (Table 6). Examination of blood smears indicated increased numbers of the immature erythrocytes. Basophilic stippling of erythrocytes was not seen. Anemia and emaciation were constant findings during necropsy.

Chickens at the 640 mg/kg lead level showed a high mortality rate. The first chicken died on the sixth day after lead administration started, nine deaths occurred between days 11 and 21, one chicken died on day 28 and the last one on day 34. The clinical signs were similar to those described above. The number of erythrocytes and leukocyte differential counts remained within normal limits. The number of leukocytes was slightly increased as compared to the control chickens (Table 6). The packed cell volume was decreased in chicken number 32. Hemoglobin concentration was markedly decreased in all chickens with chicken number 32 showing a low of 2.1 gm/100 ml (Table 6). Blood smears revealed approximately 12 per cent of immature red blood cells, some of which were in various stages of mitotic division (Figure 1). Changes in cell shape and enucleated or binucleated cells were common. At necropsy chickens were found to have severe anemia and emaciation and all of them weighed 150 to 250 g in comparison to 750-1,000 g for the control group. At necropsy a few chickens showed absence of any fatty tissue and discolored, edematous liver.
Chemical analysis of tissue specimens revealed that significant quantities of lead were present in the tissues of lead-treated chickens. A wide range in tissue lead concentrations was found in chickens which received the same lead treatment. The range of tissue lead levels within the same chicken was quite broad. The highest concentrations were found in a bone tissue in all chickens. The next highest lead levels were found in the kidneys. Lead deposition in liver and lung was lower than in kidneys and the lowest concentrations of lead were found in muscle. Generally the mean of lead content of tissues was higher in all tissues of chickens which were given higher doses of lead. Few exceptions were observed. The mean lead concentration in bone tissue of chickens at the 640 mg/kg level was considerably lower than in chickens which were given 320 mg/kg and 160 mg/kg lead levels. The results are summarized in Table 7.

Histopathological examination of kidney, liver and lung H and E sections by light microscopy did not reveal any specific lesions which could be attributed to the lead poisoning. In kidney and liver tissues of some chickens of the 320 mg/kg, 160 mg/kg, 80 mg/kg and 20 mg/kg groups, accumulations of mononuclear cells were observed. No particular pattern of distribution of these RE cells was found.
Acid-fast staining did not reveal nuclear inclusions in tissues of lead-treated chickens.

Table 4. The concentration\(^a\) of lead in the blood of chickens given lead per os

<table>
<thead>
<tr>
<th>Daily dose of lead</th>
<th>Concentration of lead (ppm)(^b) days after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (no lead)</td>
<td>0.04</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>0.8</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>1.1</td>
</tr>
<tr>
<td>80 mg/kg</td>
<td>1.7</td>
</tr>
<tr>
<td>160 mg/kg</td>
<td>-</td>
</tr>
<tr>
<td>320 mg/kg(^c)</td>
<td>4.75</td>
</tr>
<tr>
<td>640 mg/kg(^d)</td>
<td>10.5</td>
</tr>
</tbody>
</table>

\(^a\)Pooled blood of 12 chickens.

\(^b\)ppm - parts per million.

\(^c\)One chicken died on the 11th day, 5 chickens died between days 21 and 30.

\(^d\)One chicken died on the 6th day, 9 chickens died between days 11 and 21 and one died on day 28.
Table 5. Effect of lead given *per os* for 30 days on hematologic changes in chickens

<table>
<thead>
<tr>
<th>Daily dose of lead</th>
<th>WBC $\times 10^3$</th>
<th>RBC $\times 10^6$</th>
<th>PCV</th>
<th>Hb (gm/100ml)</th>
<th>Lympho-</th>
<th>Eosino-</th>
<th>Baso-</th>
<th>Hetero-</th>
<th>Mono-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no lead)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken 1</td>
<td>7,700</td>
<td>2.72</td>
<td>31</td>
<td>8.6</td>
<td>67</td>
<td>3</td>
<td>1</td>
<td>26</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>7,500</td>
<td>2.65</td>
<td>30</td>
<td>8.9</td>
<td>61</td>
<td>4</td>
<td>3</td>
<td>31</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>15,350</td>
<td>2.02</td>
<td>33</td>
<td>8.9</td>
<td>80</td>
<td>1</td>
<td>2</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>15,000</td>
<td>2.84</td>
<td>39</td>
<td>11.9</td>
<td>65</td>
<td>7</td>
<td>3</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8,500</td>
<td>2.87</td>
<td>30</td>
<td>8.9</td>
<td>74</td>
<td>1</td>
<td>4</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>9,850</td>
<td>2.53</td>
<td>32</td>
<td>8.3</td>
<td>56</td>
<td>6</td>
<td>3</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>6,400</td>
<td>3.09</td>
<td>35</td>
<td>9.1</td>
<td>69</td>
<td>4</td>
<td>4</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>7,700</td>
<td>3.10</td>
<td>36</td>
<td>8.6</td>
<td>63</td>
<td>3</td>
<td>4</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td></td>
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<tr>
<td>9</td>
<td>10,400</td>
<td>2.18</td>
<td>32</td>
<td>8.0</td>
<td>51</td>
<td>8</td>
<td>3</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>19,300</td>
<td>—</td>
<td>—</td>
<td>9.1</td>
<td>46</td>
<td>7</td>
<td>1</td>
<td>44</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>5,900</td>
<td>2.94</td>
<td>29</td>
<td>8.0</td>
<td>70</td>
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<td>3</td>
<td>23</td>
<td>2</td>
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<tr>
<td>12</td>
<td>7,000</td>
<td>2.98</td>
<td>29</td>
<td>8.0</td>
<td>70</td>
<td>1</td>
<td>3</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>80 mg/kg</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>13</td>
<td>12,950</td>
<td>2.85</td>
<td>—</td>
<td>8.0</td>
<td>53</td>
<td>1</td>
<td>1</td>
<td>43</td>
<td>2</td>
</tr>
<tr>
<td>14</td>
<td>15,700</td>
<td>3.05</td>
<td>—</td>
<td>8.9</td>
<td>51</td>
<td>3</td>
<td>2</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>10,250</td>
<td>2.11</td>
<td>28</td>
<td>7.2</td>
<td>50</td>
<td>7</td>
<td>4</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>7,300</td>
<td>2.84</td>
<td>32</td>
<td>7.7</td>
<td>61</td>
<td>3</td>
<td>6</td>
<td>29</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 6. Effect of lead given per os for 30 days on hematologic changes in chickens

<table>
<thead>
<tr>
<th>Daily dose of lead</th>
<th>WBC &lt;sup&gt;x103&lt;/sup&gt;</th>
<th>RBC &lt;sup&gt;x10&lt;sup&gt;6&lt;/sup&gt;&lt;/sup&gt;</th>
<th>PCV</th>
<th>Hb gm/100ml</th>
<th>Lymphocytes</th>
<th>Eosinophils</th>
<th>Basophils</th>
<th>Heterophils</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no lead)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Chicken 17</td>
<td>12,800</td>
<td>2.72</td>
<td>35</td>
<td>9.8</td>
<td>65</td>
<td>4</td>
<td>5</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Chicken 18</td>
<td>8,700</td>
<td>3.10</td>
<td>30</td>
<td>10.1</td>
<td>59</td>
<td>2</td>
<td>4</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Chicken 19</td>
<td>9,850</td>
<td>2.25</td>
<td>35</td>
<td>8.9</td>
<td>72</td>
<td>5</td>
<td>3</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Chicken 20</td>
<td>7,600</td>
<td>2.28</td>
<td>31</td>
<td>8.6</td>
<td>51</td>
<td>8</td>
<td>6</td>
<td>35</td>
<td>0</td>
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<tr>
<td>160 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken 21</td>
<td>14,350</td>
<td>3.07</td>
<td>32</td>
<td>8.3</td>
<td>55</td>
<td>3</td>
<td>6</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>Chicken 22</td>
<td>8,200</td>
<td>2.54</td>
<td></td>
<td>8.2</td>
<td>69</td>
<td>3</td>
<td>10</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Chicken 23</td>
<td>8,450</td>
<td>2.82</td>
<td>32</td>
<td>8.3</td>
<td>60</td>
<td>5</td>
<td>7</td>
<td>25</td>
<td>3</td>
</tr>
<tr>
<td>Chicken 24</td>
<td>9,650</td>
<td>2.56</td>
<td>34</td>
<td>9.8</td>
<td>77</td>
<td>1</td>
<td>8</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>320 mg/kg&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken 25</td>
<td>9,000</td>
<td>2.52</td>
<td>33</td>
<td>7.7</td>
<td>63</td>
<td>3</td>
<td>12</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>Chicken 26</td>
<td>5,650</td>
<td>2.50</td>
<td>35</td>
<td>6.9</td>
<td>40</td>
<td>6</td>
<td>15</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>Chicken 27</td>
<td>15,000</td>
<td>2.28</td>
<td>24</td>
<td>4.7</td>
<td>54</td>
<td>6</td>
<td>1</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>Chicken 28</td>
<td>9,250</td>
<td>2.25</td>
<td>30</td>
<td>7.4</td>
<td>56</td>
<td>2</td>
<td>9</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>640 mg/kg&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken 29</td>
<td>13,800</td>
<td>2.49</td>
<td>30</td>
<td>5.1</td>
<td>48</td>
<td>6</td>
<td>2</td>
<td>41</td>
<td>3</td>
</tr>
<tr>
<td>Chicken 30</td>
<td>12,600</td>
<td>2.53</td>
<td>30</td>
<td>5.4</td>
<td>56</td>
<td>2</td>
<td>2</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>Chicken 31</td>
<td>17,280</td>
<td>2.20</td>
<td>31</td>
<td>7.2</td>
<td>50</td>
<td>3</td>
<td>7</td>
<td>38</td>
<td>2</td>
</tr>
<tr>
<td>Chicken 32</td>
<td>15,000</td>
<td>2.33</td>
<td>27</td>
<td>2.1</td>
<td>54</td>
<td>2</td>
<td>2</td>
<td>38</td>
<td>4</td>
</tr>
</tbody>
</table>

<sup>a</sup>Chicken number 26 died on day 21 and hematologic changes given are those observed after 15 days of treatment.

<sup>b</sup>Chickens number 29, 31 and 32 died between days 15 and 21 after initiation of treatment. Results reflect observations made after 15 days of treatment.
Table 7. The concentration of lead in tissues of chickens given lead per os for 35 days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>5.4</td>
<td>0.0-11.5</td>
<td>8.9</td>
<td>5.0-15.0</td>
<td>7.8</td>
<td>3.5-15.0</td>
</tr>
<tr>
<td>Lung</td>
<td>1.9</td>
<td>0.0-5.0</td>
<td>8.1</td>
<td>2.8-12.5</td>
<td>4.4</td>
<td>2.5-7.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.3</td>
<td>0.0-3.8</td>
<td>5.1</td>
<td>1.3-9.1</td>
<td>3.1</td>
<td>0.0-4.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.9</td>
<td>0.0-10.0</td>
<td>10.8</td>
<td>6.3-14.1</td>
<td>11.2</td>
<td>6.2-17.5</td>
</tr>
<tr>
<td>Liver</td>
<td>2.1</td>
<td>0.0-7.5</td>
<td>9.5</td>
<td>4.7-20.0</td>
<td>7.7</td>
<td>2.5-12.5</td>
</tr>
<tr>
<td>Bone</td>
<td>65.7</td>
<td>17.0-112.5</td>
<td>166.9</td>
<td>105.8-294.6</td>
<td>282.6</td>
<td>211.5-338.8</td>
</tr>
</tbody>
</table>

*Four chickens per treatment group.*
Table 7. (Continued).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160 mg/kg</td>
<td></td>
<td>320 mg/kg</td>
<td></td>
<td>640 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>7.3</td>
<td>3.1-13.8</td>
<td>14.8</td>
<td>12.5-16.3</td>
<td>15.4</td>
<td>10.4-25.0</td>
</tr>
<tr>
<td>Lung</td>
<td>6.3</td>
<td>1.9-12.5</td>
<td>16.9</td>
<td>7.5-25.0</td>
<td>77.3</td>
<td>8.3-222.2</td>
</tr>
<tr>
<td>Muscle</td>
<td>4.2</td>
<td>0.0-9.1</td>
<td>10.6</td>
<td>0.0-34.7</td>
<td>13.7</td>
<td>3.8-22.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>11.2</td>
<td>10.0-14.3</td>
<td>49.8</td>
<td>10.7-141.7</td>
<td>152.1</td>
<td>47.2-345.8</td>
</tr>
<tr>
<td>Liver</td>
<td>7.2</td>
<td>3.1-13.9</td>
<td>18.9</td>
<td>7.1-44.4</td>
<td>78.9</td>
<td>71.3-91.6</td>
</tr>
<tr>
<td>Bone</td>
<td>438.1</td>
<td>290.9-512.0</td>
<td>494.8</td>
<td>290.5-650.0</td>
<td>310.5</td>
<td>190.0-396.9</td>
</tr>
</tbody>
</table>

bChickens of this treatment group died between days 12 and 18 after initiation of lead treatment.
Figure 1. Blood smear of a chicken which received lead acetate (640 mg/kg) for 30 days. Note immature erythrocytes and mitotic figure. x1680
Experiment III: The Effect of Lead on Interferon and Antibody Production in Chickens

Interferon induction in chickens

Statolon, polyI-polyC and the viruses PRV-DR, SIV and NDV-B₁ were used as interferon inducers.

Nine groups consisting of three ten-week-old chickens per group were inoculated using different routes and doses of interferon inducers (Table 8). Chickens were bled at 0, 2, 6, 24 and 48 hours after induction. Blood of the chickens from the same treatment group was pooled and serum was assayed for interferon using the plaque-reduction method.

Effect of lead on interferon and antibody production

Statolon (500 mg/kg) and NDV-B₁ (1024 HA/kg) were administered I.V. as interferon inducers. Twelve ten-week-old chickens from each group that previously received lead at the rate of 20 mg/kg, 40 mg/kg, 80 mg/kg or 160 mg/kg for 30 days per os were divided into two equal groups. Six chickens were inoculated with statolon and six with NDV-B₁. Only NDV-B₁ was used as an interferon inducer in the group of six chickens which received lead at the 320 mg/kg level for 30 days.

Lead treatment was continued at the previous regimen until termination of this experiment.

Chickens inoculated with statolon were bled at 0, 2, 6, 24, 48, 72 and 100 hours postinduction. Chickens which
received NDV-B₃ were bled at 0, 6, 24, 48, 72 and 100 hours after virus inoculation. Blood of chickens from the same treatment group was pooled and serum was assayed for interferon using the plaque-reduction method.

The viral inhibitor produced in chickens was examined for dialysability, sensitivity to trypsin, acid and heat sedimentation by centrifugation at 100,000g for 60 min. and species specificity.

Chickens which were inoculated with NDV-B₃ were bled 14 days after virus inoculation. Blood samples were collected for lead determination and serum was tested for NDV antibodies using the HI test.

Results

Interferon induction in chickens

Titers of interferon in serum were determined after the administration of the interferon inducers by various routes and the results are summarized in Table 8. PolyI·polyC, SIV and PRV-DR were not effective inducers of interferon at the doses used. Statolon at the 50 mg/kg dose level induced low titer interferon at six hours postinduction. Statolon at the 500 mg/kg dose level and NDV-B were effective interferon inducers.

Effect of lead on interferon and antibody production

Lead treatment of chickens at the 20 mg/kg, 40 mg/kg, 80 mg/kg and 160 mg/kg levels did not affect interferon
production induced by statolon. Neither interferon concentration nor duration of detectable interferon in serum were markedly changed as compared with the controls.

Interferon production in response to NDV-B₁ was not affected in chickens treated at the 20 mg/kg, 40 mg/kg and 80 mg/kg lead dosage levels. Lead treatment of chickens at the 160 mg/kg level slightly decreased interferon concentration induced by NDV-B₁.

All but one of the chickens in the 320 mg/kg group showed some signs of lead poisoning at the time of inoculation of interferon inducer. Interferon concentrations and duration in serum were markedly decreased in chickens of this group. The results are summarized in Tables 9 and 10.

The viral inhibitor produced in chickens by statolon and NDV-B₁ stimulation was characterized as being acid-resistant, non-dialyzable, non-sedimentable at 100,000g for 60 min., trypsin sensitive, heat stable and species specific. The results are summarized in Table 13.

Long-time lead exposure had no marked effect on antibody production to NDV-B₁ in chickens. No consistent correlation was observed between blood lead concentration and antibody titers.

Three chickens out of six which were on the 320 mg/kg lead treatment died between days 35 and 40 after initiation of lead treatment. All three surviving chickens showed
signs of lead poisoning and only chicken number 71 had a markedly lower antibody titer than the untreated chickens. The results are summarized in Tables 11 and 12.

### Table 8. Induction of interferon in chickens by various interferon inducers

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Dosage</th>
<th>Route</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>PolyI·polyC</td>
<td>0.1 mg/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td>&lt;8</td>
</tr>
<tr>
<td></td>
<td>0.05 mg/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td></td>
<td>1 wing web</td>
<td>I.V.</td>
<td>0</td>
<td>8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td></td>
<td>5 wing web</td>
<td>I.V.</td>
<td>0</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td>Statolon</td>
<td>50 mg/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td></td>
<td>500 mg/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>256</td>
<td>128</td>
<td>8</td>
</tr>
<tr>
<td>PVR-DR(^a)</td>
<td>1 ml/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>&lt;8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td>SIV</td>
<td>640 HA/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>8</td>
<td>&lt;8</td>
<td>&lt;8</td>
</tr>
<tr>
<td>NDV-B(_1)</td>
<td>2048 HA/kg</td>
<td>I.V.</td>
<td>0</td>
<td>-</td>
<td>256</td>
<td>256</td>
<td>32</td>
</tr>
</tbody>
</table>

\(^a\)PRV-DR titer 3.2 x 10^5 pfu/ml.
Table 9. Effect of lead on interferon production in chickens

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Daily dose of lead for 30 days</th>
<th>Interferon titer hours postinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Statolon</td>
<td>Control (no lead)</td>
<td>0</td>
</tr>
<tr>
<td>500 mg/kg I.V.</td>
<td>20 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>NDV-B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1024 HA/kg I.V. Control (no lead)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>80 mg/kg</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 10. Effect of lead on interferon production in chickens

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Daily dose of lead for 30 days</th>
<th>Interferon titer hours postinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Statolon</td>
<td>Control (no lead)</td>
<td>0</td>
</tr>
<tr>
<td>500 mg/kg I.V.</td>
<td>160 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td>NDV-B&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1024 HA/kg I.V. Control (no lead)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>160 mg/kg</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>320 mg/kg</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 11. Effect of low lead levels on antibody titer to NDV-B₁ in chickens

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Daily dose of lead</th>
<th>HI antibody titer&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Lead concentration in blood (ppm)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>Control (no lead)</td>
<td>128</td>
<td>0.05</td>
</tr>
<tr>
<td>34</td>
<td></td>
<td>128</td>
<td>0.25</td>
</tr>
<tr>
<td>35</td>
<td></td>
<td>512</td>
<td>-</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>256</td>
<td>-</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td>512</td>
<td>-</td>
</tr>
<tr>
<td>38</td>
<td></td>
<td>512</td>
<td>-</td>
</tr>
<tr>
<td>39</td>
<td>20 mg/kg</td>
<td>128</td>
<td>2.00</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td>256</td>
<td>1.45</td>
</tr>
<tr>
<td>41</td>
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<td>256</td>
<td>3.27</td>
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<td>42</td>
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<td>2.40</td>
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<td>43</td>
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<tr>
<td>44</td>
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<td>256</td>
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</tr>
<tr>
<td>45</td>
<td>40 mg/kg</td>
<td>512</td>
<td>3.20</td>
</tr>
<tr>
<td>46</td>
<td></td>
<td>128</td>
<td>2.50</td>
</tr>
<tr>
<td>47</td>
<td></td>
<td>128</td>
<td>1.95</td>
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<tr>
<td>48</td>
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<td>256</td>
<td>2.25</td>
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<td>49</td>
<td></td>
<td>64</td>
<td>3.65</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>256</td>
<td>2.40</td>
</tr>
<tr>
<td>51</td>
<td>80 mg/kg</td>
<td>128</td>
<td>3.30</td>
</tr>
<tr>
<td>52</td>
<td></td>
<td>256</td>
<td>4.45</td>
</tr>
<tr>
<td>53</td>
<td></td>
<td>64</td>
<td>4.75</td>
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<td>5.55</td>
</tr>
<tr>
<td>55</td>
<td></td>
<td>64</td>
<td>8.00</td>
</tr>
<tr>
<td>56</td>
<td></td>
<td>128</td>
<td>4.40</td>
</tr>
</tbody>
</table>

<sup>a</sup>On the 14th day after NDV-B₁ inoculation.

<sup>b</sup>After 44 days of lead administration per os.
Table 12. Effect of high lead levels on antibody titer to NDV-B$_1$ in chickens

<table>
<thead>
<tr>
<th>Chicken No.</th>
<th>Daily dose of lead</th>
<th>HI antibody titer$^a$</th>
<th>Lead concentration in blood (ppm)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>57</td>
<td>Control (no lead)</td>
<td>128</td>
<td>0.25</td>
</tr>
<tr>
<td>58</td>
<td>128</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>59</td>
<td>128</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>128</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td>64</td>
<td>-</td>
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<tr>
<td>62</td>
<td>256</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>63</td>
<td>160 mg/kg</td>
<td>128</td>
<td>6.55</td>
</tr>
<tr>
<td>64</td>
<td>160 mg/kg</td>
<td>128</td>
<td>6.55</td>
</tr>
<tr>
<td>65</td>
<td>256</td>
<td>-</td>
<td>5.25</td>
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<td>66</td>
<td>256</td>
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<td>6.40</td>
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<td>67</td>
<td>128</td>
<td>-</td>
<td>5.70</td>
</tr>
<tr>
<td>68</td>
<td>64</td>
<td>-</td>
<td>11.05</td>
</tr>
<tr>
<td>69</td>
<td>320 mg/kg</td>
<td>128</td>
<td>9.00</td>
</tr>
<tr>
<td>70</td>
<td>128</td>
<td>-</td>
<td>10.85</td>
</tr>
<tr>
<td>71</td>
<td>32</td>
<td>-</td>
<td>13.64</td>
</tr>
</tbody>
</table>

$^a$On the 14th day after NDV-B$_1$ inoculation.

$^b$After 44 days of lead administration per os.
Table 13. Properties of chicken interferon

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Results induced by statolon</th>
<th>Results induced by NDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Dialysis for 48 hours at 4 C</td>
<td>Non-dialyzable</td>
<td></td>
</tr>
<tr>
<td>2. Acidification at pH 2</td>
<td>Activity retained</td>
<td></td>
</tr>
<tr>
<td>3. Trypsin sensitivity</td>
<td>Activity destroyed</td>
<td></td>
</tr>
<tr>
<td>Control titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin (2 ug/ml) for 2 hours at 37 C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64 . . 128</td>
<td></td>
<td>64 . . 128</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Heat stability</td>
<td>Heat stable</td>
<td></td>
</tr>
<tr>
<td>Control titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titer after heating at 56 C for 60 min.</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td></td>
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DISCUSSION

There is good evidence that interferon and interferon inducers exert an antitumor effect in tumors caused by oncogenic viruses (73, 105, 108, 125, 130, 134, 160, 175), transplantable tumor cells (74) or chemical carcinogens (111). Neoplastic diseases caused either by DNA (125, 160) or RNA (73, 105, 130, 134, 175) viruses have been reported to be repressed by interferon or interferon inducers.

Very little work has been reported in the literature on the effect of interferon and interferon inducers on oncogenic viruses in chickens. Nemes et al. (123) reported that repeated inoculations of polyI·polyC partially protected chickens against Rous sarcoma virus (RSV) but not against MDV. Exogenous interferon inhibits replication of RSV and lymphoid leukemia virus in vitro and inhibits development of tumors induced by these viruses in chickens (105). Lampson et al. (106) reported that crude and purified chicken interferon produced in chick embryos was active in suppressing Rous sarcoma development but was without therapeutic effect once infection was established.

In this study (Experiment I) the prophylactic and therapeutic effect of interferon and statolon in chickens given 39 LD$_{50}$ of MD-JMV I.P. was investigated.

The repeated administration of crude exogenous
interferon preparations increased the percentage of surviving chickens when administration was initiated simultaneously with virus. A survival rate of 17.3 per cent was observed. When interferon treatment was started 72 hours after viral administration, only 7.4 per cent of the chickens survived. If interferon administration was initiated 168 hours after virus, at the time when chickens started to show clinical signs of the disease, interferon neither protected the chickens nor prolonged their survival when compared with controls. Fewer than 4 per cent of the birds survived in these two groups.

A statistical analysis by the chi-square test indicated no significant difference ($P<0.05$) between survival in the interferon-treated and control groups. A survival rate of 17.3 per cent was observed in chickens when administration of interferon was initiated simultaneously with virus. The survival of one additional chicken in this group would have made the survival rate in the interferon-treated group statistically significant. Individual variation in the weight of chickens and their susceptibility to MDV, as well as possible minute variations of administered virus dosage, could have affected their survival.

Generally the percentage of surviving chickens decreased as interferon treatment was initiated at successively longer periods after virus inoculation. The biological data collected in this experiment show that interferon at the
concentrations used may have prophylactic value but is without any effect once infection is established.

Statolon has previously been shown to be an effective inducer of interferon in chickens (56, 132). In this study (Experiment I) statolon induced low levels of circulating interferon at six hours postinoculation in four-day-old chicks. A maximum titer was detected in serum 24 hours after induction and then decreased. No circulating interferon was found at 96 hours postinoculation.

Statolon is known to be effective in protecting mice against Friend-virus leukemia even after the disease is established (175, 176). Statolon treatment tends to convert leukemia into a persistent, inapparent infection (176). Statolon given systemically protects chickens against avian influenza (132) and has a prophylactic effect against MDV when inoculated into baby chicks (16).

In this study chicks infected with MD virus exhibited various degrees of resistance depending upon the time of statolon administration. Statolon given at 24 and 6 hours before virus protected fewer chicks than when statolon was administered simultaneously with virus. In the first trial statolon inoculated six hours after virus protected more chicks than when given simultaneously with virus. However, in the second trial simultaneous administration of virus and statolon resulted in a higher percentage of surviving chicks than in a group when statolon was given six hours
after virus inoculation. In both trials the maximum protection was achieved when statolon was given 24 hours after virus. The survival rate was 56.8 and 51.5 per cent respectively as compared with five and zero per cent in the control groups. The protective effect decreased when statolon was administered 48 hours or more after virus inoculation.

The lack of protection against MDV resulting from repeated administration of polyI•polyC in chickens (123) can be attributed to the fact that polyI•polyC is a very poor interferon inducer in chickens (56, 132). PolyI•polyC also showed very weak protection against RSV (123), whereas exogenous interferon suppressed Rous sarcoma development (105, 106). However, the presence of lesser amounts of interferon or the absence of detectable circulating interferon is not always indicative of protection or lack of protection (56). Synthetic polynucleotides have been shown to induce cellular resistance in the absence of detectable interferon production (169). Viral resistance conferred by stimulation of the interferon system depends upon the interferon sensitivity of the test virus, the target cells or organ infected and the nature of the interferon inducer used (56). The route of administration of the inducer is also a very important factor (56). Since the antitumor and antiviral mechanism of polyI•polyC is multifaceted (5, 111), it is difficult to explain why polyI•polyC does not protect
chicks against RSV and MDV. Since interferon plays a very important role in protecting chicks against these viruses, it may be concluded that the poor interferon-inducing capacity of polyI·polyC in chickens is the reason for the lack of protection.

It is likely that the protective effect of statolon against MD-JMV in chicks is due not only to interferon. Detectable circulating interferon was present only for three days after statolon administration and yet the protective effect of statolon persisted beyond this time.

Recent findings show that the mechanism of antitumor and antiviral action of interferon and interferon inducers may be multifaceted (5, 111). Wheelock et al. (177) indicated that the antitumor effect of statolon might be due to its capacity to restore immuno-competence in mice infected with Friend virus. It is possible that statolon-induced interferon delays MD virus replication briefly permitting some chicks to mobilize an effective immune response. The crucial factor determining the outcome of the MD-JMV infection is the time of statolon administration. Since the kinetics of MD virus replication and the mechanism by which it causes symptoms and death are not elucidated, it is difficult to state why statolon is effective when administered over a wide range of time before or after virus inoculation. Statistical analysis of the experimental data shows that
inoculation of the inducer from 24 hours before virus inoculation up to 72 hours after virus inoculation significantly protects chicks against MD-JMV.

It is known that Marek's disease virus is a typical cell-associated virus (36, 121, 155) which spreads slowly and in cell cultures appears to spread directly from cell to cell (36). It seems possible that interferon induced by statolon effectively interferes with virus replication at the early stages of infection and prevents virus spread to vital organs. Experimental data indicates that statolon is most effective when administered from 6 to 24 hours after virus inoculation. It is obvious that some unknown step of virus replication is very sensitive to statolon at this time. Interferon, which is at maximal concentration at 24 hours postinduction, prevents further replication and spread of virus. The fact that statolon-induced interferon is detectable in serum for three days explains the protection of chicks inoculated with statolon even 72 hours after virus inoculation. Statolon inoculated at 96 hours after virus injection did not protect chicks probably because MD virus had already reached and damaged vital organs. The first chicks started to die on the seventh day (168 hours) after virus inoculation.

The biological data collected in this experiment show that statolon has a prophylactic value in protecting chicks against MD-JMV. The therapeutic value of statolon at the
dose used was insignificant. Reports in the literature (99) implicate other herpesviruses as oncogenic agents in humans. In view of the above findings statolon at higher doses may be of therapeutic value in treatment of neoplastic disease in man.

There is very little published information on the effects of lead in chickens. Signs of poisoning including drowsiness, thirst, loss of appetite and diarrhea ascribed to the feeding of lead arsenate were described in 1932 (159). Salisbury et al. (144) reported lead poisoning in adult chickens caused by feeding a grit which contained lead oxide. These authors observed a marked loss of weight in all birds, cessation of egg production, severe anemia and high mortality. Most of the chickens were found to have a severe necrosis of the gizzard lining. Experiments with young chickens showed that the susceptibility to lead intoxication decreases with age (144). Simpson et al. (151) described abnormalities of erythrocytes and renal tubules of chickens poisoned with high doses of lead. Approximately 24 per cent of erythrocytes were immature and approximately two per cent of the immature red blood cells were in various stages of mitotic division. By light microscopy, necrosis of the epithelium of the proximal convoluted tubules of the kidney was observed and by electron microscopy, the nuclei of these cells were seen to contain
irregularly shaped, electron-dense inclusions (151).

Most published reports on lead poisoning in birds have been about its toxicity in wild fowl (6, 7, 38, 90).

The effects of lead given per os to chickens for 35 consecutive days was investigated in this study (Experiment II).

Chickens tolerated levels of lead as high as 160 mg/kg without exhibiting clinical signs or hematological changes in spite of very high levels of lead in the blood.

Analysis of lead in the blood is generally considered to be the best single test for the antemortem diagnosis of lead poisoning. Lead concentrations of pooled blood samples of twelve chickens which were given 160 mg/kg of lead for 30 days was 6.2 ppm. It is apparent from this data that chickens are more resistant to lead poisoning than humans, horses, dogs and wild fowl such as ducks. The upper limit for lead in human blood is subject to some disagreement. Some believe it to be approximately 0.8 ppm, but others believe it to be less especially in young children (31, 143). Lead poisoning has been confirmed in cattle having blood lead levels as low as 0.35 ppm in both experimental and naturally-occurring cases (20). Horses appear more susceptible to lead poisoning than cattle (3). The upper limit for blood lead in dogs is 0.35 ppm (181). Wild water-fowl are very susceptible to lead poisoning. Daily
administration of aqueous lead nitrate solution per os at the dosage level of 12 mg/kg killed all experimental ducks within a month (38). No data was found in the literature describing blood lead levels in wild gallinaceous birds. Pheasants that died of lead poisoning were reported to have 168 ppm of lead in their livers and 42 ppm in the breast muscles (90). Tissue lead levels reported in Canada geese poisoned by lead were much lower (6).

The evidence cited above suggests that gallinaceous birds are relatively resistant to lead poisoning, however, to confirm this statement, more detailed comparative studies are needed.

The present study suggests that chickens can tolerate a 6.2 ppm level of blood lead without evidence of intoxication, hematological changes or death.

Fifty per cent of chickens treated at a lead level of 320 mg/kg died within 30 days. All but one of the chickens receiving 640 mg/kg lead died within 30 days. The clinical signs included weakness, depression, anorexia, anemia and loss of weight. A marked peripheral paralysis was observed in most chickens a day or two prior to death. In contrast to other reports (159), no diarrhea was observed. Hemo­globin concentration was markedly decreased in all chickens of the 320 mg/kg and 640 mg/kg lead-treatment groups. Examination of blood smears revealed increased numbers of
immature erythrocytes, some of which were in various stages of mitosis. Poikilocytosis, anisocytosis and enucleated or binucleated cells were common. Simpson et al. (151) reported similar changes in erythrocytes of chickens poisoned with high doses of lead. Basophilic stippling of erythrocytes was not observed. This finding is in agreement with other published reports about lead poisoning in birds (38, 144, 151). Since lead interferes with heme synthesis (31, 171), anemia is the functional effect of lead poisoning on blood. The decrease in heme synthesis leads at first to a decrease in the life-span of RBC and the amount of hemoglobin per cell (171). In compensation the blood-forming tissues step up their production of RBC and immature forms of cells appear in the circulation (31, 171). The slight increase observed in the white blood cell counts may be due to high numbers of immature red blood cells which closely resemble leukocytes.

At necropsy chickens were found to have severe anemia, emaciation and a marked loss of weight. Chickens of the 640 mg/kg lead level group weighed 150-250g compared to 750-1,000g for the control group. Absence of fatty tissue and discolored, edematous liver were found in some chickens. No macroscopic lesions were observed in the gizzard. Apparently if lead is given in a form which would not be retained in the gizzard, no severe necrosis develops in
Chemical analysis of tissue specimens revealed that significant quantities of lead are present in tissues of lead-treated chickens. Deposition of lead in the tissues showed variation in chickens which were in the same lead treatment group as well as within the same chicken. The highest lead concentrations were found in bone (femur). The next highest lead levels were in the kidney. The lowest concentrations of lead were found in muscle. Generally the mean lead content was higher in all tissues of chickens which were given higher doses of lead. However, the mean lead concentration of femur in chickens receiving the 640 mg/kg lead dose was 310.5 ppm. The mean lead concentration of bone tissue in chickens which were given 320 mg/kg and 160 mg/kg of lead was 494.8 ppm and 438.1 ppm respectively. Lead concentrations in liver, kidney and lung were at least three times higher in the 640 mg/kg group than in the 320 mg/kg treatment group. It was reported (7) that the degree of active exposure to lead is indicated by the concentration of the metal in liver while chronic exposure is reflected by relatively higher concentrations of lead in bone. Apparently the concentrations of lead in the bone of 320 mg/kg and 160 mg/kg chickens reflect the longer exposure to lead. Tissues of chickens from these groups were collected after 35 days of lead treatment, whereas, chickens
of the 640 mg/kg group died of acute lead poisoning between 12 and 18 days after initiation of lead treatment.

Examination of kidney, liver and lung sections by light microscopy did not reveal any specific lesions which could be attributed to lead poisoning. Neither necrosis of the proximal convoluted tubules of the kidney (151) nor intranuclear inclusions were found. Intranuclear inclusions in kidney and liver have been reported in other species (6, 72) following lead poisoning. Inclusions were not found even in acid-fast-stained tissue sections of lead-treated chickens in this study.

A number of different inducers were reported to stimulate production of interferon in chickens. Both DNA (87, 111) and RNA (11, 113) viruses as well as statolon (56, 132) have been reported as potent inducers. In contrast tilorone hydrochloride (132) and polyI-polyC have been reported as poor inducers. A number of interferon stimulators were used in this study (Experiment III). Pseudorabies and swine influenza viruses, which induce circulating interferon in pigs (166), were not effective in inducing interferon in chickens at the doses used. PolyI-polyC at the several doses and routes of inoculation used induced very low titers of circulating interferon. This finding agrees with reports in the literature (56, 132). In contrast statolon at the 5000 mg/kg dose level and NDV-B1 I.V. were effective in
producing high levels circulating interferon of long duration. Because of these findings statolon and NDV were chosen for use in Experiment III.

Recent studies suggest that the immunologic system and the interferon system may be linked (43, 75). It has been reported that a variety of agents and environmental factors like X-irradiation (43) and corticosteroids limit antibody and interferon production. Freund's adjuvant on the other hand helped promote the production of antibodies and interferon (44).

There is considerable evidence that lead exerts adverse effects upon the resistance of the body to disease by influencing immunologic mechanisms. Lead poisoning has been associated with the increased susceptibility of mice to Salmonella infection (81) and increased sensitivity to bacterial endotoxin in rats (147) and chickens (161). Lead poisoning has also been associated with a decrease in the production (59) and inactivation (178) of antibodies.

It has been reported that arsenicals inhibit interferon production and action in mice and rabbit kidney cells (62). Recently it was reported that lead acetate increases the \textit{in vivo} interferon-inducing capacity of polyI•polyC when injected simultaneously into mice (45). The mechanism by which lead acetate increases the interferon response to polyI•polyC is not clear (45).
In this study long-term lead treatment of chickens at the lead dosage levels used did not affect interferon response to statolon. Neither interferon concentration in the blood nor duration of detectable interferon in serum were changed as compared with the controls. Lead treatment of chickens at the 160 mg/kg level slightly decreased blood interferon concentration induced by NDV. The duration of circulating interferon was not affected.

Only NDV virus was used as an interferon inducer in the 320 mg/kg chickens since 50 per cent of the treated birds died before administration of inducer. All chickens but one in this group showed some clinical signs of lead poisoning at the time of interferon stimulation. Interferon response to NDV was markedly changed in concentration and duration. Control chickens had a maximal titer of 512 at 24 hours postinduction and a titer of 16 at 100 hours postinduction. The interferon titer of chickens of the 320 mg/kg treatment group reached a maximum interferon titer of 64 at 24 hours and no circulating interferon was found at 72 and 100 hours postinduction.

It appears that subclinical doses of lead of up to 160 mg/kg for 30 days did not affect interferon production in chickens in response to statolon or NDV. The slight decreases in interferon concentration of the NDV-inoculated chickens could be due to variation in the ability of
individual chickens to produce interferon. Another possi-
bility is that lead-treated chickens do not support NDV
replication as well as untreated chickens. Replicating
virus in chickens has the potential to stimulate more
interferon-producing cells and for a longer period of time.
It is known that high interferon titers are often found at
the tissue site of greatest viral replication (168). On
the other hand circulating interferon titers were not
affected by lead treatment when statolon was used as an
inducer. Since statolon is a non-replicating inducing sub-
stance, its interferon-inducing capacity is limited to the
initial dose.

The decrease in interferon concentration and duration
observed in chickens of the 320 mg/kg group could be due to
lead interference with many biological functions in the
body. Lead decreases nicotinic acid (21) and many amino
acids (17), inhibits biosynthesis of DNA and RNA (103) and
interferes with the activities of numerous enzymes (41, 101,
170, 171). It is obvious that interference with any of these
biologically important functions may lead to suppression of
interferon production. The decrease in interferon concen-
tration and duration could be due to the inability of
lead-treated cells to support NDV replication.

The results of this experiment indicate that subclinical
doses of lead do not markedly suppress interferon production
in response to statolon and NDV in chickens. However, the
effect of lead on interferon action should be further in­
vestigated. It is possible that the antiviral action of
interferon could be suppressed or inhibited by lead poisoning
since the mechanism of antiviral action is an involved
process and requires synthesis of a new protein (60, 84,
93). Without studying the effect of lead poisoning on inter­
feron action one cannot disprove or prove the statement that
metals, by suppressing interferon, activate or induce the
oncogenic viruses already present in the host. It is very
possible that the suppression of interferon action is one of
the mechanisms by which metals cause tumors.

Long-time lead exposure had no marked effect on anti­
body production to NDV in chickens. No consistent correla­
tion was observed between blood lead concentration and anti­
body titer. Even two chickens out of three of the 320 mg/Kg
group, which showed clinical signs of lead poisoning and had
blood lead concentrations of 9.0 and 10.85 ppm, did not have
decreased antibody titers to NDV. The third chicken of this
group had a blood lead concentration of 13.64 and its anti­
body titer was markedly decreased.

The results of these studies indicate that long term
subclinical lead intake suppresses neither interferon nor
antibody production in chickens. One should not forget
the fact that chickens are very resistant to lead poisoning,
as shown in these studies, and thus these findings may not be applicable to other species.
SUMMARY

The effect of interferon and statolon on Marek's disease in chickens and the effect of lead on interferon production in chickens were studied.

Four-day-old chickens free of MDV antibodies were inoculated intraperitoneally with the JMV strain of MDV. Exogenous interferon induced by NDV in chicken embryos was administered I.P. daily for 14 days into three groups of chickens. Interferon administration was initiated simultaneously with virus in the first group, 72 hours after virus inoculation in the second group and 168 hours following virus inoculation in the third group. A survival rate of 17.3 per cent was observed when interferon administration was initiated simultaneously with virus. When exogenous interferon administration was started 72 hours after virus inoculation, only 7.4 per cent of chicks survived. Interferon treatment at 168 hours after virus inoculation, at the time when first chicks started to show clinical signs, neither protected the chicks nor prolonged their survival.

A statistical analysis by the chi-square test indicated no significant difference (P<0.05) between survival in the interferon-treated and control groups. The percentage of surviving chicks decreased as interferon treatment was initiated at successively longer periods after virus inoculation.
The biological data collected in this experiment show that interferon at the concentration used may have prophylactic value when administered at the time of infection, but is without any effect once infection is established.

The experiment on the effect of statolon on MDV in chickens was performed twice. Four-day-old chickens were inoculated with MD-JMV and a single dose of 500 mg/kg statolon I.P. was given at different times. The effect of statolon on the survival of chicks was marked and the time of statolon administration was critical. In both trials the maximum protection was achieved when statolon was given 24 hours after virus. The survival rate was 56.8 and 51.5 per cent respectively as compared with five and zero per cent in the control groups. A statistical analysis by the chi-square test indicated significant difference (P<0.05) between chicken survival in statolon-treated and control groups except when statolon was administered 96 hours or later after the virus.

The data show that statolon has a prophylactic value in protecting chicks against MD-JMV. The therapeutic value of statolon at the dosage used was insignificant.

The effect of aqueous lead acetate given per os to chickens for 35 consecutive days was investigated. Chickens were found to tolerate levels of lead as high as 160 mg/kg without exhibiting clinical signs or hematological changes in spite of very high levels of lead in the blood. The
lead concentration in pooled blood samples of twelve chickens which were given 160 mg/kg of lead for 30 days was 6.2 ppm. It is apparent from these findings that chickens are more resistant to lead poisoning than humans, horses, dogs and wild fowl such as ducks.

Fifty per cent of the chickens treated at a lead level of 320 mg/kg died within 30 days. All except one of the chickens receiving 640 mg/kg lead died within 30 days. The clinical signs included weakness, anemia and loss of weight. A marked peripheral paralysis was observed in most chickens a day or two prior to death. Hemoglobin concentration was markedly decreased in all chickens of the 320 mg/kg and 640 mg/kg lead-treatment groups. Examination of blood smears revealed increased numbers of immature erythrocytes, some of which were in various stages of mitosis.

Statolon (500 mg/kg) and NDV-B₁ were used I.V. as interferon inducers in chickens which previously received lead at the rate of 20 mg/kg, 40 mg/kg, 80 mg/kg or 160 mg/kg for 30 days per os. Only NDV-B₁ was used as an interferon inducer in the group of chickens which received lead at the 320 mg/kg level for 30 days. In this study lead treatment of chickens at the dosage levels used did not affect interferon response to statolon. Lead treatment of chickens at the 160 mg/kg level slightly decreased blood interferon concentration induced by NDV. Interferon
concentrations and duration in serum were markedly decreased in chickens which received lead at the 320 mg/kg level.

The viral inhibitor produced in chickens by statolon and NDV stimulation was characterized as being acid-resistant, non-dialyzable, non-sedimentable at 100,000g for 60 min., trypsin sensitive, heat stable and species specific. Possession of these characteristics qualifies the inhibiting substance as an interferon.

Long-time lead exposure had no marked effect on antibody production to NDV in chickens. No consistent correlation was observed between blood lead concentration and antibody titer.

The results of these studies indicate that long-term subclinical lead intake suppresses neither interferon nor antibody production in chickens. One should not forget the fact that chickens are very resistant to lead poisoning, as shown in these studies, and thus these findings may not be applicable to other species.
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