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IMMUNOPATHOLOGY OF 8-WEEK-OLD RING-NECKED PHEASANTS (*PHASIANUS COLCHICUS*) EXPOSED TO MALATHION

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Abstract—In addition to their acute neurotoxic effects, some organophosphorus compounds have been shown to have immunotoxic properties. Alterations in the immune system may lead to chronic morbidity and/or mortality that is not readily apparent at the time of initial exposure. Because it often inhabits land that is intensely farmed, the ring-necked pheasant (*Phasianus colchicus*) may be a suitable biomonitor of agroecosystems, especially those used in the production of corn. We examined immunopathologic effects of a widely used organophosphate insecticide, malathion, on 8-week-old, cage-reared ring-necked pheasants 3 d after a single oral dose. No differences were seen in hematologic parameters, body weight, or weight of the bursa of Fabricius. Birds given a high dose of malathion (230 mg/kg) displayed significant decreases in absolute and relative thymic and splenic weights ($p \leq 0.05$). Significant changes were also seen in thymic and splenic histomorphometry ($p \leq 0.05$). The high-dose group and, in some cases, the low-dose group (92 mg/kg) displayed significant histologic lesions. These findings indicate that a single dose of malathion, at the LD50 level (230 mg/kg) and occasionally at 40% of the LD50 level (92 mg/kg), is capable of inducing quantitative and qualitative changes in the lymphoid organs of the ring-necked pheasant that may affect immune function.

Keywords—Ring-necked pheasant Biomonitor Malathion Chronic Immunopathology

INTRODUCTION

The use of wildlife species as monitors of environmental integrity has become increasingly important, particularly in the area of ecological risk assessment [1]. The ring-necked pheasant (*Phasianus colchicus*) may be useful as a biomonitor in evaluating agroecosystems, especially those used in the production of corn. Because they extensively use cornfields and adjacent habitat, ring-necked pheasants may be preferable to northern bobwhite quail (*Colinus virginianus*) as biomonitors in these environments [2].

Organophosphates represent the largest known category of insecticides and have been used effectively for over 40 years [3]. Their low environmental persistence has made them much more acceptable than some other insecticide groups [4]. Although they are best known for their acute neurotoxic effects in insects and nontarget species, they also have been associated with other conditions such as delayed neuropathy [5], teratogenicity [6], rhabdomyopathy [7], pneumotoxicity [8], and immunotoxicity [9–11].

In the past, end points of ecological experiments involving wildlife often have been limited to measurement of death or reproductive variables. Because of the relatively recent increase in the number and power of investigative techniques and the opportunities they offer to expand the scientific database, a greater variety of end-point choices now exists, some of which include immune parameters. The importance of the immune system in maintaining homeostasis has become a focus of intense interest [12]. An impaired immune system can lead to increased incidence of death and disease caused by a variety of opportunistic pathogens and neoplasias. In this regard, the adverse effects of organophosphate insecticides may extend beyond acute neurotoxicity and encompass significant, subacute to chronic morbidity and mortality.

On a broad scale, immunopathology has been defined as the study of lesions induced by or resulting from immune mechanisms [13]. For purposes of this study, immunopathology involves the study of disease in different organs of the immune system and can include such parameters as hematology, body and lymphoid organ weights and ratios, lymphoid histomorphometry and lymphoid organ histopathology [14]. Many studies have shown that histopathologic end points can be quite useful in assessing the potential immunotoxicity of a chemical [15,16]. Histopathology allows examination of lymphoid organs and cells as they are affected under in vivo conditions [17]. Immunopathology represents only a portion of a battery of tests designed to assess immunotoxicity.

For this study, modifications of the National Toxicology Program's guidelines for immunotoxicity evaluation in mice [18] were adapted to the pheasant with primary focus on the bursa of Fabricius (BOF), thymus, and spleen. The BOF and thymus are chiefly nonantigen driven, primary lymphoid or-

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gans responsible for B-lymphocyte (humoral immunity) and T-lymphocyte (cell-mediated immunity) selection, expansion, and maturation. These organs contrast with the spleen, which is highly antigen driven and is involved with rapid humoral and cellular response to circulating antigen. The objective of this controlled laboratory study was to examine the lymphoid system of the ring-necked pheasant for immunopathologic changes following a single oral dose of malathion (dimethyl mercaptosuccinate, *S*-ester with *O,O*-dimethyl phosphorodithioate), a moderately toxic organophosphate insecticide.

MATERIALS AND METHODS

Animals

Day-old, mixed-gender, outbred pheasant chicks were obtained from a local supplier (Oakwood Game Farm, Princeton, MN) and raised in suspended wire cages in limited-pathogen-exposure facilities. Birds were maintained on granular feed (T-10 Turkey Starter, University of Minnesota Feed Center, Rosemount, MN). Food and water were available at all times. Serology was performed on 10 randomly selected birds after loss of maternal antibody (12 weeks). Antibodies to hemorrhagic enteritis virus were measured using agar gel immunodiffusion. Antibodies to *Mycoplasma gallisepticum* and *Mycoplasma synoviae*, Newcastle disease virus, and infectious bronchitis virus were measured using hemagglutination inhibition. The medial lethal dose (LD50) range of malathion in pheasants was reported to be 120 to 230 mg/kg [19]. Preliminary observational studies indicated that for our 7- to 8-week-old pheasants, the LD50 was approximately 230 mg/kg. When they reached 7 to 8 weeks of age, birds were randomly divided into four groups: a control group ($n = 10$), a low-dose group ($n = 10$), a high-dose group ($n = 20$), and an immunosuppressed group (IMS) ($n = 4$). All control, low-dose-, and high-dose-group birds were dosed by gavage using a semirigid polypropylene tube attached to a syringe. The control-group birds received 2.5 ml of fresh corn oil. The low-dose-group birds received malathion in corn oil at 40% of the LD50 (92 mg/kg). The high-dose group received malathion in corn oil at the LD50 (230 mg/kg). Final volumes given to malathion-dosed birds were between 2 and 3 ml. IMS-group birds received either cyclophosphamide ($n = 2$) at 12 mg/kg once/d intraperitoneally for 3 days or dexamethasone ($n = 2$) at 2 mg/kg once/d intramuscularly for 3 d. Birds in the 7- to 8-week-old category were selected to correlate with conditions in the field where, at this age, a significant portion of the pheasant diet still consists of insects, the primary mode of toxicant exposure. A single oral dose of malathion was used, also to mimic conditions in the field. Because malathion has a relatively short half-life in soils [20], chronic exposure is less likely.

Brains of birds in the high-dose group that died acutely (within 4 h of exposure) ($n = 7$) were harvested and frozen at −80°C. Three days after dosing, blood was drawn for hematology, and the animals were euthanized using CO_2. Body weights and lymphoid organ (BOF, thymus, spleen) weights were measured. These tissues were then fixed in fresh 10% neutral buffered formalin for histomorphometric and histopathologic evaluation. To verify the extent of malathion exposure via acetylcholinesterase (AChE) activity levels, brains were also harvested from birds in all groups and frozen at −80°C. Brain cholinesterase activity was measured according to a modification [21] of the colorimetric procedure of Ellman and expressed as μmol acetylthiocholine iodide hydrolyzed/min/g of wet tissue.

Chemicals

Technical-grade malathion (Cythion® ULV) was supplied by the American Cyanamid Company (Princeton, NJ) as a 95% solution. Neutral buffered formalin (10%) was obtained from University Stores, University of Minnesota (Minneapolis, MN). Dexamethasone (4 mg/ml as the sodium phosphate) was obtained from the Butler Company (Columbus, OH). Cyclophosphamide (Neosar 100 mg/vial) was purchased from Adria Labs (Columbus, OH). Unless otherwise mentioned, all other reagents were purchased from Sigma Chemical Company (St. Louis, MO).

Immunopathology

Blood for hematology, obtained via brachial (cutaneous ulnar) venipuncture, was collected in heparinized syringes. White blood cell counts were made manually using the eosinophil Unopette 5877 system (Becton Dickinson Company, Rutherford, NJ) and a Neubauer hemocytometer and were rounded to the nearest 100. Differential counts were made from blood smears stained with Diff Quik (American Scientific Products, McGraw Park, IL) according to the recommendations of Campbell [22].

Morphometric evaluations of BOF, thymus, and spleen were made using a 21-mm-diameter Chalkley 25 cross reticle (KR-822, Klarman Rulings Inc., Manchester, NH). Four-micron-thick sections of each organ from all birds (prepared as for histopathology) were examined according to the point-counting method [23,24]. Within the BOF and thymus, the cortex, medulla, stroma, and clear-space regions were measured. In the spleen, diffuse lymphoid tissue, germinal center, ellipsoid, red pulp, and stromal areas were quantified. Foci of examination occurred where four equidistant radii were bisected, except in the thymus where two radii were used. In each field, the organ region under each of the 25 crosses of the reticle was classified, and the total percentage of each region was calculated from the respective sums from four fields.

For histopathologic evaluation, lymphoid tissue specimens from all birds were prepared for staining using an automated processor (MVP II, Instrumentation Laboratory, Minneapolis, MN). After processing, tissues were embedded in paraffin. Four-micron-thick sections were mounted and stained with Harris Hematoxylin (Fisher Scientific Co., Pittsburgh, PA) and Eosin Y (Mallinckrodt Corp., Chesterfield, MO). Sections were then evaluated for histopathologic changes by examining four randomly selected sites within specified micro-anatomic regions. The average of four fields was used as the final value for each bird. Lesions characterized by necrosis of individual cells and the presence of increased numbers of macrophages were quantified using the central four squares of the point-counting reticle. The primary indices used for describing degenerative change were numbers of degenerating cells and the extent of their cyto-

Table 1. Lymphoid organ and body weights and ratios in 8-week-old ring-necked pheasants 3 d following a single oral dose of malathion

Group	n	Body wt. (g ± SD)	BOF wt. (g ± SD)	BOF:bw ($\times 10^{-4}$ ± SD)	Thymus wt. (g ± SD)	Thymus:bw ($\times 10^{-4}$ ± SD)	Spleen wt. (g ± SD)	Spleen:bw ($\times 10^{-4}$ ± SD)
Control	10	429 ± 88	0.62 ± 0.32	14.0 ± 5.5	1.45 ± 0.54	33.3 ± 8.5	0.48 ± 0.21	10.8 ± 3.1
Malathion								
92 mg/kg	10	402 ± 50	0.50 ± 0.17	12.4 ± 2.8	1.37 ± 0.61	33.2 ± 11.2	0.48 ± 0.20	11.8 ± 4.9
230 mg/kg	13	405 ± 82	0.48 ± 0.16	11.7 ± 2.6	0.95 ± 0.38*	22.9 ± 8.6*	0.31 ± 0.11*	7.7 ± 2.5*
IMS[a]	4	485 ± 66	0.31 ± 0.09	6.4 ± 2.0*	0.68 ± 0.44*	14.0 ± 10.3*	0.41 ± 0.15	8.5 ± 3.8

BOF, bursa of Fabricius; bw, body weight.
Weight and ratio values represent group means ± SD.
[a]IMS equals immunosuppressed with either cyclophosphamide or dexamethasone.
*Significantly different from the control group ($p \leq 0.05$).

plasmic vacuolar (hydropic) change. These alterations were measured using a degree scale (0 = no change to 10 = severe change).

Data analysis

The software system STATISTIX, Version 4.0 (Analytical Software, St. Paul, MN) was used to conduct all statistical analyses. Unless otherwise mentioned, among groups, determination of significant differences concerning the same parameter was measured using one-way ANOVA at level $\alpha = 0.05$, with means separated using the least-significant difference (LSD) method [25]. When data were nonparametric as determined by Bartlett's test of equal variance ($p \leq 0.05$), the Kruskal–Wallis test was used.

RESULTS

Immunopathology

Hematologically, no significant differences ($p > 0.05$) were found among groups concerning the following parameters: total serum protein, packed cell volume, white blood cell count (WBC), lymphocyte and heterophil percentages, and absolute numbers of lymphocytes and heterophils. The high-dose group displayed markedly increased variability compared to the control group concerning WBC values (11,892 ± 5,987 vs. 11,378 ± 2,250). IMS group hematology was not performed.

No significant differences ($p > 0.05$) existed between control and malathion-dosed groups in body weight (bw), BOF weight, and BOF:bw ratios. Significant differences ($p \leq 0.05$) were seen between the control and high-dose (230 mg/kg) groups in thymic and splenic weights and their corresponding body weight ratios. The IMS group was significantly different ($p \leq 0.05$) from the control group in BOF:bw, thymus weight, and thymus:bw parameters (Table 1).

No significant histomorphometric differences ($p > 0.05$) were seen between control and low-dose groups in any parameter. The control, high-dose, and IMS groups differed significantly ($p \leq 0.05$) in several parameters. Regarding the BOF, the high-dose and IMS groups exhibited increased clear space. In the thymus both groups displayed relatively decreased cortical area and correspondingly increased medullary area. In the spleen, both groups displayed significantly increased ellipsoidal area. Only the high-dose group showed a significant decrease in red pulp area (Table 2).

Histopathology revealed significant lesions ($p \leq 0.05$) in the lymphoid organs (Table 3). Compared to the control group (Fig. 1), bursae in the high-dose group displayed increased medullary reticular epithelial (RE) cell vacuolation (Fig. 2). This was also true in the IMS group. In the bursal cortex the high-dose and IMS groups displayed increased numbers of necrotic lymphocytes. In contrast to the control group (Fig. 3), thymi from high-dose (Fig. 4) and IMS groups displayed increased numbers of vacuolated cortical reticular epithelial cells. Increased numbers of cortical macrophages and necrotic lymphocytes were observed in low-dose, high-dose, and IMS groups. In the spleen, compared to the con-

Table 2. Histomorphometric changes in lymphoid organs of 8-week-old ring-necked pheasants 3 d following a single oral dose of malathion

Group	n	BOF clear space (% ± SD)	Thymic cortex (% ± SD)	Thymic medulla (% ± SD)	Splenic ellipsoid (% ± SD)	Splenic red pulp (% ± SD)
Control	10	3.0 ± 1.5	56.4 ± 6.7	38.2 ± 6.7	15.3 ± 3.5	32.4 ± 4.8
Malathion						
92 mg/kg	10	2.6 ± 1.3	54.4 ± 4.2	37.6 ± 3.8	18.6 ± 2.7	30.6 ± 2.7
230 mg/kg	13	5.7 ± 2.7*	44.0 ± 6.2*	44.9 ± 4.2*	28.9 ± 7.6*	24.1 ± 9.9*
IMS[a]	4	8.5 ± 3.1*	48.0 ± 1.6*	46.5 ± 2.5*	25.3 ± 4.5*	28.5 ± 9.1

Percentage values represent group means ± SD.
[a]IMS equals immunosuppressed with either cyclophosphamide or dexamethasone.
*Significantly different from control group ($p \leq 0.05$).

Table 3. Histopathology of lymphoid organs of 8-week-old ring-necked pheasants 3 d after a single oral dose of malathion

		BOF		Thymus			Spleen
Group	*n*	Med. RE cell vac.[a] (deg. scale ± SD)[f]	Cor. lymph. nec.[b] (avg. no./ field ± SD)[g]	Cor. RE cell vac.[c] (deg. scale ± SD)	Cor. macrophages[d] (avg. no./ field ± SD)	Cor. lymph. nec. (avg. no./ field ± SD)	EAC vac.[e] (deg. scale ± SD)
Control	10	1.8 ± 0.79	3.5 ± 1.3	0.80 ± 0.79	0.71 ± 0.42	0.75 ± 0.49	1.0 ± 1.0
Malathion							
92 mg/kg	10	2.6 ± 1.2	4.0 ± 1.3	1.7 ± 1.1	2.5 ± 0.99*	2.4 ± 0.94*	1.9 ± 1.5
230 mg/kg	13	3.1 ± 0.95*	5.5 ± 1.8*	3.6 ± 1.5*	5.6 ± 1.8*	4.3 ± 1.5*	2.9 ± 1.4*
IMS[h]	4	5.3 ± 1.7*	5.2 ± 0.72*	4.3 ± 1.3*	3.5 ± 1.2*	2.9 ± 0.25*	2.5 ± 0.58*

Lesion values represent group means ± SD.
[a]Medullary reticular epithelial cell vacuolation.
[b]Cortical lymphocyte necrosis.
[c]Cortical reticular epithelial cell vacuolation.
[d]Cortical macrophages.
[e]Ellipsoid-associated cell vacuolation.
[f]Degree scale: (no lesion)—0 10—(severe lesion).
[g]Average number of cells per field based on evaluation of 4 fields.
[h]IMS, immunosuppressed with either cyclophosphamide or dexamethasone.
*Significantly different from the control group ($p \leq 0.05$).

trol group (Fig. 5), the high-dose group (Fig. 6) and the IMS group displayed increased ellipsoid-associated cell (EAC) vacuolation.

Controls

Antibody titers of 10 randomly selected, 12-week-old birds were negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and the agents of infectious bronchitis, Newcastle disease, and hemorrhagic enteritis. None of the birds in the low-dose (92 mg/kg) group died. In the high-dose (230 mg/kg) group, seven birds (35%) died acutely within 4 h of exposure. There were significant differences ($p \leq 0.05$) among all groups regarding activity of brain AChE (Table 4).

DISCUSSION

Results of these experiments indicated that a single oral dose of malathion induced significant changes in weight, histomorphometric, and histologic parameters in the lymphoid organs of the ring-necked pheasant. These changes may have deleterious implications concerning lymphoid ontogeny and antigen neutralization. Several possibilities could be included when considering the cause(s) of the immunotoxic changes induced by malathion exposure. They include direct effects of native malathion; direct effects of a metabolite of malathion [26]; direct effects of an impurity in the malathion solution (e.g., *O,O,S*-trimethyl phosphorothioate) [27]; indirect effects mediated through endogenous neuroendocrine mechanisms including the autonomic nervous system, the hypothalamic-pituitary axis, neuropeptides and transmitters, and neuroimmunopeptides [28]; a combination of these possibilities. Determination of the definitive cause(s) of changes observed is complex and incidental to this experiment, as our main objective was to determine if measurable immunopathologic changes occurred following malathion ex-

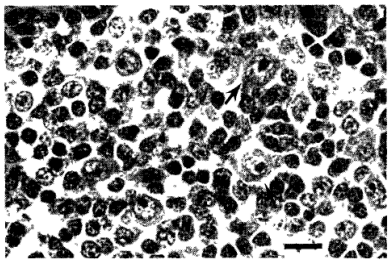

Fig. 1. Bursa of Fabricius medulla from control pheasant showing normal, stellate, reticular epithelial cells (arrows). These cells are surrounded by smaller B-lymphocytes. Bar = 10 μm.

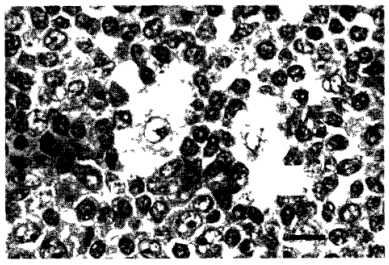

Fig. 2. Vacuolated bursal medullary reticular epithelial cells (arrows) in pheasant exposed to malathion (230 mg/kg). Increased cytoplasmic clear space is consistent with hydropic degeneration. Bar = 10 μm.

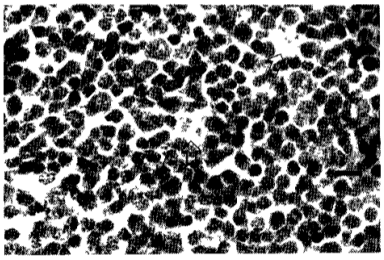

Fig. 3. Thymic cortex from control pheasant showing normal reticular epithelial cells (open arrows). Because of the adjacent closely packed T-lymphocytes, only the nuclei and small amounts of cytoplasm are visible. Bar = 10 μm.

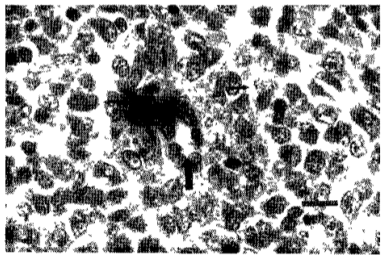

Fig 5. Normal splenic ellipsoid with central penicillar capillary (large arrow) and peripheral ellipsoid associated cells (smaller arrows) in a control pheasant Bar = 10 μm.

posure. However, based on this study and others, it seems most likely that malathion exerts its immune-altering effects through multiple events.

In all groups, differences in body weight and BOF weight were not statistically significant (Table 1). The lack of difference in BOF weight likely was related to high variability within groups and to a level of refractoriness of the organ due to its involutionary physiologic status. During involution, bursal epithelial cells are less sensitive to the effects of extraneous agents. Nevertheless, BOF weights did display a downward trend as increasing levels of malathion or the immunosuppressive agents were used. When adjusted for body weight, the BOF:bw ratio did exhibit a dramatic decrease in the IMS group. Both the high-dose and IMS groups showed significant decreases in thymus weight and thymus:bw values. These findings may reflect the more sensitive nature of the pheasant thymus, which probably does not begin to involute until 15 to 17 weeks of age, as occurs in the chicken [29]. The splenic weight and spleen:bw ratio were both significantly decreased in the high-dose group, but not in the IMS group. This finding may reflect cholinergic-induced contraction of the spleen mediated through autonomic stimulation.

Significant histomorphometric changes were seen only in the high-dose and IMS groups (Table 2). Increased clear space in the BOF was likely secondary to shrinkage of the organ due to uniform loss of lymphocytes. There was significant loss of thymic cortical area with a corresponding relative increase in medullary area. This finding also reflected loss of cortical lymphocytes. In the spleen the ellipsoidal area surrounding the penicillar capillaries was increased in both high-dose and IMS groups. Normally, the poorly phagocytic ellipsoid cells are clustered about the mid- and distal portions of the penicillar capillaries and are 2 to 3 cell layers thick.

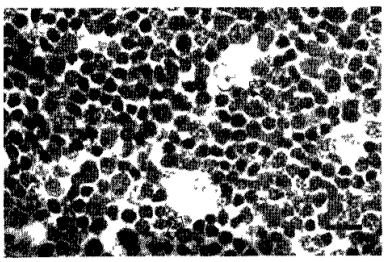

Fig. 4. Vacuolated reticular epithelial cells (open arrows) in thymic cortex of pheasant exposed to malathion (230 mg/kg) Cytoplasmic clear spaces are often separated by fine strands of protein. Bar = 10 μm.

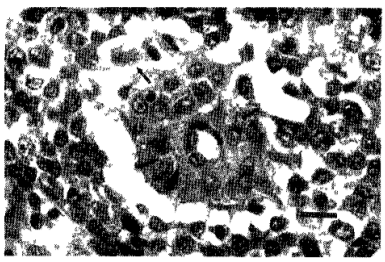

Fig. 6. Vacuolated splenic ellipsoid-associated cells (arrows) surrounding ellipsoid sheath in pheasant exposed to malathion (230 mg/kg). Bands of these cells often create a clear, segmented halo around the sheath Bar = 10 μm

Table 4. Activity of brain AChE in 8-week-old ring-necked pheasants 3 d after a single oral dose of malathion

Malathion (mg/kg)	n	$\bar{X}^{a,b}$	SD
0	10	18.40	1.62
92	10	15.56	1.99
230	13	13.54	1.95
230[c]	7	5.23	1.81

[a]Measures μmol acetylthiocholine hydrolyzed/min/g wet tissue.
[b]All groups were significantly different from each other ($p \leq 0.05$).
[c]Acute deaths (~4 h after dosing).

They are usually oval or round with scant intercellular substance. Particulate antigen and antigen–antibody complexes percolate around these cells to reach the more peripheral and highly phagocytic ellipsoid-associated cells (EAC) [30]. The increase in ellipsoidal area occurred, in part, because of the variable size and disorganization of the ellipsoid cells.

Additionally, in the high-dose group, this increase may have represented gain due to relative decrease in red pulp area secondary to cholinergic-induced adrenergic splenic contraction, perhaps mediated by vascular smooth muscle. Pheasant spleen appears similar to chicken spleen [29] in that it contains scant parenchymal smooth muscle with very poorly developed trabeculae. The red pulp consists of multiple venous sinusoids that are found in loosely arranged lymphoid tissue. Contraction of these vascular sinusoids could lead to significant loss of splenic mass. Interestingly, in the IMS group, which was not exposed to direct cholinergic stimulation, the splenic red pulp area was not significantly different from the control group. This also corresponds with a previously shown lack of significant difference between IMS group splenic weight and spleen:bw ratios. These findings imply that high levels of cholinergic stimulation are associated with splenic contraction and diminished red pulp area, perhaps autonomically mediated.

Histopathologic changes were present in all lymphoid organs in high-dose and IMS groups and in the thymus of the low-dose group (Table 3). A common lesion in all organs was cytoplasmic vacuolar change in certain nonlymphoid cells involved with such functions as lymphocyte development and antigen processing. This type of change was most consistent with cytoplasmic hydropic degeneration. This process can be reversed and is usually caused by mitochondrial toxicosis or hypoxia, leading to diminished adenosine triphosphate (ATP) production [31]. A prominent lesion in the BOF was cytoplasmic vacuolation of medullary RE cells (Fig. 2). These rather large dendritic cells are involved in maintaining a suitable microenvironment in which bursal lymphocytes can mature [32]. They do so through secretory and direct cell-to-cell mechanisms [33]. Developmentally, the medullary reticular epithelial cells appear to be most active near the time of hatch when the available antibody repertoire is markedly expanded through the mechanisms of gene conversion and somatic mutation in lymphocytes residing within the BOF [34,35]. Because the BOF is destined to begin involution at around 4 to 10 weeks of age [36], it seems reasonable to assume that lesions in these cells (at 7 to 8 weeks of age) may not be as critical to normal lymphocyte ontogeny. Another prominent bursal lesion was lymphocyte necrosis in the cortex. These cells usually were individual and randomly distributed. The significant increase in the number of these cells in the high-dose and IMS groups implied treatment effect additional to physiologic deletion.

The thymus, in contrast to the BOF, displays greater functional longevity and appears quite active and vital at 7 to 8 weeks of age. High-dose- and IMS-group birds displayed significant thymic cortical reticular epithelial cell vacuolar change (Fig. 4). The thymus receives lymphocytes via the bloodstream and provides a suitable microenvironment for survival of T-cells whose receptors only recognize foreign antigen (positive selection). Associated with this process is the deletion of cells whose receptors recognize self-antigen (negative selection). It is highly likely that the thymic cortical RE cells are crucial to these ontogenic processes through humoral and direct cell-to-cell mechanisms [37–41]. The degenerative change observed in these cells raises questions concerning their functionality, with possible sequelae consisting of impairment of T-cell-mediated immune responses or initiation of autoimmune processes. Also noticed in the thymic cortex in low-dose, high-dose, and IMS groups were significantly increased numbers of necrotic lymphocytes and macrophages. These findings are similar to those observed with "accidental involution" commonly associated with sudden stress [38] and may later lead to altered immune function. Cyclophosphamide and dexamethasone are both known to cause lysis of T-cells [42].

The most prominent lesion in the spleen of high-dose and IMS groups was vacuolar change in the ellipsoid-associated cells (Fig. 6). The EACs are oval or dendritic cells found on the outer surface of the ellipsoid sheath and are subjacent to the peri-ellipsoidal lymphoid sheath (PELS) [30]. Extensive studies in the chicken [30,43] have shown that EACs are highly phagocytic to circulating particulate and smaller protein antigens that leave the penicillar capillary and diffuse through the ellipsoid sheath. Soon after phagocytosis, EACs detach from the ellipsoid surface and migrate to the border of the T-dependent area where they interact with T- and B-lymphocytes to induce the formation of germinal centers and antibody production [30,36]. Significant hydropic degeneration was observed in many EACs following malathion exposure, potentially obtunding phagocytosis and subsequent immune responsiveness.

Regarding experimental controls, serologic examination and daily observation throughout the experiment confirmed that the birds were disease-free and not affected by extraneous infectious agents that could severely alter the lymphoid organs. All groups (IMS not tested) had significantly different brain AChE activity. These findings verified that malathion had been delivered and metabolized in each subject. Values in low- and high-dose groups represented AChE activity levels 3 d after exposure to malathion, with the high-dose group representing survivors of an LD50 dose ($n = 13$). Behaviorally, birds that died acutely ($n = 7$) were nearly always quiet and stuporous with only rare excitability. Seromucoid oral discharge, ptosis, and piloerection were oc-

casionally seen These signs appeared to be consistent with acute organophosphate toxicity To varying degrees, it was evident that even the control-group birds displayed some immunopathologic changes This finding illustrates that unavoidable stressors (e g , confinement, handling, bleeding, euthanasia) common to all subjects were inherent in this experiment and likely mediated their effects through neuroendocrine mechanisms

The results of this study demonstrated that malathion can induce significant morphometric and histologic changes in the lymphoid organs of the pheasant and point to the need for further studies to determine the degree to which functional immunological impairment is associated with these immunopathologic changes These investigations could include T- and B-lymphocyte response tests, monocyte/macrophage function assays, and/or challenge with a pathogenic agent Also shown was the fact that immunopathologic end points in a terrestrial avian species are practical and quantifiable Finally, this study helped to address the issue of potential subacute to chronic morbidity and/or mortality due to immune alteration long after initial exposure to an acute-acting chemical such as malathion

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