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Effect of Nucleopolyhedrosis Virus on Selected Mammalian Predators of the Gypsy Moth

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Effect of Nucleopolyhedrosis Virus on Selected Mammalian Predators of the Gypsy Moth[1]

Abstract

Nucleopolyhedrosis virus (NPV) of the gypsy moth was fed to three mammalian predators of the insect: the white-footed mouse, the short-tailed shrew, and the Virginia opposum in the form of NPV-infected 5th instar gypsy moth larvae, polyhedral inclusion bodies (PIB's) mixed in dog food and PIB's mixed in a standard spray formulation. The total amount of NPV consumed by each treated mouse and shrew was equivalent to more than a 40-ha exposure for a 70kg person, assuming the NPV was applied at the rate of 5.0×10^{11} PIB's/ha. Analyses of general body condition, weight, and reproductive efficiency, as well as necropsy and microscopic examination of tissues, indicated that the ingestion of NPV had no short-term effect on these predators of the gypsy moth.

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INTRODUCTION

The nucleopolyhedrosis virus (NPV) of the gypsy moth *(Lymantria dispar* L.) is being developed by the U.S. Department of Agriculture as a biological control agent. During experimental field spraying of NPV for control of the gypsy moth, approximately 5.0 x 10^{11} polyhedral inclusion bodies (PIB's) of this virus are delivered per hectare. The spraying of large woodland areas with NPV presents a potential hazard to resident mammals because they may consume contaminated food, NPV-infected gypsy moth larvae, or both. Our observations indicate that white-footed mice *(Peromyscus leucopus),* short-tailed shrews *(Blarina brevicauda)* and Virginia opposums *(Didelphis marsupialis)* readily ingest gypsy moth larvae that are in early stages of NPV infection.

Published reports have indicated that other nucleopolyhedrosis viruses have no deleterious effects on either laboratory animals (Aizawa 1954a, Smirnoff and MacLeod 1964, Ignoffo and Hiempel 1965, Ignoffo et al. 1970, Meinecke et al. 1970) or man (Aizawa 1954b).

The NPV of the gypsy moth has been shown to produce no adverse effects when tested against a variety of laboratory animals[2,3,4], but there have been no reports of its effects on the wild mammalian predators of the insect. The following is a report on the responses of the white-footed mouse, the short-tailed shrew and the Virginia opossum to the consumption of gypsy moth NPV

MATERIALS AND METHODS

In March 1975, 40 white-footed mice, 6 short-tailed shrews and 2 Virginia opossums were collected in the field. Mice and shrews were caged on absorbent granules and wood chips in 38-liter glass aquaria with screen tops. Mice were caged in pairs (1 female and 1 male), with cotton as nesting material, but shrews were caged individually. Opposums were housed individually on wood chips in 0.6 x 0.6 x 1.2-m rabbit cages. As a normal diet, mice were fed Charles River[5] mouse chow and sunflower seeds, shrews were fed Alpo dog food and sunflower seeds and opossums were fed Alpo dog food. All animals were allowed at least a 1-week adjustment period. At the end of this period animals were weighed, alloted to test and control groups, and then denied food for 24 hours.

On day 1, each test animal was offered 30 5th instar gypsy moth larvae that had been infected with NPV by allowing 4th instar larvae to feed on a diet containing 1.0 x 10^6 PIB's/ml. These larvae were in their 8th day of infection when fed to the test animals and each contained between 3.5 x 10^8 and 6.0 x 10^8 PIB's. Test animals were fed their normal diet on day 2, again offered 30 NPV-infected larvae on days 3 and 4, and then fed their normal diet through day 11. Control animals were fed healthy 5th instar larvae and normal diet on the same schedule.

On day 12, purified PIB's (Breillatt et al. 1972) were mixed in 1 g of lactose, then were mixed with dog food and fed to test animals at the rate of 1.0 x 10^9 PIB's/animal per day for 3 days. Control animals were fed the same amount of lactose and dog food minus the PIB's. On day 15, all animals resumed eating their normal diet.

On day 18, test animals were offered dog food mixed with the standard spray formulation (CIB) for gypsy moth NPV; each liter contains: Cargil Insecticide Base[5], 123 ml; Chevron Spray Sticker, 23 ml; IMC 90-001, 58.6 g; H_2O, 845 ml and 1.0 x 10^8 PIB's. Test animals were fed at the rate of 1.0 x 10^5 PIB's/animal per day for 3 days. Control animals were fed dog food mixed with CIB minus PIB's. All animals resumed eating a normal diet on day 21.

All animals were weighed at least twice a week during the experiment, and were checked daily for mortality and signs of disease. Male mice were checked regularly for descent of testes, an indication of reproductive activity. On day 32, all animals were killed and fixed whole in 10 percent neutral buffered formalin. After necropsy, the following organs and tissues were

[2] Litton Bionetics, Inc. 2-year Carcinogenicity Study in Rats. Nucleopolyhedrosis Virus (NPV) of the gypsy moth. Unpublished report 1975.

[3] Litton Bionetics, Inc. Acute Toxicity Tests. Nucleopolyhedrosis Virus of the gypsy moth. Unpublished report 1975.

[4] Litton Bionetics, Inc. Subacute Toxicity Tests. Nucleopolyhedrosis Virus of the gypsy moth. Unpublished report 1975.

[5] Mention of a proprietary product in this paper does not constitute an endorsement by the U.S. Department of Agriculture or the Forest Service.

removed for histopathological examination: brain, spinal cord (mice and shrews), trachea, lung, heart, liver, kidneys, urinary bladder, spleen, thyroid, adrenals, salivary glands, esophagus, stomach, small intestine, pancreas, testes, epididymis, accessory sex organs, ovaries, uterus, mammary glands of lactating females, skin, bone marrow, and lymph nodes. These organs and tissues were processed routinely, sectioned at 6 μm, stained with hematoxylin and eosin, and examined by light microscopy.

RESULTS AND DISCUSSION

Both test and control mice ate at least 90 percent of the larvae offered during the experiment. Shrews ate about 33 percent of the larvae, whereas the opossums ate all of the larvae offered. Test animals ate more than 90 percent of both the dog food mixed with lactose and PIB's and the dog food mixed with CIB and PIB's. The total amount of NPV consumed by each test mouse and shrew was equivalent to more than a 40-ha exposure for a 70-kg person, assuming that NPV was applied at the rate of 5.0×10^{11} PIB's/ha.

Table 1 shows the numbers of experimental animals. The test mice produced five litters during the experiment, compared with only one litter from the control mice. This difference probably occurred because some of the test mice were captured several days before the controls and were better cage-adapted at the start of the experiment. At necropsy, the two groups did not differ significantly in number of pregnant females or in number of embryos per pregnant mouse (table 2.)

For analysis of body weight of the mice and shrews and the number of male mice with descended testes, the experiment was divided into four periods: 1) before feeding of PIB's; 2) after feeding of infected larvae; 3) after feeding of PIB's and 4) after feeding of PIB's and CIB.

There were no significant differences between groups in body weights of mice and shrews (table 3) or in the number of male mice with decended testes (table 4). No differences in general condition of the two groups of each species were seen during the experiment or at necropsy.

Histopathological examination revealed no significant differences between adult animals of the control and test groups. Control mice had higher incidences of periportal lymphocytic infiltration of the liver than test mice (table 5). However, these lesions were minimal in 6 of 10 control mice, whereas 4 control mice and 4 test mice were found to have mild to moderate lesions. This finding indicated an equal distribution between groups of the more severe forms of this lesion.

The test mice had a slightly higher incidence of splenic follicular hyperplasia than the controls, but the difference was not statistically significant. Splenic plasmacytosis occurred more often in control mice than in mice exposed to NPV. This lesion may be related to age, for it also occurred in 5 of 20 mice in the test group born during the experiment. The age of the field-collected mice was not known, but there

Table 1.—Numbers of experimental animals

Group	Treated animals	Born during experiment
Mice:		
Test	17 (8 males, 9 females) [a]	20 (12 males, 8 females) [b]
Control	20 (10 males, 10 females)	4 (2 males, 2 females) [c]
Shrews:		
Test	3 (2 males, 1 female)	0
Control	3 (2 males, 1 female)	0
Opossums:		
Test	1 (adult, male)	-
Control	1 (adult, female)	0

[a] Three of the 20 mice originally in this group escaped during the experiment.

[b] Five litters

[c] One litter; all animals died shortly after birth.

Table 2.—Pregnancy status of mice at necropsy

Group	Number of females	Number pregnant	Number of embryos per pregnant female [a]
Test	9	5	4.6 ± 1.3
Control	10	6	5.2 ± 1.2

[a] Mean and standard deviation

Table 3.—Body weights of mice and shrews (in grams) [a]

Group	Number of animals	Before NPV feeding	After feeding of:		
			Larvae	PIB's	PIB's + CIB
Mice:					
Test males	8	24.3 ± 1.0	23.9 ± 0.9	22.6 ± 0.8	22.6 ± 0.7
Control males	10	25.7 ± 0.6	24.3 ± 1.0	23.9 ± 1.1	23.0 ± 1.1
Test females	9	23.4 ± 2.1	22.7 ± 1.8	23.3 ± 2.6	21.6 ± 2.7
Control females	10	22.8 ± 1.4	22.1 ± 1.1	21.9 ± 0.9	21.2 ± 0.8
Shrews:					
Test	3	19.5 ± 1.6	19.7 ± 0.8	22.1 ± 1.0	21.7 ± 1.5
Controls	3	21.8 ± 1.2	22.7 ± 0.7	23.9 ± 1.0	24.8 ± 1.1

[a] Mean and standard error

Table 4.—Frequency of descent of testes in mice

Group	Before NPV feeding	After feeding of:		
		Larvae	PIB's	PIB's + CIB
Test:				
Number of mice	10	10	9	8
Observations/mouse	2	4	3	5
Total observations	20	40	27	40
Frequency of descended testes at each observation [a]	0.30 ± 0.26	0.15 ± 0.13	0.41 ± 0.34	0.55 ± 0.33
Controls:				
Number of mice	10	10	10	10
Observations/mouse	2	4	3	3
Total observations	20	40	30	30
Frequency of descended testes at each observation	0.30 ± 0.26	0.23 ± 0.14	0.23 ± 0.27	0.58 ± 0.24

[a] Mean and standard deviation

Table 5.—Numbers of microscopic lesions in white-footed mice

Organ	Lesion	Control animals			Test animals: Fed NPV			Test animals: Born during experiment		
		Male	Female	Total	Male	Female	Total	Male	Female	Total
Brain:	Hemorrhage, meniges, mild	0	0	0	0	1	1	0	0	0
Lung:	Interstitial pneumonia, mild	0	0	0	0	0	0	0	1	1
	Parasitic granuloma, mild nematode larvae	0	1	1	0	0	0	0	0	0
	Parasitic arteritis, mild, nematode larvae	0	0	0	0	1	1	0	0	0
Liver:	Fatty change, mild to moderate	1	3	4	3	2	5	2	0	2
	Capillaria hepatica, moderate	2	0	2	0	0	0	0	0	0
	Periportal lymphocytic infiltration minimal to moderate	6	4	10	3	1	4	0	1	1
Spleen:	Follicular hyperplasia, moderate to severe	5	4	9	6	6	12	0	2	2
	Plasmacytosis, moderate	3	2	5	0	2	2	1	4	5
Kidney:	Glomerulosclerosis, moderate	0	0	0	0	1	1	0	0	0
Adrenal Gland:	Lipidosis, cortical, mild to moderate	0	0	0	2	0	2	0	0	0
	Cortical degen- mild	0	1	1	0	0	0	0	0	0
Uterus:	Metritis, suppurative, moderate	-	1	1	-	0	0	-	0	0
Skeletal Muscle:	Sarcosporidiosis, mild	1	1	2	2	1	3	0	0	0
Number of animals examined		10	10	20	8	9	17	12	8	20

may have been a greater number of young animals in the control group.

Other lesions occurred with equal frequency in both groups of mice or were so infrequent as to be considered unimportant. Lymphoid hyperplasia seen in both groups indicated an active immune system in most of these wild mice.

The groups of shrews and opossums were not large enough to reveal any significant differences unless the lesions were very severe. The lesions in these two species (tables 6 and 7) appear to have been spontaneous, with no major differences between control and NPV-treated animals. All the shrews and opossums were heavily parasitized, as were several of the mice, and it appeared that they had adjusted well to these infestations.

In this experiment, only mice were available in large numbers. However, we consider the results for shrews and opossums significant, in

Table 6.—Numbers of microscopic lesions in short-tailed shrews

Organ	Lesion	Control animals			Test animals		
		Male	Female	Total	Male	Female	Total
Liver:	Fatty change, mild to moderate	0	0	0	2	0	2
	Fatty change, severe	1	0	1	0	0	0
	Centrilobular congestion, mild	0	0	0	0	1	1
	Telangiectasis, mild	0	0	0	1	0	1
Spleen:	Extramedullary hematopoiesis, moderate	0	1	1	0	0	0
	severe	1	0	1	1	0	1
Kidney:	Tubular lipidosis, mild to moderate	1	0	1	2	0	2
	Hemosiderosis, moderate	0	0	0	0	1	1
Esophagus:	*Gongylonema sp.*, moderate to severe	2	1	3	2	1	3
Small Intestine:	Nematodiasis, mild	1	1	2	1	0	1
Skin:	Fat necrosis, mild Sebaceous gland hyperplasia, mild	1	0	1	0	0	0
Number of animals examined		2	1	3	2	1	3

Table 7.—Numbers of microscopic lesions in opossums

Organ	Lesion	Control animal	Test animal
Brain:	Meningitis, chronic, mild	0	1
Liver:	Fatty change, mild	1	0
	Congestion, mild	0	1
Spleen:	Extramedullary hematopoiesis, mild to moderate	1	1
	Congestion, moderate	1	0
Kidney:	Glomerulosclerosis, mild	0	1
	Hemosiderosis, mild	0	1
Stomach:	Ulcerative gastritis, moderate to severe, due to *Physaloptera turgida*	1	1
Small Intestine:	Larval cestodiasis, *Taenia sp.*	1	1

light of the massive dose of NPV consumed by the test animals. The gypsy moth is available as a food source to small mammals for only 3 to 4 weeks each year, beginning with 4th instar larvae and ending with pupae. Our observations have indicated that small mammals avoid insects that are heavily infected with NPV. This, coupled with the short life span of both the white-footed mouse and the short-tailed shrew, leads us to believe that the NPV dosage was much higher than these mammals would naturally consume in the wild.

NPV appeared to have no short-term effect on physical condition or reproductive efficiency and caused no consistent differences in the occurrence of gross or microscopic lesions in the white-footed mouse, the short-tailed shrew, or the Virginia opossum, the mammals most likely to ingest nucleopolyhedrosis virus in the wild.

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