The infectivity of polyhedra of nuclear polyhedrosis virus (N.P.V.) after passage through gut of an insect-predator

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Summary. Polyhedral inclusion bodies (P.I.B.) of a nuclear polyhedrosis virus of *Heliothis punctigera* (Lep.) were found in excreta of *Nabis tasmanicus* (Het.), a predator of *H. punctigera.* An immunofluorescent counting method was used to estimate numbers of P.I.B. Bioassays demonstrated that no loss of infectivity occurred during passage through the gut.

The possibility that predators play a role in disseminating insect viruses is suggested in a number of publications[2-6]. Little work has been done on the effect on infectivity of nuclear polyhedrosis virus (N.P.V.) of passage through the gut of predators belonging to the class Insecta.

Materials and methods[7]. Partially purified polyhedral suspensions were obtained by repeated differential centrifugation and sonication[8]. Purified N.P.V. was the antigen used for production of antiserum in a female goat. Antiserum to polyhedra was conjugated with fluorescein isothiocyanate by the method of Nairn[9]. 8-mm-diameter rings of waterproof ink were impressed on gelatin coated slides, using a thin walled tube. 0.02 ml of suspension of polyhedra in 0.05% Tween 80 were confined by these rings. Slow drying overnight gave an even spread of the polyhedral inclusion bodies (P.I.B.). Polyhedra were stained with fluorescein conjugated antiserum. Tests showed that this staining was specific. Slides were washed with sodium-carbonate buffer (0.05 M, pH 9.1) and mounted in glycerol buffered to pH 9.1[10]. Counts of P.I.B. were made with a Zeiss fluorescence microscope with incident illumination (excitation wavelength 290–400 nm, emission observed at 520 nm). Six 5th instar nymphs and 5 adults of the predator *N. tasmanicus,* were fed once on larvae of *H. punctigera* that had died of N.P.V. Their excreta were sampled daily for 10 days during which time they were maintained on healthy larvae. 55 *N. tasmanicus* were caught in fields of lucerne (*Medicago sativa* L.). Excreta produced within 24 h after capture were sampled. All samples of excreta were diluted in 0.2 ml of 0.05% Tween 80, sonicated for 20 min and examined for P.I.B. of N.P.V. of *H. punctigera* by the immunofluorescent method. To generate a standard dose-mortality curve, bioassays were made as follows. 2 ml plastic vials were partly filled with artificial diet[11] (minus formalin), and 0.015 ml of polyhedral suspension of known concentrations was applied to surface of diet. For each of 6 dose levels of polyhedral suspension, 30 neonate larvae of *H. punctigera* were allowed to feed on the diet in separate vials for 10 days, after which mortality was recorded. 2 parallel bioassays were made, after 0.5 min and after 20 min sonication, to ascertain if prolonged sonication would effect the infectivity of P.I.B. Polyhedra in suspensions of *N. tasmanicus* excreta were counted by the immunofluorescent method and dilutions then made to give an expected bioassay mortality of 56%, using the standard dose-mortality curve generated by the earlier bioassays. Comparison of the expected and observed mortality after 10 days gave information about eventual loss of infectivity due to passage through the gut.

Results and discussion. Adults of *N. tasmanicus* voided excreta containing polyhedra 2–4 days and nymphs 1–3 days, after feeding on infected *H. punctigera* larvae. 9% of *N. tasmanicus* caught in lucerne fields passed excreta with P.I.B. of N.P.V. of *H. punctigera.* (table 1). No retention of food residues (and P.I.B.) occurs in gut of *N. tasmanicus* nymphs, in contrast to *Oncopeltus fasciatus*[12]. 20 min sonication had no effect on the infectivity of P.I.B. used in these experiments. This finding was important, because P.I.B. were clustered in the excreta and 20 min sonication gave a more homogenous suspension required for an accurate estimate of numbers of P.I.B. by the immunofluorescent method. The LD_{50} for neonate larvae of *H. punctigera* is approximately 0.24 P.I.B. per mm^2 diet. There was no statistically significant difference ($p = 0.01$) between infectivity of P.I.B. of N.P.V. of *H. punctigera* in excreta of *N. tasmanicus* and in partly purified suspension (table 2). Even P.I.B. in excreta voided after 4 days, were still highly infective. This is in marked contrast with data found for *Oechalia schellenbergii,* another predator of *H. punctigera.* The numbers of P.I.B. in the excreta of field-caught insect c were very near the threshold, below which an accurate estimate of numbers P.I.B. is impracticable. This may be an explanation for the observed 7% bioassay mortality for this insect, which

Table 1. Numbers of polyhedra of N.P.V. of *H. punctigera* in excreta of *N. tasmanicus*, estimated with the immunofluorescent counting method

	Days since meal on virus-killed larva	Mean No. P.I.B.	SE
Adults*	1	1.36×10^7	(1.42×10^7)
	2	6.15×10^5	(1.04×10^6)
	3	2.08×10^4	(2.88×10^4)
	4	1.63×10^4	(3.64×10^4)
	5–10	0.00	
Nymphs**	1	9.16×10^6	(9.07×10^6)
	2	8.55×10^4	(9.25×10^4)
	3	7.07×10^3	(13.5×10^3)
	4–10	0.00	
Field-caught*** insects	a	1.68×10^4	(4.27×10^3)
	b	3.09×10^4	(6.40×10^3)
	c	1.28×10^4	(1.04×10^4)
	d	8.74×10^3	(4.05×10^3)
	e	9.32×10^3	(1.44×10^3)

* Mean for 5 insects sampled per day; ** Mean for 6 insects sampled per day; *** P.I.B. in excreta sampled within 24 h after capture.

Table 2. Expected vs observed bioassay mortality of neonate larvae of *H. punctigera* caused by polyhedra in excreta of adult *N. tasmanicus*

	Days since meal on virus-killed larva	Expected* mortality (%)	Observed** mortality (%)
Predators fed in laboratory	1	56 (23–85)	28 (21)
	2	56 (23–85)	76 (7)
	3	56 (23–85)	52 (9)
	4	56 (23–85)	27 (7)
Field-caught predators***	a	56 (23–85)	33 (19)
	b	56 (23–85)	48 (11)
	c	56 (23–85)	7 (3)
	d	56 (23–85)	68 (20)
	e	56 (23–85)	53 (15)

* Estimated with immunofluorescent method and standard bioassay. Between brackets the 0.05-confidence interval; ** Mean of 3 replicates of 30 bioassay larvae per sample ± SE. *** Day(s) since meal unknown.

differs significantly ($p = 0.01$) from the expected 56% mortality. *N. tasmanicus* was observed to defecate 6–20 small drops of excreta per day. This habit, plus the finding that P.I.B. remain highly infective in the feces of *N. tasmanicus*, suggests that the predators may be important as virus disseminators in the field.

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The adequacy of thiamine in liquid diets used in animal models of alcoholism

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Summary. The effects of chronic administration of 2 types of liquid diets on brain thiamine pyrophosphate (TPP) levels have been investigated. With the Lieber-DeCarli diet, rats in the control group had significantly lower TPP levels compared with those of the ethanol group. The Nutrament diet used in mice was apparently adequate in the thiamine supply.

The development of credible animal models of alcohol intake for the study of the mechanisms of tolerance and physical dependence has occurred only within the last 10–15 years[2,3]. One of these involves the administration of a liquid diet in which ethanol provides as much as 30–40% of the calories[4–7]. Although the liquid diets are generally considered nutritionally adequate, it has not been definitively established that they necessarily contain adequate levels of all nutrients or that these are fully available for utilization at the subcellular level[8].

Conflicting evidence exists in animal studies as to whether the longterm administration of alcohol alters thiamine and thiamine pyrophosphate levels in tissues. Kiessling and Tilander[9] reported that rats which were forced to drink an aqueous solution of ethanol for 7 months had significantly lower thiamine and thiamine pyrophosphate levels in the liver compared to controls given water and sucrose solution. However, other investigators have reported no changes in tissue levels of TPP in rats exposed chronically to dietary alcohol[10,11].

Methods. Male Wistar rats (23–25 days old) and C57BL/6J mice (63–70 days old) were purchased from Woodlyn Laboratories Ltd., Guelph, Ontario, Canada and the Jackson Laboratories, Bar Harbor, ME, respectively. Rats were divided into 4 groups, with $N = 10$ in each: group A received the Lieber-DeCarli ethanol liquid diet (Bio-Serv Co., Inc., Little Silver, N.J.) ad libitum as the sole source of food and water; group B was pair-fed the isocaloric control diet (carbohydrate substituted for ethanol); group C was pair-fed the same diet as group A except that an equal volume of water was substituted for ethanol; group D received Teklad pellets and water ad libitum. The diets were administered for 6 weeks. In another experiment, mice were divided into 4 groups: group A received ad libitum a chocolate flavored Nutrament diet (Mead Johnson Nutritionals) containing 6% (v/v) ethanol[4]. The same diet, except that an isocaloric sucrose solution or an equal volume of water was substituted for ethanol, was pair-fed to groups B and C respectively. The diets were administered for 11 days. Another group of mice (group D) was fed ordinary food pellets and water.

Rats were sacrificed by decapitation and the heads were immediately dropped into liquid N_2 remaining there for at least 2 min. Mice were sacrificed by dropping them into liquid N_2. Procedures for sampling the cerebellum and for preparing perchloric acid extracts were the same as those previously described[12,13]. TPP was measured by the enzymatic-fluorometric method of Seltzer and McDougal[14].

Results and discussion. It is seen from Table 1 that rats fed the Lieber-DeCarli ethanol diet (group A) for 6 weeks did not have significantly lower TPP content in the cerebellum compared to those fed ordinary food pellets and water (group D), or the liquid diet with water substituting for ethanol (group C). However, significantly lower TPP levels were found in rats fed the isocaloric control diet (group B). The decrease in TPP in this group could be the result of an

Table 1. TPP contents in rat cerebellum after chronic administration of Lieber-DeCarli liquid diet

Diet	TPP (μmoles/kg wet wt)*
Ethanol (group A)	10.38 ± 0.99
Isocaloric control (group B)	8.11 ± 0.83**
Water control (group C)	11.03 ± 0.49
Ordinary food pellets and water (group D)	11.04 ± 0.66

* Mean values ± SD; $N = 10$. ** Significantly different from groups A, C and D; $p < 0.001$.

Table 2. TPP contents in mouse cerebellum after chronic administration of Nutrament liquid diet

Diet	TPP (μmoles/kg wet wt)*
Ethanol (group A)	14.06 ± 2.52
Isocaloric control (group B)	14.65 ± 2.39
Water control (group C)	14.32 ± 2.05
Ordinary food pellets and water (group D)	13.31 ± 1.08

* Mean values ± SD; $N = 8$.