PASSAGE OF INFECTIOUS NUCLEAR POLYHEDROSIS VIRUS
BY MICE AND CHICKENS (1)

A. GRÖNER (2) & GABRIELE DÖLLER (3)

(2) Biologische Bundesanstalt für Land - und Forstwirtschaft, Heinrichstrasse 243, D-6100 Darmstadt, Federal Republic of Germany.
(3) Bundesforschungsanstalt für Viruskrankheiten der Tiere, Paul-Ehrlich-Strasse 28, D-7400 Tübingen, Federal Republic of Germany.

After ingesting inclusion bodies of a nuclear polyhedrosis virus by mice and chickens, the feces of these test animals showed virus activity, caused in the case of mice by virions liberated from inclusion bodies and in the case of chickens by unaltered inclusion bodies.

For a reduced environmental contamination by agrochemicals the use of selective biological agents for controlling pest insects is increasing in importance. Within the different groups of insect pathogens, nuclear polyhedrosis viruses (Baculoviridae, Subgroup A) are effective agents against certain lepidopterous pests. The active ingredients of such a biopreparation are the enveloped virions, included in a proteinic crystal. The formation of these so-called inclusion bodies, or polyhedra, may be an adaptation to discontinuous infection circles in nature, protecting the occluded virus in the natural environment. This property of the inclusion body also facilitates the handling (purification, formulation, and storage) of a biopreparation. The virions are liberated from the polyhedra after ingestion by target organisms in order to become infectious. No health or environmental hazards have been demonstrated with baculoviruses by safety tests, conducted mainly with respect to toxicological data (SUMMERS et al., 1975).

We have studied the pathogenicity of a nuclear polyhedrosis virus (NPV), isolated from Mamestra brassicae (L.) (Lep. : Noctuidae) after application to mice and chickens. The first step was to determine, whether virions from polyhedra were liberated into the alimentary tract of vertebrates as a prerequisite of a hypothetical infection in these non-target organisms.

Each of 140 white mice (strain NMRI, mean weight of 25 g) and 15 chickens (mean weight of 400 g) received a single feeding dose of $1 \times 10^8$ polyhedra per g body weight, applied on a peace of bread. The polyhedra were suspended in 0.07M phosphate buffer pH 7.25 with a titer of $2.5 \times 10^9$ polyhedra per ml. The same number of animals received buffer-treated bread as a control. The feces from mice were collected and combined daily for 8 days post feeding (p.f.) and that from chickens for 6 days with day 5 and 6 pooled. These feces, and also feces from the control animals collected and pooled for day 1 to 3 (separated for mice and chickens) were lyophilized.
and homogenized in a mill. An aliquot of dried feces equal to 16.7% of each sample was incorporated into *M. brassicae* semisynthetic diet tempered to 40°C (BATHON & GRÖNER, 1977). This amount was calculated to produce a virus concentration 10 times the LC$_{95}$ for 2nd instar *M. brassicae* larvae (i.e. $1 \times 10^7$ polyhedra per ml diet), assuming all ingested polyhedra were passed unaffected within that day the sample was taken. These contaminated diets were tested in a standardized bioassay with 4 replications of 50 larvae per treatment.

In order to determine the form of the virus in the feces (i.e. free or occluded), an aliquot of feces from each species, equal to 10% of the feces collected at the 1st day, was suspended in a volume of water : chloroform (9 : 1, v/v) equal to 10 times the sample weight. Chloroform inactivates free virions of *M. brassicae* NPV but has less effect on virions occluded within the polyhedra (GRÖNER, 1978). After incubation for 5 h at 4°C, the chloroform was removed and these samples were incorporated into diet and assayed as above. Purified polyhedra treated in the same manner and incorporated into diet served as the control.

The results showed that virus activity in the collected feces could be detected until 5 and 6 days p.f. in the case of mice and chickens, respectively. Because the virus activity in the chloroform treated feces of mice had dropped drastically (fig. 1), the demonstrated activity could be attributed to liberated virions in the feces (inactivated by chloroform). It was calculated that more than 90% of the polyhedra in the feces had been solubilized and had liberated virions. The treatment of the chicken feces with chloroform had no deleterious effect on the virus activity in the feces (fig. 1). Therefore, the polyhedra were presumably not solubilized in the alimentary canal of the chickens.

![Fig. 1. Mortality (in probits) of *Mamestra brassicae* larvae after ingestion of diet containing feces of mice (1) or chickens (2) that had been fed polyhedra of *M. brassicae* nuclear polyhedrosis virus. Larvae fed diet containing purified polyhedra (3) served as controls. Open columns : untreated feces ; black columns : feces treated with 10% chloroform.](image-url)