Some Biophysical Properties of Virus Present in Tissue Cultures Infected with the Nuclear Polyhedrosis Virus of *Trichoplusia ni*

*(Accepted 28 August 1973)*

**SUMMARY**

The infectious agent in culture fluids of serially passed nuclear polyhedrosis virus of the cabbage looper (*Trichoplusia ni*) sediments at 830S. Infectivity falls below detectable limits when virus concentrates are treated with either deoxycholate (DOC) or with Tween-ether, and the sedimentation coefficient becomes 325S. The buoyant density in CsCl of several virus preparations was in the range 1.25 to 1.35 g/ml and increased to 1.42 in all cases after DOC treatment. The results indicate that the non-occluded form of the virus produced in tissue culture consists of fragile enveloped particles.

A multiple embedded type (MEV) of the nuclear polyhedrosis virus of *Trichoplusia ni* (Hubner), the cabbage looper, may be serially propagated in a continuous insect cell line derived from *T. ni* (Faulkner & Henderson, 1972). Typical virus inclusion bodies form in nuclei of infected cells and these are infectious *per os* for the host insect. In culture, the virus is passed by means of an agent present in inclusion body free tissue culture fluids. This agent has not been identified and its characterization as a membranous virus is the subject of this communication. The presence of non-occluded virus and/or subviral particles in insects infected with inclusion bodies of nuclear polyhedroses has been inferred for some time, since it is well known that the disease can be transmitted by injection of filtered infected insect extracts and haemolymph. In addition, primary infection of continuous cell lines of insects has been achieved using haemolymph (Goodwin *et al.* 1970; Faulkner & Henderson, 1972; Sohi & Cunningham, 1972) or cell-free extracts from infected insects, which contain a DNase sensitive component (Ignoffo, Shapiro & Hink, 1971).

The virus used was from the fifth tissue culture passage of cells originally infected with haemolymph from insects fed inclusion bodies of the nuclear polyhedrosis disease of *Trichoplusia ni* as described previously (Faulkner & Henderson, 1972). In order to produce sufficient virus for biophysical studies, virus labelled with [³H]-thymidine was grown in Falcon plastic tissue culture flasks (250 ml size) containing approx. 2×10⁷ cells at the time of infection. The inoculum (approx. 1.5 ml) at a dose of approx. 2 TCID units/cell was added to cells from which the growth medium had been poured off. The virus was allowed to adsorb for 1 h. Fifteen ml of growth medium (Hink, 1970) was added as well as 15 μCi [³H]-thymidine ([6-³H]-thymidine, sp. act. 5 Ci/m-mol) and the culture was incubated for 3 days at 28°C. Virus was concentrated from the culture medium as follows. Fluids from infected cultures were clarified by centrifuging at 1600 g for 20 min and the supernatant fluid was recentrifuged at 23000 rev/min for 30 min (Spinco SW-27 rotor). The virus pellets were resuspended overnight in phosphate-buffered saline (PBS; 140 mm-NaCl, 27 mm-KCl, 8 mm-Na₂HPO₄, 1.5 mm-K H₂PO₄, pH 7.2). The resuspended virus was layered over a 15 to 30% sucrose gradient in PBS and was centrifuged for 30 min at 30000 rev/min.
The infectivities of the concentrated virus and virus fractions were assayed using a quantal response assay in tissue culture which depended on scoring the proportion of uninfected cells as determined microscopically after 24 h of infection (Faulkner & Henderson, 1972).

When unlabelled concentrated virus was subjected to rate zonal centrifuging in the presence of a vesicular stomatitis virus (VSV) marker labelled with [$^3$H]-uridine, the polyhedrosis virus sedimented as a band with a peak of infectivity at 830 S with respect to the 625 S B particle of VSV (Fig. 1). The sedimentation profile indicates that the virus was in the form of a single nucleocapsid, since aggregates of nucleocapsids such as those seen in sections of inclusion bodies (Heimpel & Adams, 1966), would be expected to have much higher sedimentation coefficients.

The infectivity of virus concentrates was abolished when the virus was treated with 0.2 % sodium deoxycholate (DOC) or with Tween-ether (shaking with 0.5 % Tween 80 for 15 min, followed by shaking with an equal vol. of ether for 1 h). Following treatment with 0.2 % DOC in PBS, the sedimentation coefficient of [$^3$H]-labelled virus decreased to 325 S (Fig. 2). The infectivity of the virus preparation fell from 10^7 units/ml to below the detectable level at 5 × 10^4 units/ml. The buoyant density of the infectious 825 S particle in CsCl was determined after fixation in 4 % formaldehyde (0.1 ml 40 % formaldehyde added slowly with shaking to 0.9 ml virus concentrate obtained by pooling peak fractions from a sucrose
Fig. 3. Distribution of radioactivity after CsCl density gradient centrifuging of (A) untreated and of (B) DOC-treated [³H]-nuclear polyhedrosis virus from tissue culture fluid. ■, density.

velocity gradient) and found in one experiment to be 1.26 g/ml (Fig. 3A). However, the value was variable and densities in the range 1.26 to 1.35 g/ml have been noted. Electron microscope examination of the infectious 825S fraction by both phosphotungstic acid and uranyl acetate staining techniques was carried out, and although many nucleocapsids and partly enveloped virus particles were seen, no micrographs of completely enveloped nucleocapsids were obtained. This difficulty in observing completely enveloped virus particles indicates that the membrane is fragile and may account for the range of observed buoyant densities. After treatment with 0.2% DOC, all virus fractions tested were converted to a nucleoprotein of density 1.42 g/ml (Fig. 3B). This result suggests that the free virus in tissue culture fluid is an enveloped nucleocapsid. Such virus particles were seen to be released by a budding process from the plasma membrane of infected tissue culture cells (E. A. MacKinnon, J. F. Henderson & P. Faulkner, unpublished data).

These experiments show that at least two infectious forms of the nuclear polyhedrosis virus are produced in tissue culture: (a) an inclusion body which is believed to be the usual vehicle for natural transmission of the virus between insects and (b) enveloped virus particles. Non-occluded enveloped virus particles have been detected in the nuclei and cytoplasm of gut columnar cells of larvae of the tortoiseshell butterfly (Aglais urticae L.) infected with an NPV disease and it has been suggested that the virus particles subsequently infect adjacent tissues in the diseased insect (Harrap, 1970). Thus, the finding of free virus particles in tissue culture fluids is consistent with the suggestion that free enveloped virus particles are the form in which systemic dissemination occurs in the insect.

This work was supported by a grant from the National Research Council of Canada.
REFERENCES


(Received 21 June 1973)