J.Biol.Control, 5 (2), 88-92, 1991

Size and Shape of Inclusion Bodies of Nuclear Polyhedrosis Virus of *Spilosoma obliqua* (Walker)

G.S. BATTU*, N.RAMAKRISHNAN and NAM PRAKASH
Division of Entomology and Nuclear Research Laboratory
Indian Agricultural Research Institute, New Delhi

ABSTRACT

The polyhedral inclusion bodies of the NPV of *Spilosoma obliqua* were polyhedral (73.9%), cubic (11.1%), triangular (7.6%), spherical (4.1%) and oval (3.3%) and measured on an average 1.0 to 1.3, 1.2 to 1.3, 1.1 to 1.6, 0.6 to 0.8 and 0.6 to 1.4μ respectively in diameter calculated from their mean projected areas observed in electron microscope. Ultrasonic treatment of the polyhedra resulted in swelling of the polyhedra and the projected area increased by 35.3 per cent over the normal size.

KEY WORDS : NPV, *Spilosoma obliqua*, size, shape

The nuclear polyhedrosis virus (NPV) is an important mortality factor in the natural control of Bihar hairy-caterpillar, *Spilosoma obliqua* (Walker) (Arctiidae : Lepidoptera) in Northern India and its biological properties have been investigated earlier (Battu, 1982). The biological properties including host range along with morphological characters have been used by various workers for virus identification (Payne and Kelly, 1981). The characteristic feature of NPV is the formation of polyhedral inclusion bodies (PIB). These occur in different shapes and sizes and may vary in different insects or even in the same insect but tend to be of the same size in the same cell (Smith, 1976). The present contribution deals with various shapes and sizes of PIB of NPV of *S. obliqua* and the plausible explanation for their occurrence.

MATERIALS AND METHODS

The semipurified suspensions of PIB in water obtained through the usual processes of filtration and differential centrifugation were used for electron microscopy work. The PIB suspension in distilled water containing a pinch of sodium dodecyl sulphate was further purified by sonication in an ultrasonic cleaner (Vibronics Model VS-250) for half an hour. The sonication was done at 25 KHz and 250 watts. The PIB were mounted on copper grids (3.5 mm, 300 mesh) previously coated with formvar film, air-dried and then examined under an electron microscope (Phillips, EM-300). A total of 368 PIB were examined and the shape and size were studied. The projected area was measured by tracing the boundary of the photographic image of the PIB. The paper clippings equivalent to the traced area were cut and fed to an automatic area meter to determine the projected area.

RESULTS AND DISCUSSION

The data on the various shapes and sizes of the PIB observed are presented in Table 1 and Plate 1, while Table 2 and Plate 2, include the data on the effect of sonication on the shape and size of the polyhedra.

The PIB of the nuclear polyhedrosis virus of *S. obliqua* were of five different shapes *viz.*, spherical, cubic, oval, triangular and polyhedral (Plate 1). Some polyhedra with irregular surface were rarely seen. The majority of the inclusion bodies (73.9%) were polyhedral in shape and the distribution percentage of the other shapes was 11.1 (cubic), 7.6 (triangular), 4.1 (spherical) and 3.3 (oval). The cubic PIB are in fact, hexahedral and tetrahedral PIB which are seen in different orientations, while the triangular polyhedra are tetrahedral.

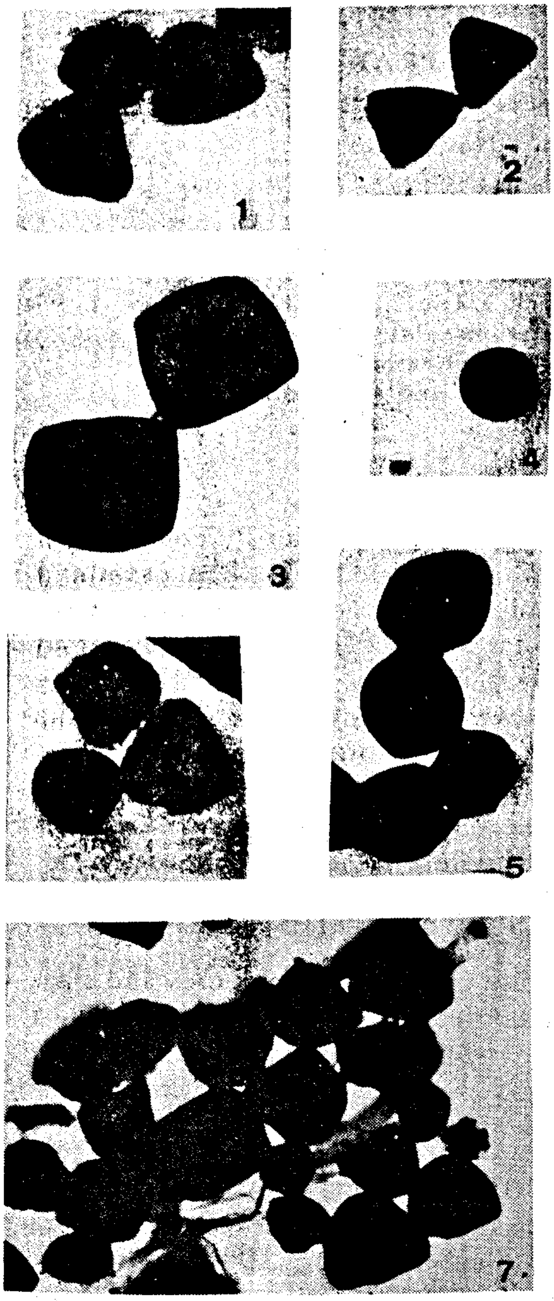

Plate 1. Electron micrographs showing shapes of inclusion body of the nuclear polyhedrosis virus of *S. obliqua*

1. Polyhedral x 16280
2. Triangular x 12720
3. Cubic x 20350
4. Spherical x 33580
5. Oval x 16280
6. Irregular x 16280
7. General View x 12720

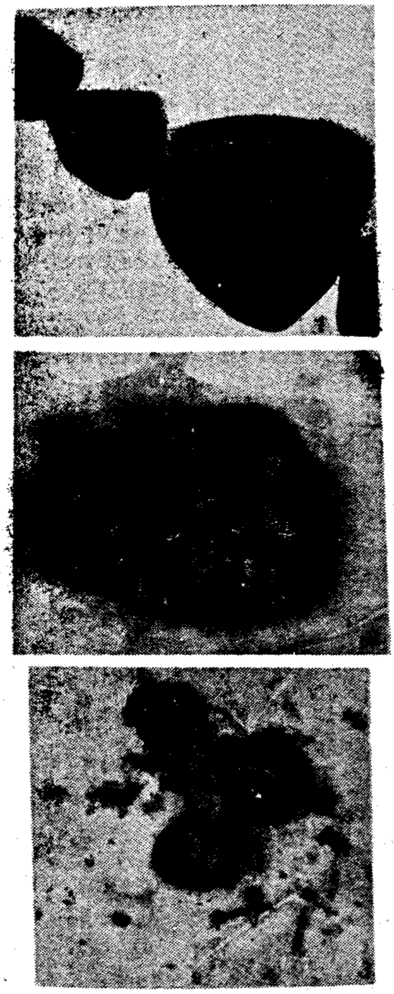

Plate 2. Electron Micrographs showing the effect of ultrasonic treatment on inclusion body

1. Swollen Inclusion Body x 27470
2. Disintegration of Inclusion Body protein x 42470
3. Ruptured Inclusion body and release of virions (V) x 12720

The majority of the inclusions were polyhedral in shape. Polyhedral particles have been observed earlier in virus infection of *D. obliqua* (Jacob and Thomas, 1974). Cubic polyhedra were seen in nuclear polyhedrosis of *P. demoleus* (Smith, 1976), *H. cunea* (Vago *et al.*, 1970) and *Trabala vishnu*

Table 1. Shape and size of the inclusion body of the nuclear polyhedrosis virus of *S. obliqua*

Shape	No. of IB	% of the total PIB examined	Projected area (μ^2) of the various shapes of the PIB		
			Number examined	Mean± S.D.	Range
Spherical	15	4.1	9	0.6 ± 0.2	0.3 - 1.0
Cubic	41	11.1	40	1.2 ± 0.4	0.6 - 2.0
Oval	12	3.3	6	1.0 ± 3.0	0.3 - 1.6
Polyhedral	272	73.9	239	1.1 ± 0.4	0.2 - 2.5
Triangular	28	7.6	25	1.2 ± 0.3	0.6 - 1.6
Total No. of polyhydra examined	368	-	319	1.2 ± 0.3	-
Overall mean	-	-	-	1.1 ± 0.4	0.2 - 2.5

Table 2. Effect of sonication on the size of the inclusion body of the nuclear polyhedrosis virus of *S. obliqua*

Details of PIB examined	Number of PIB examined	Projected area μ^2	
		Mean± S.D.	Range
Sonicated PIB	10	1.7 ± 0.5	0.9 - 2.6
PIB without sonication	368	1.1 ± 0.4	0.2 - 2.5

(Lefevere) (Battu, 1986). Triangular / tetrahedral polyhedra are known from *Orgyia thyellina* Butler (Sato, 1979) and *T. vishnu* (Battu, 1986).

Edges of many of the observed polyhedra were rounded. This rounding might have given rise to, in some cases, oval or spherical particles. Thus the oval and spherical particles are in fact the polyhedral particles with rounded edges. Rounding has also been observed by some earlier workers in the polyhedra of *H. zea* (Gregory *et al.*, 1969) and *S. litura* (Ramakrishnan *et al.*, 1975). In very rare cases, a few inclusion bodies were also seen having irregular surface boundaries. These boundaries are in fact the projections of the virus bundles inside the polyhedral matrix, wherein, the crystallization of the polyhedral proteins has not taken place to the fullest extent and the polyhedral proteins have not covered the virus bundles adequately. These observations indicate that the formation of the virus bundles and the polyhedra are independent processes occurring separately inside the nucleus and later on, the virions singly or in bundles get occluded by some biochemical pathways, where, if the polyhedral proteins are insufficient, formation of particles with irregular surfaces will occur. Pawar (1974) and Summers and Arnott (1969) while working with *S. litura* and *Trichoplusia ni* (Hubner) respectively have observed in ultra thin sections, the formation of polyhedra from a fibrous matrix nearby the virions. Even the process of occlusion of the virus bundles inside the polyhedral matrix has been shown by these workers as occurring independently by some enzymatic activity and physico-chemical forces.

The projected area of the polyhedra varied between 0.2 and 2.5μ^2 (Table 1). Based on 368 polyhedra seen, the mean projected area was found to be 1.1 ± 0.4μ^2 which is equivalent to a particle of diameter 1.2 ± 0.7μ. The majority (39.2%) of the polyhedra had the projected area of 0.9 to 1.3μ^2. The projected area varied within each shape. The frequency distribution of the size, within each shape, are presented in Fig.1. The highest frequency within each class interval was taken as the representative of the size. Thus, equivalent diameter of the various polyhedra were in the following order : cubic (1.2 to 1.3μ), triangular (1.1 to 1.5μ), polyhedral (1.0 to 1.3μ), oval (0.6 to 1.4μ) and circular (0.6 to 0.8μ), as calculated from their respective projected area.

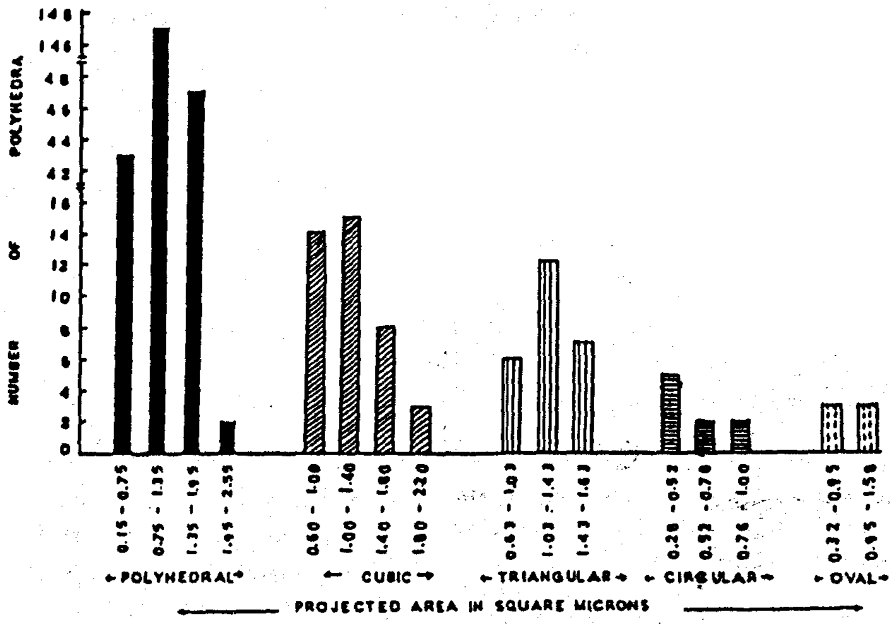

Fig 1. Frequency distribution of the projected area (μ^2) of different shapes of the inclusion body of the nuclear polyhedrosis virus of *S. obliqua*

In the present study, the ultrasonic treatment of the polyhedra resulted in the swelling of the polyhedra (Plate 2) as well as the virions, just like the swelling of the polyhedra due to alkali treatment. Alkali treatment increased the projected area by 62.5% of the average projected area of the untreated polyhedra. The virions inside the polyhedra also showed the swelling due to alkali treatment (Battu, 1982). The mean projected area of such swollen polyhedra increased by 35.3 per cent of the average size of unsonicated polyhedra.

The average diameter of the polyhedral particles was found to be 1.2 ± 0.7μ. The smallest particles which were about 40% in the present observations had the diameter varying from 1.06 to 1.29μ. Jacob and Thomas (1974) have also reported the NPV of *S. obliqua* to have irregular polyhedral particles with a mean diameter of 2.34μ which is much larger as compared to our observations. As they have mentioned that their polyhedra were alkali-treated before examination, the alkali treatment might have given rice to the increase in the size by the swelling of the particles.

On sonication, there was swelling of the polyhedra as well the partial disintegration of the polyhedral proteins. In some cases, virions could also be seen coming out. Smirnoff and Ackermann (1977) have also seen virus particles of NPV of *Thymelicus lineola* Ochs. with their damaged envelopes and bizarre shapes of the virions as well as of polyhedra after sonication at 80 KHz. In our observations, no such deformities of the viral particles of the polyhedra were seen when polyhedra were sonicated at 25 KHz. This may indicate the mechanical rigidity of the polyhedral particles which may vary from species to species.

REFERENCES

BATTU, G.S. 1982. Studies on the Baculovirus - A Nuclear polyhedrosis virus of *Diacrisia obliqua* (Walker). Ph.D. Thesis, P.G. School, IARI, New Delhi, 222 pp.

BATTU, G.S. 1986. New record of the nucleopolyhedrosis virus of *Trabala vishnu* (Lefevere) from India. *Curr. Sci.*, **55**, 1005-1006.

GREGORY, B.G., IGNOFFO, C.M. and SHAPIRO, M. 1969. Nucleopolyhedrosis of *Heliothis*-Morphological description of inclusion bodies and virions. *J. Invertebr. Pathol.*, 14, 186-193.

JACOB, A. and THOMAS, M.J. 1974. Nature of inclusion bodies of a nuclear polyhedrosis virus of *Diacrisia obliqua* (Wlk.). *Agric. Res. J. Kerala*, **12**, (1) 82-93.

PAWAR, V.M. 1974. Structure, development and physico-chemical properties of the nuclear polyhedrosis virus of *Spodoptera litura* (Fabricius) and associated biochemical changes in the haemolymph of the host. Unpubl. Ph.D. Thesis, P.G. School, IARI, New Delhi, 112 pp.

PAYNE, C.C. and KELLY, D.C. 1981. Identification of insect and mite viruses. In "*Microbial control of pests and plant diseases. 1970-1980*", H.D. Burges, (ed.). Academic Press, London p.p 61-62.

RAMAKRISHNAN, N., PAWAR, V.M. and NAM PRAKASH, 1975. Nuclear polyhedrosis of *Spodoptera litura* (Fabricius) : Description of inclusion bodies and virions. *Curr. Sci.*, **44**, 316-317.

SATO, T. 1979. Nuclear and cytoplasmic polyhedrosis viruses of the white- spotted tussock moth, *Orgyia thyellina Butler*. *Bull. Fruit. Tree. Res. Stn., A.* (Japan) No. 6, 43-58.'

SMIRNOFF, W.A. and ACKERMANN, H.W. 1977. A baculovirus of the European skipper, *Thymelicus lineola* (Lepidoptera : Hesperiidae). *J. Invertebr. Pathol.*, **30**, 224-31.

SMITH, K.M. 1976. Virus-insect relationships. Longman, London.pp. 291.

SUMMERS, M.D. and ARNOTT, H.J. 1969. Ultrastructural studies on inclusion formation and virus occlusion in nuclear polyhedrosis and granulosis virus-infected cells of *Trichoplusia ni* (Hubner). *J. Ultrastr. Res.*, **28**, 462-480.

VAGO, C., QUIOT, J.M., VINCKIER, D., DULHOIT, J.L., MEYNADIER, G. and INJAC, M. 1970. Etude des corps d'inclusions polyhedriques d' Invertebres en microscopic electrinique a balayage. *C. R. Acad. Sci. Paris*, **271**, 1053-1055.