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FLUORESCENCE MICROSCOPIC STUDIES ON RNA OF CYTOPLASMIC POLYHEDROSIS VIRUS OF TASAR SILKWORM, *ANTHERAEA MYLITTA* (DRURY)

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POLYHEDRAL inclusion bodies (PIBs) of occluded insect viruses are important for identification of the viruses and their strains[1-10]. PIBs have also been used as source for extraction of cytoplasmic polyhedrosis virus (CPV) virions and their RNA. In order to characterize the viral RNA, several methods have been used, including X-ray diffraction, melting point analysis, base composition analysis and electron microscopy[11-13]. The use of acridine orange staining to visualize RNA of *Antheraea mylitta* CPV virions occluded within polyhedra is reported here.

Samples were taken in the form of thin tissue smears from highly infected midgut epithelium of larvae that had been fed orally 120 h earlier with a lethal dose of purified polyhedra (0.02 ml of 5×10^6 polyhedra/ml). Larvae were cooled on ice before samples were taken. Slides were fan-dried, washed gently in basal salt solution, then stained with acridine orange (G) without fixing or after fixing with Carnoy's acid alcohol following the methods of Lea[14] and Primose and Dimmock[15]. The slides were examined immediately under short-wave UV light (source, Philips HOB-202 mercury lamp) in a Zeiss phase fluorescence microscope fitted with dark-field condenser.

Polyhedra in unfixed samples gave yellowish-orange fluorescence while those in fixed samples gave green fluorescence, suggesting the high molecular weight of the viral RNA[14,15]. The green fluorescence of the fixed polyhedra faded out, however, on molybdic acid treatment (figure 1a, b). This suggests double-stranded nature of the viral RNA[15]. The polyhedra in all cases did not give any fluorescence without the dark-field condenser, in transmitted or in incident light. This suggests that

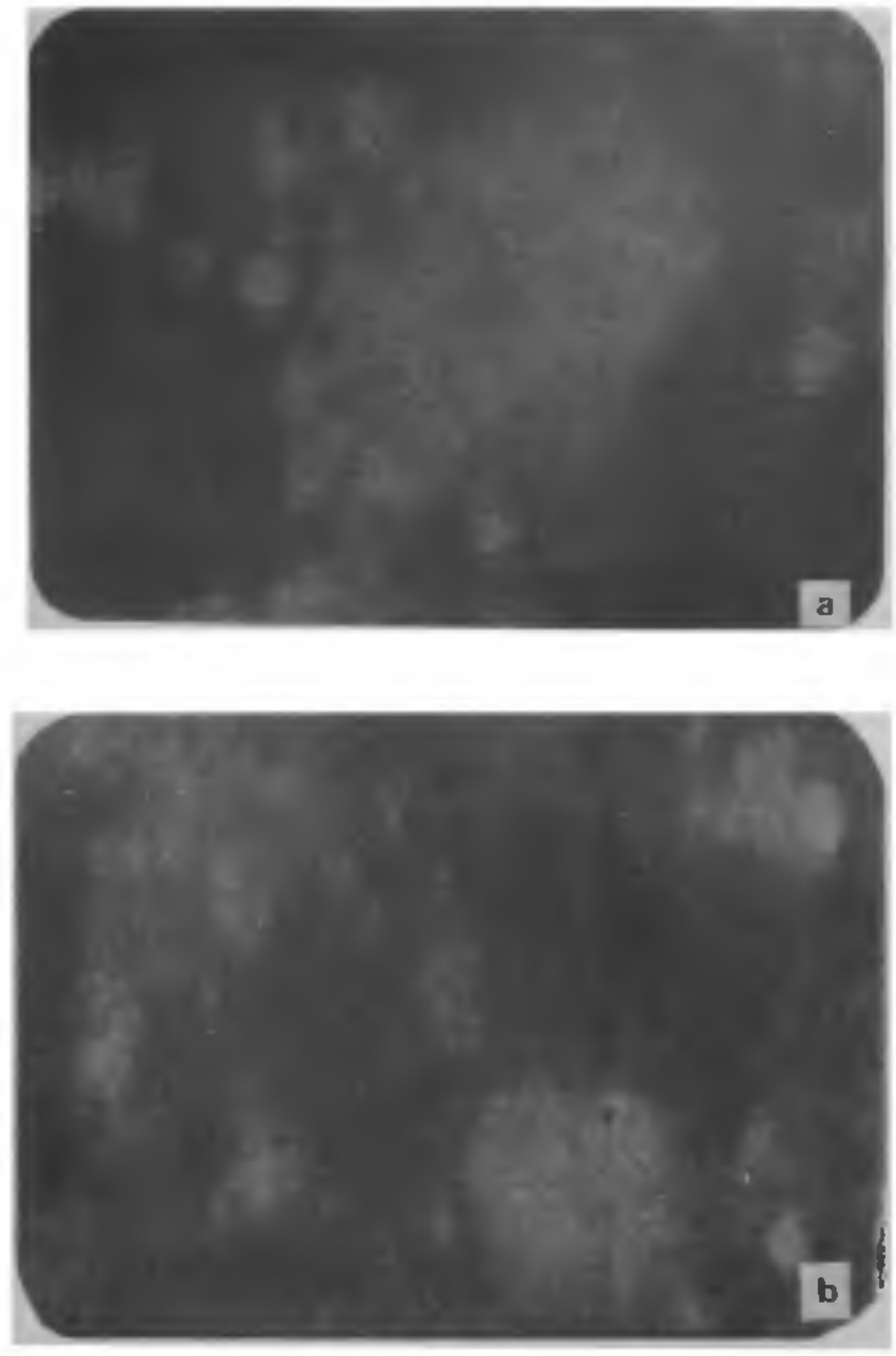

Figure 1a, b. **a.** Phase-fluorescence micrograph of polyhedra of CPV from highly infected midgut epithelium of *Antheraea mylitta*, fixed with acid alcohol and stained with acridine orange (× 800); **b.** Phase-fluorescence micrograph of polyhedra as in **a** on molybdic acid treatment, showing faded fluorescence of the polyhedra (× 800).

the polyhedra were fluorescing because of the presence of naked virions on the surface of the polyhedra.

The direct fluorescence technique has been used extensively for visualizing nucleic acids of the naked viruses[14-17]. The fluorescence of CPV polyhedra under dark-field and transmitted UV light clearly indicates that naked virions are present on the surface of polyhedra. The presence of naked virions on the surface of CPV polyhedra was also reported by other workers[18-22]. They further suggested that the virions of CPV undergo maturation (maturation of viral nucleic acid) before occlusion within PIBs.

This report of high molecular weight double-stranded RNA as the nucleic acid of the CPV of

Antheraea mylitta is in agreement with the earlier studies. Hayashi and Kawase[23] confirmed the double-stranded nature of the high molecular weight RNA of the CPV of *Bombyx mori* by showing that the RNA had equimolar amounts of adenine and uracil, and guanine and cytosine. Miura *et al*[11] obtained a sharp melting profile and showed that the RNA was resistant to ribonuclease. Identical results were obtained by Hayashi[13] for the RNA of the CPV of *Orgyia leucostegma*. Nishimura and Hosaka[12] obtained the same result by electron microscopy, and Kawase and Furusawa[24] after radiolabelling CPV RNA in the presence of actinomycin D, which selectively blocks RNA synthesis of the host cells.

The CPV of *Antheraea mylitta* thus belongs to the group of reoviruses[25-29]. The advantage of phase in the present study is that it reveals the more or less hexahedral shape of the PIBs. This may be useful for identification of the viral strain as a hexahedron CPV. It must, however, be confirmed by scanning electron microscopy or by shadowing in transmission electron microscopy.

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