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A New Permissive Cell Culture Obtained from *Latoia viridissima* (Lepidoptera, Limacodidae)

Two viruses were isolated on *Latoia viridissima* (Lepidoptera, Limacodidae), defoliator of oil palm in Côte d'Ivoire, a picornavirus (G. Fédière, R. Philippe, J. C. Veyrunes, and P. Monsarrat, *Entomophaga* 37, 354–357, 1990) and a nuclear polyhedrosis baculovirus (N. K. Kouassi, *Oléagineux,* in press). No lines were known from this family and as no permissive cell culture existed to multiply these viruses, we have attempted to obtain a permissive cell line from hemocytes of this insect.

Larvae from four different field samples were used for the culture of 75 C35 petri dishes. Hemolymph obtained by the "dropping" method (J. M. Quiot, Doctorat, Montpellier, France, 1975) was put directly into petri dishes with Grace medium containing 10% fetal bovine serum (FBS), modified form (X. Léry and G. Fédière, *J. Invertebr. Pathol.* 55, 47–51, 1990), with the addition of 5‰ NaN_3 and incubated at 28°C (all chemicals from Sigma).

After 4 hr of culture, most of the hemocytes adhered to the flask walls. During the first days of the culture, the different types of hemocytes described by T. J. Kurtti and M. A. Brooks (*J. Invertebr. Pathol.* 15, 341–350, 1970) were observed. After 1 to 2 weeks, small fibroblast-like cells (prohemocytes) were the only cells remaining in the cultures. They occurred in two forms, fibroblast-like adherent cells and small rounded suspension cells representing mitotic prohemocytes (Fig. 1).

During the first 10 days, in 40% of the cultures, no multiplication occurred and cells died. In a few cultures, characteristic polyhedra were found in the cells. After purification and analysis they appeared to be nuclear polyhedrosis viruses and represented endogenous infections.

During the following weeks, a low rate of multiplication was observed in 50% of the remaining cultures. The vigorous multiplication was generally observed af-

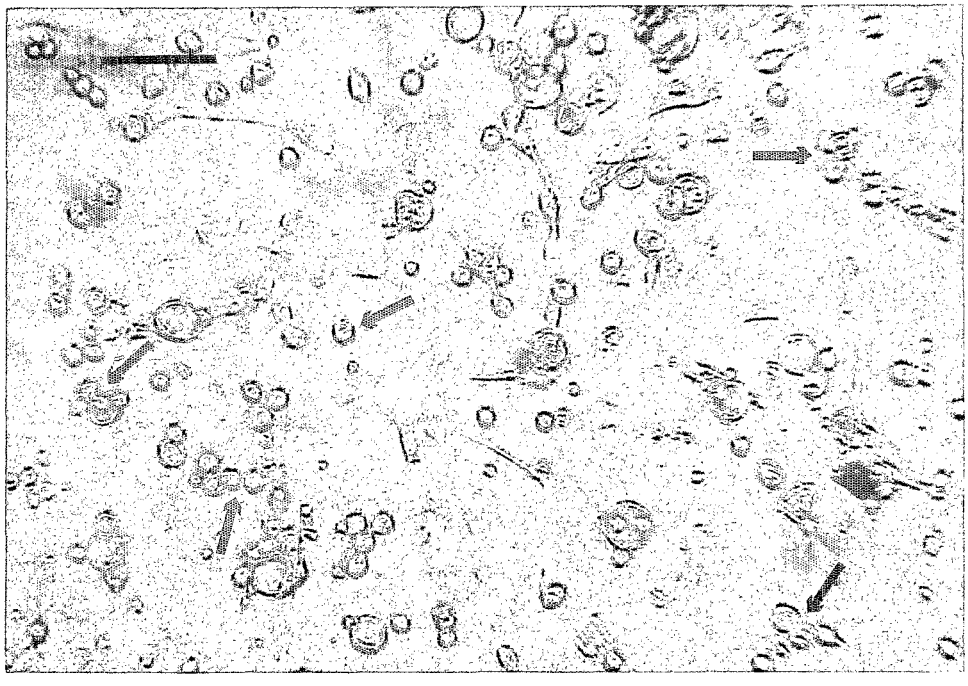

FIG. 1. Culture of *Latoia viridissima* hemocytes at the fifth passage after 3 days. Fibroblast-like adherent cells and small rounded suspension cells. Bar, 100 μm. Arrows, mitotic cells.

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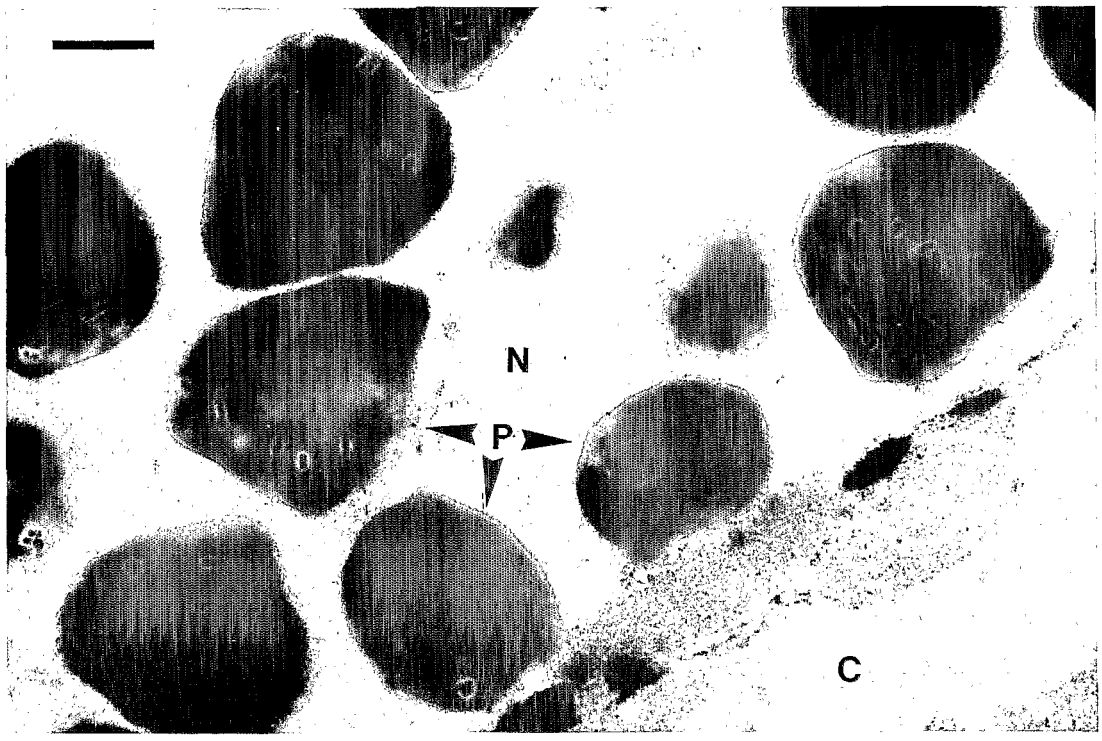

FIG. 2. Electron micrograph of a culture of hemocytes from *Latoia viridissima* infected with nuclear polyhedrosis virus. Bar, 0.5 μm. N, nucleus; C, cytoplasm; P, polyhedra containing baculoviruses.

ter 3 or 4 weeks, but the best results were obtained when this phase began after 2 or 3 months.

A suspension of purified polyhedra (0.1 OD/ml) isolated from larvae was used for experimental infections (N. K. Kouassi, *Oléagineux* **46**, 53–59, 1991). Polyhedra were dissolved by alkali treatment and Grace medium without FBS added. Ten percent of the noninfected primary cultures were infected. After 6 hr, the virus was removed and fresh medium containing 3% FBS was added. Ten days later, cells that showed polyhedra in their nuclei were collected and ultrastructural studies were performed (Fig. 2).

At this stage, when monolayers attained full confluency, subcultures were attempted every 1 or 2 weeks. Adherent and supernatant cells were placed in new flasks (1:2) with fresh medium. After 10 months, it was possible to maintain 2 of the 75 primary cultures. This is an improvement upon the 1% obtained by J. Mitsuhashi and A. Shozawa (*Dev. Growth Differ.* 27, 599–606, 1985) under the same conditions with a different lepidopteran species. Twenty subcultures were achieved during this period and experimental infections of cells remained possible.

The larvae used were taken directly from the field, not from laboratory reared stock (the rearing of *L. viridissima* was impossible). This explained the rapid decrease in the number of primary cultures and the endogenous infections with nuclear polyhedrosis virus observed. The final successful results (3%) obtained are encouraging.

Even when the cell line was not established, because of the low number of passages, subcultures of *L. viridissima* cells were always accomplished and the virus multiplied. This represents a great hope for the production of all the viruses isolated on this lepidopteran for use in biological control and molecular biological studies.

KEY WORDS: Lepidopteran cell line; *Latoia viridissima;* nuclear polyhedrosis virus.

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