

A Simple Way of Purifying Several Insect Viruses

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Various methods have been reported for purifying 'non-occluded' viruses of insects but they have seldom been compared. We find that one we use to purify acute bee-paralysis virus (crytogram† **R/1:2/(25):8/3:I/O**) and sacbrood virus (**R/*:*/(35):S/S:I/O**) (Bailey, Gibbs & Woods, 1963, 1964) is suitable for many other viruses, and is as effective but simpler than other methods. For example, we have used it to purify *Galleria dense nucleus* virus (GDNV) (**D/*:*/37:S/S:I/***) (Meynadier *et al.* 1964). Larvae of *Galleria mellonella* that had been injected with the virus and kept at 30° for 7 to 14 days were ground in a 4/1 mixture of water and carbon tetrachloride (1 larva/ml. of water) and centrifuged at 8000 g for 10 min. The supernatant fluid contained about 10¹³ virus particles/ml., which separated in the analytical centrifuge into two components with *S*_{20,w} values of 120 and 60 (Fig. 1*b*); the median lethal doses, by injection, of these two components, separated by centrifuging in sucrose density gradients, were about 10² and 10⁵ particles respectively. Extracts of 5th instar larvae (1 cm. long) that had died of GDNV infection, made using carbon tetrachloride, contained little but the virus, and almost none of the three other components plentiful in 8th instar (2 cm.) larvae (Fig. 1*a*) and reported by Longworth *et al.* (1968). The use of young GDNV-infected larvae therefore seems as important for obtaining pure virus preparations as the purification method.

The same method of extraction but with less centrifugation successfully clears preparations of viruses that sediment readily. For example, extracts of *G. mellonella* infected with *Tipula* iridescent virus (TIV) (**D/2:126/15:S/S:I/***) were clarified at 1000 g for 5 min. Such extracts contained 10¹¹ or more particles/ml. and the median infective dose by injection for *G. mellonella* was 7 to 21 particles.

To see whether preparations made by our method were less pure than those obtained with the more complex methods described by Longworth *et al.* (1968) for GDNV and Glitz, Hills & Rivers (1968) for *Sericesthis* iridescent virus (SIV) (**D/2:134/17.6:S/S:I/***), we extracted virus-infected larvae ground together with uninfected larvae that had been injected with ¹⁴C-labelled amino acids, and measured the radioactivity of the virus preparations at various stages of the purification methods. Each uninfected larva was injected with 5 μl. (77,000 counts/min.) of ¹⁴C reconstituted algal protein hydrolysate (Schwarz Biochemical Inc., N.Y.) and kept for 5 days at 30° on honeycomb before use. Of the 40% of the injected radioactivity recovered from uninfected labelled larvae, 13% was in particles that sedimented readily, 71% in water-soluble material that did not pass through a dialysis sac, 5% in water-soluble dialysable material, and 11% was soluble in carbon tetrachloride. The labelled larvae were mixed with larvae infected with either GDNV or SIV and extracted by the different methods. GDNV was sedimented from the extracts by centrifuging at 150,000 g for 70 min. and SIV at 17,500 g for 40 min., and the radioactivity of samples at different stages of the procedures was measured by Geiger-Müller or liquid scintilla-

† As proposed by Gibbs *et al.* (1965).

tion methods. Table 1 shows that the radioactivity of the virus preparations purified by our method differed little from that of the preparations made by the more complex methods; Glitz *et al.* (1968) used 12 periods of centrifugation to purify SIV before a further eight periods of centrifugation to fractionate the preparation, whereas our

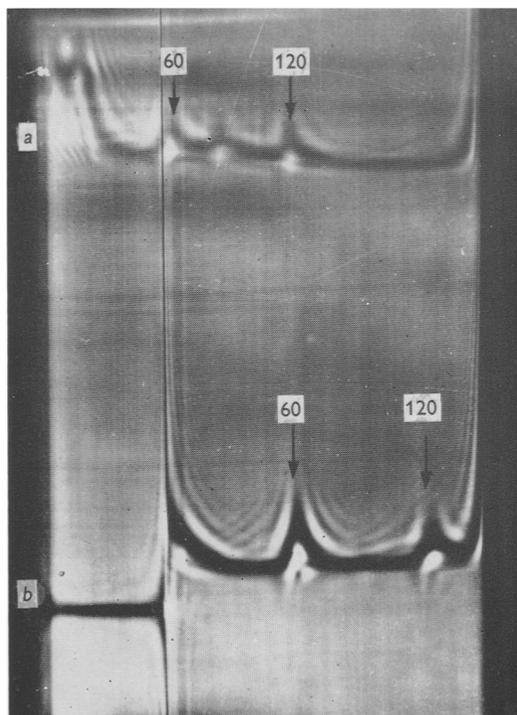


Fig. 1. Schlieren pattern of a centrifugal analysis of clarified extracts of old (*a*) and young (*b*) wax-moth larvae (*G. mellonella*) killed by injected virus. Approximately 100 mg. of tissue of each kind of larva were extracted/ml. water. The numbers indicate sedimentation coefficients ($S_{20,w}$).

Table 1. Radioactivity of virus preparations contaminated with extracts of ^{14}C -labelled larvae

Virus	Purification method	% of radioactivity in the initial extract remaining in:		
		Whole preparation	Small S material*	Virus
GDNV	Longworth <i>et al.</i> (1968)	5.3 (0.8)†	1.7	3.6
	As described in this note	6.2 (1.5)	2.2	4.0
SIV	Glitz <i>et al.</i> (1968)	8.2, 3.7, < 1.0‡	—	—
	As described in this note	4.3	2.0	2.3

* The final preparation was fractionated in a sucrose density gradient, and the radioactivities of the zone containing material with an S value of less than 30 and of the virus zone (s) was measured.

† In parentheses, the results of a second experiment.

‡ The radioactivity of the virus preparation after the first, second and third cycle of centrifugation.

tests of their method show that there was no detectable host material contaminating the preparation after the first six periods of centrifugation.

Our method also gives good results with a virus of *Antherea* (Grace & Mercer, 1965), *Chilo* iridescent virus, a nuclear polyhedrosis virus of *G. mellonella*, a granulosis virus of *Pieris rapae*, and with viruses similar to acute bee paralysis virus isolated from termites (A. J. Gibbs, unpublished) and crickets (C. Reinganum, unpublished). Hence it would probably also be useful for purifying many other viruses.

Our method renders virus preparations sufficiently free from micro-organisms to be injected directly into the haemocoel of host insects. Thus, there is no need to add antibiotics to the inoculum (e.g. Glitz *et al.* 1968), or to infect insects orally (e.g. Longworth *et al.* 1968), which is an inaccurate and unhygienic method compared with injection.

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