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NOTES

Infectivity Degradation by Ribonuclease and Table Sugar of a Nonoccluded Virus Inoculum Prepared from the Honey Bee

Hairless-black syndrome in the honey bee has been described previously (J. M. Kulinčević, G. R. Stairs, and W. C. Rothenbuhler, *J. Invertebr. Pathol.* **14,** 13–17, 1969; R. J. Horvath and W. C. Rothenbuhler, *J. Invertebr. Pathol.* **20,** 255–263, 1972). Attempts to isolate a pathogen associated with this syndrome have yielded a nonoccluded virus (Fig. 1) similar in morphology to chronic bee-paralysis virus (CBPV) described by L. Bailey, A. J. Gibbs, and R. D. Woods (*Virology,* **21,** 390–395, 1963). Infectivity tests with this nonoccluded virus fed to bees in a water solution of table sugar yielded positive but variable results. In refining the infectivity test, the possible effects of the table sugar and ribonuclease on the inoculum were considered. This experiment evolved from those considerations.

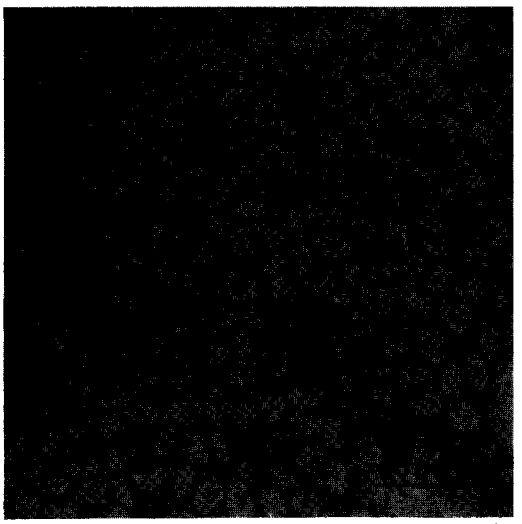

FIG. 1. Nonoccluded virus particles of varied dimensions negatively stained with phosphotungstic acid.

Virus suspensions were obtained by grinding heads of sick bees with a mortar and pestle, and mixing the resulting slurries with sterile distilled water. These suspensions were processed with one cycle of differential centrifugation. After the resulting pellets were resuspended in sterile distilled water, particle concentrations were determined with a Zeiss EM9-S electron microscope, using a methodology similar to that of R. C. Williams and R. L. Backus (*J. Amer. Chem. Soc.* **81,** 4052–4057, 1949). The virus suspensions were then added to either saturated solutions of ribonuclease-free sucrose,[1] saturated solutions of table sugar, or saturated solutions of ribonuclease-free sucrose to which bovine pancreatic ribonuclease[1] was added to a concentration of 2000 ppm.

These three types of virus-containing suspensions were fed in 2-ml aliquots every 24 hr for three consecutive days to groups of 50 bees housed in cages identical to those described by J. M. Kulinčević, W. C. Rothenbuhler, and G. R. Stairs (*J. Invertebr. Pathol.* **21,** 241–247, 1973). Each test bee received a total of approximately 3×10^8 particles. Groups of control bees received the same sugar suspensions as test bees, but without added virus. After virus feeding, experimental groups had continual access to both their respective sugar suspension and sterile distilled water. For 14

[1] Schwarz/Mann, Orangeburg, New York.

TABLE 1

AVERAGE PERCENT MORTALITY OF HONEY-BEE ADULTS FED VIRUS IN SUGAR SIRUP CONTAINING VARIOUS CONCENTRATIONS OF RIBONUCLEASE

Sugar	Virus concentration per bee	Number of replicates per treatment[a]	Average % mortality ($\bar{X} \pm s$)
Ribonuclease-free sucrose	3×10^8	10	79.8 ± 8.66**
	0[c]	8	5.5 ± 2.33
Table sugar	3×10^8	10	49.2 ± 14.76**
	0[c]	8	4.3 ± 3.11
Ribonuclease-free sucrose + ribonuclease[b]	3×10^8	10	33.4 ± 9.80**
	0[c]	8	5.5 ± 3.66

[a] Bees per replicate = 50.
[b] 2000 ppm ribonuclease.
[c] Control groups.
** Significantly different from all other means ($P < 0.01$).

days, including the days inoculum was fed, each cage was observed, dead bees were removed, and mortality was recorded. The data were analyzed by a least-squares analysis of variance and a least significant difference test.

Feeding virus in ribonuclease-free sugar, table sugar, and ribonuclease-free sugar to which ribonuclease was added resulted in averages of 79.8%, 49.2%, and 33.4% mortality, respectively (Table 1). Average mortality in control groups was, in all cases, below 6%. Differences among means of control groups are not significant, whereas differences among means of groups receiving virus are highly significant ($P < 0.01$). It is concluded that compounds associated with table sugar (probably ribonuclease) reduce mortality when compared to ribonuclease-free sucrose, and that the addition of 2000 ppm of ribonuclease to ribonuclease-free sucrose decreases mortality to an even greater degree.

Certain speculations can be made concerning the events leading to these differences in mortality. The results are most reasonable if the viral nucleic acid is RNA, as is the case in CBPV (L. Bailey, *J. Gen. Virol.* **2**, 251–260, 1968). There is a strong possibility that the preparation contains naked, infectious RNA which is destroyed by ribonuclease, thereby reducing the infectious dosage and consequent mortality. It is also possible that during the infectious process in the honey-bee gut, the RNA of the particles themselves becomes exposed (possibly by proteases) and thus susceptible to ribonuclease degradation.

Experimentation now under way will hopefully uncover the precise mechanisms and design practical applications.

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