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Serological Relationship between Chronic Bee Paralysis Virus and the Virus Causing Hairless-Black Syndrome in the Honeybee

Hairless-black syndrome of the honeybee, *Apis mellifera,* is in certain respects similar, and in other respects dissimilar, to chronic bee paralysis. The two maladies result in apparently different histopathologies (R. J. Horvath and W. C. Rothenbuhler, *J. Invertebr. Pathol.* **20,** 255–263, 1972) and different behavior (K. D. Waddington and W. C. Rothenbuhler, *J. Apic. Res.* in press). The syndrome is caused by a non-occluded virus (T. E. Rinderer, *J. Invertebr. Pathol.* **24,** 120–121, 1974; T. E. Rinderer and W. C. Rothenbuhler, *J. Invertebr. Path.* **27,** 215–219, 1976) similar in morphology to the chronic bee paralysis virus (CBPV) described by L. Bailey, A. J. Gibbs, and R. D. Wood (*Virology* **21,** 390–395, 1963). This morphological similarity of the two viruses suggests that there may be a relationship between the two maladies and the two viruses. In order to answer the question of the relationship of the viruses, we studied them serologically by immunodiffusion techniques.

Antiserum to hairless-black syndrome virus (HBSV) antigenic material, obtained from the heads of diseased bees, was prepared in two adult female rabbits. Rabbits were injected intramuscularly, three times at weekly intervals, with virus preparations. Each preparation was made from 1 ml of normal saline, 1 ml of Freund's complete adjuvant, and 4 mg of density-gradient-purified HBSV (T. E. Rinderer and W. C. Rothenbuhler, loc. cit.). Following a 3-week rest period that ended in a 24-hr fast, rabbits were bled and antiserum was collected, stored in 1.0-ml aliquots at −20° C and used undiluted in immunodiffusion experiments.

Antiserum to uninfected bee-head antigenic material was also prepared. The uninfected material was obtained as a pellet from a suspension of ground bee-heads after differential centrifugation at 480, 9750, 9750, and 73,950*g* for 15, 60, 60, and 120 min, respectively. Antiserum to this material was prepared in the same way used to prepare antisera to HBSV antigenic material.

Lyophilized CBPV antigenic material and lyophilized unabsorbed antiserum were obtained for this investigation.[1] These materials were reconstituted in distilled water and used immediately.

Ouchterlony gel immunodiffusion experiments were performed using a microtechnique (H. Markowitz, *J. Bacteriol.* **87,** 232, 1964). For each experiment, an agar matrix, 1 × 22 × 22 mm, was formed by placing a plastic template over a dry, agar-coated microscope slide and filling the template with 1.1% Noble-agar in 1.2% Tris–barbital–sodium barbital buffer of *p*H 8.8. The cooled matrix received 50 μl of antigen or antisera in appropriate wells and was held in a moist chamber, maintained at 4° C, for 72 hr. After this period, the matrix was treated for 6 hr and then 16 hr in 1.0% NaCl solutions. After NaCl treatment, templates were removed, wells were rinsed, and the matrix was soaked in distilled water for 1 hr, covered with wet bibulous paper, and dried for 24 hr at 25° C. The matric was fixed and stained for 5 min in 0.6% amido black dissolved in 45% methanol: 10% acetic acid: 45% distilled water. Excess stain was removed by four 15-min washes in solvent lacking stain.

The experiments shown in Figures 1 and 2 were replicated three times and twenty other confirming experiments were also performed.

Figure 1 shows the immodiffusion patterns of CBPV with antisera to healthy bee tissue antigens and to CBPV and HBSV. Two precipitin patterns are present. The outer coalescent precipitin pattern of triangular

[1] Obtained from L. Bailey, Rothamsted Experimental Station, Harpenden, Hertfordshire, England.

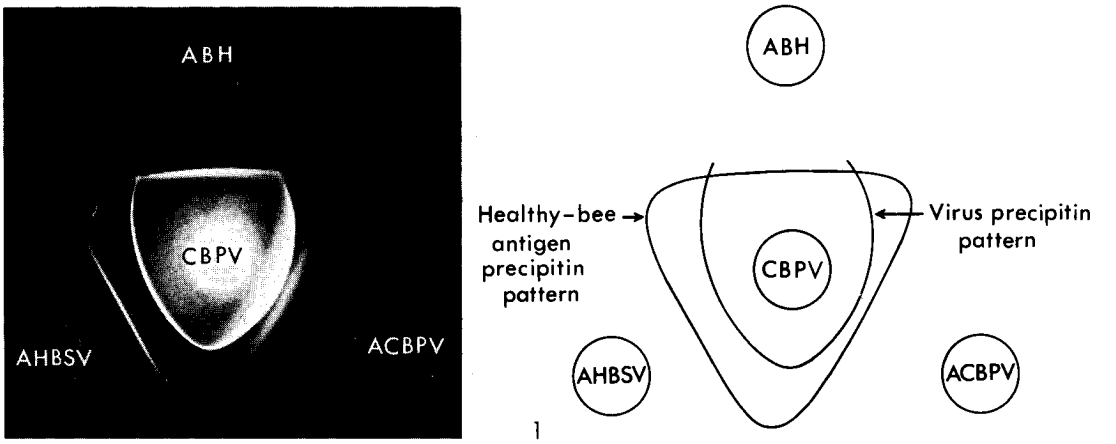

FIG. 1. Photograph and drawing of chronic bee paralysis virus (CBPV) antigen reacting with antiserum to healthy bee-head antigen (ABH), antiserum to chronic bee paralysis virus (ACBPV), and antiserum to hairless-black syndrome virus (AHBSV).

shape indicates the presence of serologically related nonviral antigen in CBPV and HBSV preparations used in antisera production as well as in normal tissue. The inner precipitin pattern formed by the reaction of CBPV with CBPV and HBSV antisera shows deviation and complete fusion and indicates identical antigenic specificity between the two viruses. The lack of interference where the virus-related pattern crosses the nonviral precipitin patterns confirms the lack of any serological relationship between the two patterns.

Figure 2 shows HBSV tested with antiserum to healthy bee tissue, CBPV and HBSV. The HBSV preparation contains too little healthy bee antigen to allow a precipitin reaction with antisera to healthy bee tissue. Thus, the outer coalescent pattern seen in Figure 1 is not seen in Figure 2. The HBSV did react with antisera to both CBPV and HBSV. The precipitin pattern of these reactions shows deviation with complete fusion, confirming that there was identical antigenic specificity in the two viruses.

On the basis of these serological investigations, and similar virus morphology, we conclude that hairless-black syndrome is caused by CBPV or a serologically indistinguishable genetic variant.

The sources of variation in histopatholo-

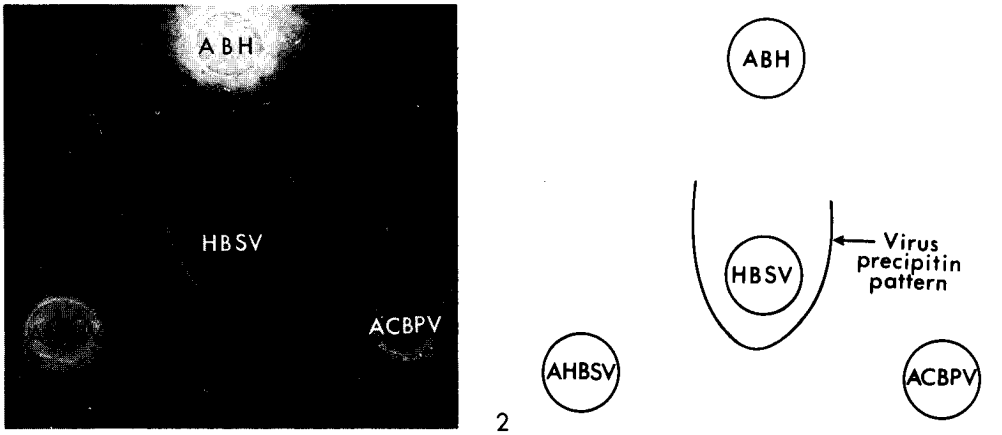

FIG. 2. Photograph and drawing of hairless-black syndrome virus (HBSV) antigen reacting to the same three antisera used in Fig. 1.

gies and disease-elicited behavior remain hidden. All the components of this disease which may differ, such as genotypes of bees, genotypes of virus, and environments, could account, singly or in concert, for the observed differences. Whatever the sources may be, their elucidation is important to a fuller understanding of chronic bee paralysis.

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