Comparison of Bee Chronic Paralysis Virus with Mouse Lactic Dehydrogenase Virus

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Bee chronic paralysis virus (cryptogram R₁:*/*:X/X:I/O.) is common in honey bees (Apis mellifera L.) throughout the world (Bailey, 1967). Its ellipsoidal particles contain ribonucleic acid and are of three sizes; 22 nm. wide and about 41, 54 and 64 nm. long, and have sedimentation coefficients ($S_{20,W}$) of 97S, 110S and 125S respectively (Bailey, Gibbs & Woods, 1968). The particles of bee chronic paralysis virus (CBPV) seem unlike those of any other described virus, except perhaps mouse lactic dehydrogenase virus ((R)/*:*/*:X/*:V/O), (Notkins, 1965). The particles of lactic dehydrogenase virus (LDHV) may contain RNA, and are elliptical in outline, 36 to 42 x 45 to 75 nm. when sectioned but 60 to 65 x 70 to 85 nm. in suspension (De Thé & Notkins, 1965). In this note some properties of the particles of LDHV are compared with those previously reported for CBPV.

LDHV was grown in multicoloured, out-bred, low-pathogen mice (Mus musculus L.) derived from the Walter and Eliza Hall Institute strain. These mice have been found to be free of lymphocytic choriomeningitis virus (R/*:*/*:S/*:V/O.) and ectromelia virus (D/2:*/*:X/X:V/O†) and also Eperythrozoon coccoides Schilling, and tapeworm. For each virus preparation a group of 100 to 300 mice was infected by intraperitoneal injection with an Australian isolate of LDHV (Pope & Rowe, 1964) kindly supplied by Dr J. H. Pope of the Queensland Institute for Medical Research. The mice were bled from the heart 24 to 48 hr after infection, and the pooled blood, after 10 min.

at 37°, was centrifuged at 8,000 g for 10 min. The virus in the clarified serum was sedimented at 75,000 g for 1 hr, resuspended, clarified at 8,000 g for 5 min., then centrifuged at 50,000 g for 1 hr in a 10 to 40 %, w/v, sucrose density gradient, and finally pelleted at 100,000 g for 45 min. and resuspended. Gelatin saline (8.5 % sodium chloride, 0.5 % gelatin, 0.2 M borate, 0.02 M magnesium chloride, 0.0025 M calcium chloride; pH 7.0) was used as diluent during purification, and the final pellet of purified virus was resuspended in 0.1 M ammonium acetate, pH 7.0.

Purified preparations made in this way had the ultra-violet absorption spectrum of a nucleoprotein with a small nucleic acid content ($E_{260}/E_{230} \approx 1.18$). They contained a single sedimentable component with a sedimentation coefficient ($S_{20,w}$) of 235S; in three experiments the sedimentation coefficient (230S to 237S) was independent of a fourfold range of virus concentration, near the limit of detection by the schlieren optical system of the analytical centrifuge.

For electron microscopy purified virus preparations were mixed with an equal volume of Regaud's solution and kept at room temperature for 30 min. One volume of the fixed virus preparation was then mixed with one tenth volume of 0.1 % bovine serum albumin and one volume of negative stain, which was either neutral 4 % sodium phosphotungstate or 2 % uranyl acetate, pH 5.0. The mixture was sprayed on a
carbon film supported on an electron microscope grid. Specimens were examined in a Siemens Elmiskop I at a fixed instrument magnification of $\times 76,600$. The sizes of the images of the particles were measured on the electron micrographs. They were variable in shape and size, but most were approximately elliptical in outline (average dimensions $47 \times 56$ nm. (Fig. 1); the size and appearance of the particles in the two types of negative stain were indistinguishable. Despite the variability there was no evidence for more than one type of particle as there was with the similarly variable particles of CBPV (see Fig. 2 and 3 in Bailey et al. 1968).

Fig. 1. Number of particles of different sizes in a fixed preparation of LDHV in neutral sodium phosphotungstate. The greatest linear dimension of each particle was recorded as its length, and the width at right angles to this. The area of each spot is proportional to the number of particles of that size, the smallest spots correspond to single particles, the largest spot to seven particles; 217 particles were measured in all.

LDHV is known to be affected by ether. Notkins (1965) showed that treating LDHV with ether destroyed the infectivity of crude preparations of the virus, but not purified preparations. Preparations of LDHV were shaken with a small volume of cold di-ethyl ether for 5 min., and aliquots were either fixed and mounted for electron micrography, or were examined in the analytical centrifuge. The treated preparations contained no recognizable LDHV particles, but an amorphous granular material with a sedimentation coefficient of about 20 S, that was not present in the untreated preparations.

LDHV and CBPV preparations were also tested with an antiserum to purified CBPV by an Ouchterlony gel diffusion method (Mansi, 1958) using 0.75 % agar. In the tests a purified CBPV preparation gave a single clear band of precipitate, but LDHV preparations gave none.

Thus the particles of LDHV differ from those of CBPV, in that they are of one type, whereas those of CBPV are of three sizes; they are larger and have a larger sedimentation coefficient; they are disrupted by ether, whereas those of CBPV are not; and they are serologically unrelated to those of CBPV.

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REFERENCES


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