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Some Physico-chemical Properties of two Honey-bee Picornaviruses

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Acute bee-paralysis and sacbrood viruses, which multiply in the honey-bee (*Apis mellifera* L.), have some properties similar to those of the mammalian picornaviruses. Both bee viruses infect their host via the alimentary canal and are neurotropic in adult bees (Bailey & Milne, 1969; Bailey & Fernando, 1972), although they are serologically unrelated (Bailey, Gibbs & Woods, 1964) and differ fundamentally in their ecology and pathology (Bailey, 1965, 1967, 1969). The two viruses also resemble the mammalian picornaviruses in some physico-chemical properties. For example, they are isometric particles 28 nm in diameter, have sedimentation coefficients of 160 S (Bailey, Gibbs & Woods, 1963, 1964) and are resistant to ether. Lee & Furgala (1965) provided chemical evidence that sacbrood virus contains RNA. In this paper we describe other physico-chemical properties of the two viruses and compare these with those of the mammalian picornaviruses.

Sacbrood virus labelled with [^{32}P] was prepared by inoculating the food of healthy larvae each with 1 μl of a solution containing the clarified extract of 1 sacbrood larva/ml and 0·5 mCi [^{32}P]/ml when the larvae were 3 to 4 days old. Five to six days after inoculation, larvae that failed to pupate were collected.

Acute bee-paralysis virus labelled with [^{32}P] was prepared by injecting adult bees each with 1 μl of a solution containing per ml the clarified extract of three heads of acutely paralysed bees and 0·5 mCi [^{32}P]. The bees were incubated at 35 °C for 4 to 6 days and the virus then extracted from their heads.

Both viruses were purified according to the method described by Brown & Cartwright (1963) for foot-and-mouth disease virus. The aqueous extracts were clarified by centrifuging at 2000 *g* for 15 min, pelleted by centrifuging at 60000 *g* for 1 h and the pellets resuspended in 0·04 M-phosphate, pH 7·6. After removing insoluble debris, the suspensions were mixed with either 1% sodium deoxycholate or 1% sodium dodecyl sulphate and centrifuged for 3·5 h at 25000 rev/min in 15 to 45% sucrose gradients, using the Spinco SW 25.1 rotor. The fractions containing the virus were located by radioactive counting or measurement of E_{260} and the presence of virus in these fractions was confirmed by electron microscopy.

More than 90% of the [^{32}P] in each of the viruses was in the RNA. The properties of the two viruses and their RNAs are summarized in Table 1. The sedimentation coefficients were obtained by sedimentation of each virus in mixtures with [^{3}H]-labelled foot-and-mouth disease virus (Fig. 1). The sedimentation coefficients of 157S and 160S for sacbrood and acute paralysis viruses, compared with 146S for foot-and-mouth disease virus (Strohmaier, 1971), are similar to those obtained by Bailey *et al.* (1963, 1964) in the analytical ultracentrifuge. The ratios of E_{260}/E_{280} are similar to those obtained for several mammalian picornaviruses (Rueckert, 1971) and indicate that they contain approximately 30% RNA.

The buoyant density of the two bee viruses in caesium chloride was 1·33 to 1·34 g/cm^3, as found for the mammalian enteroviruses and cardioviruses by Rowlands, Sangar & Brown (1971) and Newman, Rowlands & Brown (1973). In addition, the peaks of infectivity of a mixture of acute paralysis virus (10^{10} LD_{50}/ml assayed in bees) and poliovirus type 1 (10^8 p.f.u./ml assayed in human amnion cells) coincided after centrifuging in a caesium chloride gradient.

To test whether the bee viruses shared the pH stability of the entero- and cardioviruses,

Table 1. *Some physico-chemical properties of sacbrood virus and acute paralysis virus and their RNAs*

	Sacbrood virus	Acute paralysis virus
Virus, sedimentation coefficient in S units	157	160
Diameter (nm)	28	28
Buoyant density in CsCl (g/cm^3)		
pH 7	1·33	1·34
pH 8	1·33	1·36
pH 9	1·33	1·42
E_{260}/E_{280}	1·67	1·64
Morphological stability	Stable pH 7 to 5 Unstable pH < 5	Stable pH 7 to 3
RNA, sedimentation coefficient in S units		
In 0·1 M-NaCl, pH 7	35	30
In 6% formaldehyde	15 to 16	—
Effect of RNase:		
0·01 μg/ml 0·1 M-NaCl	Hydrolysed	Hydrolysed
Base composition (%) A	32·1	30·3
C	17·9	20·5
G	19·1	18·8
U	30·9	30·4

preparations of the purified virus particles were mixed with 4 vol. of 0·1 M-acetate buffer solution at pH 2·5 to 7, kept at 20 °C for 15 min and then mixed with an equal vol. of 1% uranyl acetate before examination in the electron microscope. Acute paralysis virus was unaffected morphologically at pH 2·5 to 7, whereas sacbrood virus remained intact at pH 4 and 5 but not at pH 3 or 2·5. This was confirmed by centrifuging in sucrose gradients. Samples of the sucrose gradient purified [^{32}P]-viruses were mixed with 9 vol. 0·1 M-acetate of the appropriate pH, kept at 20 °C for 15 min and then centrifuged in 15 to 45% sucrose gradients (in 0·04 M-phosphate, pH 7·6) for 3·5 h at 25000 rev/min, using the SW 25.1 rotor of the Spinco ultracentrifuge. The distribution of radioactivity showed that particles of the acute paralysis virus were stable between pH 3 and 7. However, the sacbrood virus particles were unstable at pH 4 and gave a heterogeneous profile with a peak at about 65S, At pH 3, most of the [^{32}P] sedimented as a fairly homogeneous peak at about 65S (Fig. 2).

Since the mammalian picornaviruses unstable below pH 7 (foot-and-mouth disease virus, human rhinovirus) have a higher buoyant density at pH 9 than at pH 7, (Rowlands *et al.* 1971), the buoyant density of the two bee viruses at pH 7 to 9 was determined. The density of sacbrood virus was the same at the three pH values but the density of the acute paralysis virus increased from 1·34 g/cm^3 at pH 7·1 to 1·42 g/cm^3 at pH 9·0. Thus, in this property, the two bee viruses differ from the mammalian picornaviruses of these subgroups.

The RNA was extracted from purified virus by shaking with an equal vol. of phenol which had been saturated with water. The sedimentation coefficient in 0·1 M-NaCl, pH 7·0, was approximately 30S for acute paralysis virus RNA and 35S for sacbrood virus RNA. These values are similar to those found for several of the mammalian picornaviruses (see list in Newman *et al.* 1973). The single-strandedness of RNA of both bee viruses was indicated by their hydrolysis to slowly sedimenting molecules by incubation with 0·01 μg RNase per ml in 0·1 M-NaCl. The treatment of sacbrood virus RNA with formaldehyde under the conditions described by Fenwick (1968) reduced the sedimentation coefficient to 15 to 16S. This is lower than the value of 16 to 17S obtained for several mammalian picorna-

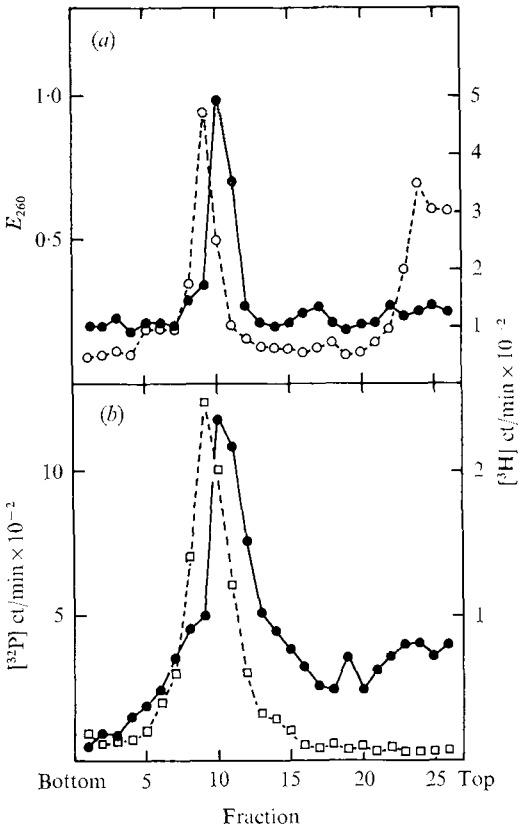

Fig. 1

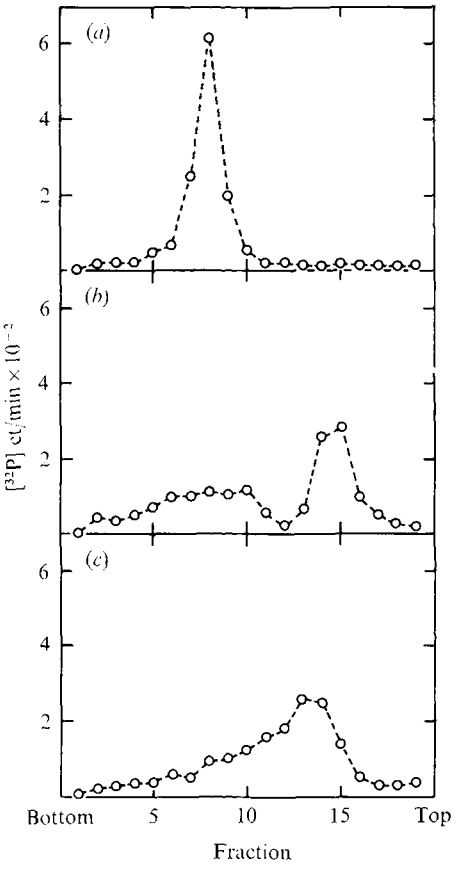

Fig. 2

Fig. 1. Sucrose gradient sedimentation of mixtures of [^{3}H]-uridine labelled foot-and-mouth disease virus (●—●) with (*a*) sacbrood virus (○ ··· ○) and (*b*) [^{32}P]-acute paralysis virus (□--□) showing the distribution of extinction and radioactivity.

Fig. 2. Distribution of radioactivity after sucrose gradient sedimentation of [^{32}P]-acute paralysis virus after mixing with 0·1 M-acetate buffer solution at (*a*) pH 7, (*b*) pH 4 and (*c*) pH 3.

viruses in sedimentation analysis on mixtures and suggests that the mol. wt. of sacbrood virus RNA is less than the value of 2·1 to 2·6 × 10^{6} currently accepted for mammalian picornaviruses. However, Newman *et al.* (1973) found that the RNA of human rhinoviruses (GC = 40%) had a smaller *s* value than foot-and-mouth disease virus RNA (GC = 52%) or poliovirus RNA (GC = 48%) after formaldehyde treatment, so it is possible that the smaller *s* value of the sacbrood virus RNA is due to a low GC content (37%) rather than to a smaller mol. wt. Our preparations of acute paralysis virus RNA sedimented very slowly (< 4S) after formaldehyde treatment, showing that there were many hidden breaks in the RNA chain.

The base compositions of the two bee virus RNAs are similar but unlike those of the mammalian picornaviruses (Newman *et al.* 1973). The two RNAs had significantly lower GC contents (37 to 39%) than that of any of the mammalian picornaviruses except the human rhinoviruses (40%).

Although the two bee viruses have several physico-chemical properties similar to those

of the mammalian picornaviruses, they are not unequivocal members of any of the subgroups proposed by Newman *et al.* (1973). A buoyant density of 1·34 g/cm^3 at pH 7 and morphological stability at pH 3 suggest that the acute paralysis virus is a typical enterovirus. However, the increase in buoyant density above pH 7 is not typical of the mammalian enteroviruses. It is not known whether the hidden breaks in the RNA account for this behaviour. The mammalian enterovirus RNAs are apparently intact since they sediment as a homogeneous band at 17S after formaldehyde treatment. Sacbrood virus, like the human rhinoviruses, is unstable below pH 5 but its buoyant density of 1·33 g/cm^3 is much lower than the value of 1·40 g/cm^3 obtained for the rhinoviruses.

Virus or virus-like particles similar in appearance and size to those of acute bee paralysis and sacbrood viruses have been found in several other insect species (Harrap *et al.* 1966; Brzostowski & Grace, 1970; Reinganum, O'Loughlin & Hogan, 1970) and also in the citrus red mite (Estes & Faust, 1965). The bee viruses also resemble Nodamura virus, which multiplies in several insect species (Scherer, Verna & Richter, 1968) and kills bees and wax-moths (Bailey & Scott, 1973). Nodamura virus shares many properties with the enteroviruses and causes paralysis and death in mice (Scherer *et al.* 1968), but is serologically unrelated to any of the enteroviruses of vertebrates with which it has been compared (Murphy *et al.* 1970).

It is important to characterize the picornaviruses of insects and other invertebrates as fully as possible so that their relatedness to the picornaviruses of mammals can be gauged, especially since some viruses, such as Nodamura virus, infect both types of host.

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