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Parvoviruses

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PARVOVIRUSES

The theme of the Symposium is the comparison of viruses with similar physical, chemical and biological properties, with no restrictions as to whether they were isolated from plants, invertebrate or vertebrate animals. It follows that one should be just as interested in the differences between viruses as in their similarities. However, to do this, the barriers created by basic disciplines must be removed as they are hindrances to the full development of the subject of virology. Further, it is very probable that the basic problem of virus specificity can be best studied by using viruses whose host ranges span wide taxonomic boundaries.

The parvoviruses (Mayor & Melnick, 1966; Andrewes, 1970) constitute a group in which the type virus was described only recently (Kilham & Olivier, 1959). The group is characterized by having isometric naked particles, 20 ± 2 nm in diameter, a buoyant density in caesium chloride (CsCl) of 1·37 to 1·45 g/cm^3, resistance to ether and chloroform and relative heat-stability (Table 1). Some of these viruses have been shown to contain single-stranded DNA with a mol. wt. of 1·2 to $2{\cdot}1 \times 10^6$. Complementary strands have been satisfactorily demonstrated with only a few of the viruses and these plus and minus strands combine *in vitro* to form a double strand. It has been suggested that the virus particles separately contain complementary strands (Rose *et al.* 1969; Barwise & Walker, 1970; Berns & Rose, 1970; Torikai *et al.* 1970; Berns & Adler, 1972) and it is possible that specific populations of positive and negative stranded particles are necessary to cause infection.

There are now six fairly definite members and at least six candidate viruses on which more information is required before full status can be conferred (Table 2). To date, the parvoviruses have been isolated only from mammals and insects as there are no reports of small single-stranded DNA viruses from plants or other animals, but this may only be a matter of time and chance.

THE VIRION

Size and structure

The name parvovirus implies small size, but the variation in sizes reported by different workers is remarkably wide. The degree of this variation in size is illustrated in Table 3. However, these size ranges more probably reflect differences in staining techniques, method of measurement, and instrument calibration, than intrinsic differences in particle size.

Hosaka (1965) and Karasaki (1966) have emphasized that the measurements of the diameters of various particles depend on their orientation and Karasaki (1966) and Hoggan (1971) measured from side to side along a three- or five-fold axis. Smith, Gehle & Thiel (1966) made two series of measurements with adeno-associated virus (AAV), the smallest and largest dimension, and found their means to be $18 \pm 1{\cdot}1$ and $21 \pm 1{\cdot}8$ nm, respectively. The application of different staining techniques for measurements with the electron microscope are a well-known source of error and Hoggan (1971) reported that parvoviruses

Table 1. *Characteristics of the parvovirus group*

Virion	Isometric particle without envelope Ether and chloroform resistant Size: 20 ± 2 nm Buoyant density (CsCl) 1·38 to 1·45 g/cm^3 Heat-stable
Nucleic acid	DNA. Single stranded – 18 to 34 % Mol. wt. 1·2 to $2{\cdot}1 \times 10^6$ Base composition (AAV-1) A T G C $22{\cdot}9 \pm 0{\cdot}1$ $22{\cdot}8 \pm 0{\cdot}1$ $26{\cdot}9 \pm 0{\cdot}2$ $27{\cdot}3 \pm 0{\cdot}3$

Table 2. *The parvoviruses*

Virus	Source	Virus	Source
Latent rat (H-1, H-2, X 14)	Rats Hamsters	Feline panleucopaenia Mink enteritis	Cats Felidae
Minute mouse	Mice	*Galleria* densonucleosis *Junonia* virus	Insecta Lepidoptera
Porcine parvo	Pigs	Adeno-associated viruses	
Avian parvo	Quail	Type I Type II	Tissue cultures of human and simian origin.
Bovine parvo (Haden)	Cattle	Type III Type IV	
Canine parvo	Dogs	.	.

Table 3. *The reported variation in size of the parvoviruses (after Hoggan, 1971)*

Latent rat	17 to 25 nm	Canine parvo	20 to 21 nm
Minute mouse	18 to 28 nm	Feline panleucopaenia	20 nm
Porcine parvo	20 to 22 nm	*Galleria*	19 to 20 nm
Avian parvo	—	*Junonia*	19 to 20 nm
Bovine parvo	22 nm	Adeno-associated	22 to 24 nm

appeared to be 12 to 15 % larger when stained with 1 % uranyl acetate than with 2 % PTA. However, it is clear that size ranges of these viruses overlap and the mean value of 20 ± 2 nm seems to be a reasonable compromise standard for this group. In many purified preparations of parvoviruses, small ring-like particles of 6 to 10 mm in diameter have been observed, but the function or significance of their structure is not known.

Vasquez & Brailowsky (1965) attempted a reconstruction of the virus capsid of the latent rat virus (LRV) and concluded that the most likely configuration was a pentagonal dodecahedron with pentagonal pyramids arranged on each face, giving a total number of 32 capsomeres. Karasaki (1966) compared LRV and its H strains and found that all particles appeared to be arranged in three- and fivefold symmetry and he agreed with Vasquez & Brailowsky (1965) that the particles had 32 capsomeres arranged either as an icosahedron or pentagonal dodecahedron. Kurstak & Côté (1969) examined the *Galleria* densonucleosis virus (DNV) and suggested that these particles had 42 capsomeres and they concluded that this was evidence of a significant difference from the LRV, H-1 and AAV viruses. Barwise (1969) dissociated empty capsids of DNV in 6 M-guanidine and 0·1 M-β mercaptoethanol. He obtained a homogenous preparation of protein and calculated its mol. wt. by equilibrium sedimentation and sedimentation velocity to be in the range 50000 to 64000. Barwise (1969) assumed that this was the basic structural unit with an average mol. wt. of

Table 4. *Buoyant densities and mean sizes of nine parvoviruses (after Hoggan, 1971)*

Virus	Mean size (nm)	Density in CsCl (g/cm³)
Latent rat	19·1 ± 1·2	1·400
Minute mouse	19·3 ± 1·2	1·417
H-1	21·7 ± 1·8	1·422
Galleria DNV	21·9 ± 2·7	1·440
Bovine parvo	22·5 ± 1·0	1·425
Adeno-associated viruses		
Type I	21·8 ± 1·3	1·395
Type II	23·8 ± 2·7	1·388
Type III	21·4 ± 2·7	1·394
Type IV	22·0 ± 1·8	1·445

60000. The mol. wt. of the DNV empty capsid was $3{\cdot}5 \times 10^6$ and the protein shell could be composed of 60 sub-units arranged as 12 capsomeres each composed of a pentamer, 20 trimers, 30 dimers or 60 monomers.

Buoyant density

Parvoviruses as a group have a relatively high buoyant density in caesium chloride (CsCl). They all band in isopycnic CsCl gradients in the range 1·38 to 1·46 g/cm³, though, as might be predicted, variations in technique have produced a range of values for buoyant density. Hoggan (1971) compared the densities of nine parvoviruses using standardized techniques and also calculated the mean diameter of particles from infectious bands after 48 h isopycnic banding in caesium chloride gradients. The results of these experiments are shown in Table 4. The density values of the infectious bands were in the range of 1·38 to 1·45 g/cm³. It is a feature of isopycnic centrifuging of parvoviruses in CsCl that several minor bands are obtained in addition to the major layer of infectious particles. Some of these contain large quantities of empty particles and are similar to the 'top component' fractions described from preparation of small plant viruses and such bands of apparently empty particles are very common with the insect parvoviruses (J. F. Longworth & T. W. Tinsley, unpublished information). Hoggan (1971) described a heavy band (1·44 to 1·47 g/cm³) which was frequently found with all the parvoviruses studied in his laboratory. This band was antigenically indistinguishable from the major infectious layer, but the particles were smaller and it was concluded that they had lost or possibly had never acquired an outer layer of protein. It is not known how many structural proteins these smaller particles possess as their analysis in SDS polyacrylamide gel systems seems not to have been investigated. It would be interesting to know which, if any, of the protein fractions is missing.

Haemagglutination

Parvoviruses have the ability to agglutinate erythrocytes from a wide range of animal species and techniques based on this property have been used in the characterization of this group. However, the results are somewhat conflicting and it is very difficult to compare data from different laboratories. However, Hoggan (1971) studied several parvoviruses under standard conditions and his results, with three types of erythrocytes, are summarized in Table 5. The canine, *Galleria* and adeno-associated viruses (AAV), I, II and III failed to agglutinate any of the three test erythrocytes and can therefore be distinguished from the

Table 5. *Agglutination of human, guinea-pig and rat erythrocytes by parvoviruses (after Hoggan, 1971)*

Virus	Human O	Rat	Guinea pig
Latent rat	−	+	+
Minute mouse	−	+	+
Porcine parvo	+	+	+
Bovine parvo	+	−	+
Canine parvo*	−	−	−
Galleria DNV	−	−	−
Adeno-associated viruses			
Type I	−	−	−
Type II	−	−	−
Type III	−	−	−
Type IV	+	+	+

* Canine virus agglutinates pig erythrocytes.

Table 6. *Haemagglutination by parvoviruses (after Hallauer et al. 1972)*

	Virus serotypes						
RBC species	I	II	III	IV	Porcine	Rat	Mouse
Human O	+++(*)	+++	+++	+++	+++	+++	++
Monkey	+++	+++	++	+++	++	++	−
Guinea pig	+++	+++	+++	+++	+++	+++	+++
Mouse	+	+	+++	++	+	++	++
Rat	+++	+++	+++	+++	+++	+++	++
Hamster	(+)	(+)	+++	+++	(+)	++	++
Sheep	(+)	++	+	+	(+)	+	−
Horse	−	++	+++	++	−	+	−
Pig	−	+++	+	++	−	(+)	−
Cat	(+)	++	++	++	+	+	−
Dog	(+)	+++	+++	+++	(+)	+	++
Chick	++	++	++	+	++	(+)	(+)
Goose	++	++	++	++	+	−	(+)

* Degree expressed by virus dilution giving partial (50 %) haemagglutination, +++ (1:1024 to 8192); ++ (1:128 to 512); + (1:2 to 8); − no reaction

other viruses where positive results were obtained fairly generally, except that the mouse, rat and bovine viruses failed to agglutinate human O and rat erythrocytes.

Hallauer, Siegl & Kronauer (1972) compared the four parvovirus serotypes isolated from human cell cultures with the porcine, H-1, H-3, X 14, rat and mouse viruses in a test system involving erythrocytes from 16 animal species. These authors in contrast to Hoggan (1971) reported that the mouse and rat viruses would agglutinate Human O cells. Other differences in the reactions of parvoviruses are shown in Table 6. All parvoviruses reacted with human O and guinea-pig blood cells in a similar manner. Haemagglutination occurred equally well at 4, 20 and 37 °C, and variations in pH from 6·6 to 8·5 had no appreciable effect on the haemagglutination titres. There was no spontaneous elution of virus particles from the blood cells when exposed to temperatures of 37 to 40 °C for 60 min. However, when agglutinated erythrocytes were resuspended in alkaline buffer of pH 9·0, the virus eluted completely within 30 min at room temperature and these treated erythrocytes were capable of reacting to fresh virus without any diminution of HA activity, so the receptor sites had obviously not been destroyed by the elution process.

Structural proteins

The knowledge of the polypeptide composition of the parvoviruses is scanty. Salzman & White (1970) showed that LRV contained three major proteins of mol. wt. 72000, 62000 and 55000 and Salzman (1971) thought that one of these protein fractions may be associated with DNA polymerase activity. Rose *et al.* (1971) compared AAV I to III on 5 % acrylamide gel systems incorporating sodium dodecyl sulphate (SDS). The samples were disrupted in 1 % SDS and 1 % β-mercaptoethanol and each serotype was found to have three structural proteins, A, B and C, with mol. wts. of 87000, 73000 and 62000, respectively. The C protein appeared to be the main unit of capsid structure, whereas A and B probably represented internal components. This is in contrast to the findings of Salzman & White (1970) that with LRV the main component was fraction B. Johnson, Ozer & Hoggan (1971) working with AAV III in 7·5 % polyacrylamide gels also found three major components which they labelled as VP 1, VP 2 and VP 3, with respective mol. wts. of 66000, 80000 and 92000. The protein VP 1 accounted for about 80 % of the total protein in the virion. This would correspond to fraction C of Rose *et al.* (1971). Macleod, Longworth & Tinsley (1971) using 5 % polyacrylamide gels showed that *Galleria* DNV and *Junonia* virus also contained three proteins, but when the same samples were run in 10 % gels four protein bands were obtained with mol. wts. of 72000, 57000, 53000 and 46000. The profiles for both the *Galleria* and *Junonia* viruses were identical. In view of this additional protein possessed by the insect parvoviruses as revealed in 10 % gel systems, it will be necessary to re-examine the vertebrate parvoviruses at this higher gel concentration.

Nucleic acid

The parvoviruses, by definition, contain DNA, but the characterization of their nucleic acid has presented many technical difficulties and so detailed knowledge is only available for a few members. The structure of the nucleocapsid is such that it is very resistant to treatments designed to extract the DNA. A further problem is provided by the making of positive and negative strains of DNA which are separately encapsidated. This phenomenon has been satisfactorily demonstrated with only a few members of this group.

The early workers established that the LRV and H-1 viruses contained DNA by showing that the intra-nuclear inclusions produced by these viruses were Feulgen positive and could be stained with acridine orange (Rabson, Kilham & Kirschstein, 1961; Bernhard, Kasten & Chang, 1963; Hampton, 1964). These findings were substantiated by Cheong, Fogh & Barclay (1965), who found that [^{3}H]-thymidine was incorporated into the DNA of LRV, whereas [^{3}H]-uridine was not. Salzman & Jori (1970) found that formaldehyde reacted with isolated DNA from LRV, and its E_{260} was increased by 13 to 16 %. Therefore, it was suggested that the formaldehyde had penetrated the protein coat of LRV and had reacted with nucleic acid bases. Sinsheimer (1959) showed that formaldehyde reacted with free amino groups of the DNA of the bacterophage øX 174, indicating that it was single-stranded as polymerized double-stranded DNA should have no free amino groups to react with formaldehyde because of hydrogen bonding between the base pairs. Crawford *et al.* (1969) showed that the DNA of the minute mouse virus manifested an increase in absorption at 260 nm of 18 % also indicating the single-stranded nature of the DNA. Salzman, White & Kakefuda (1971) exposed extracted DNA from the latent rat virus to exonuclease I, which specifically hydrolyses linear single-stranded DNA. These authors found that 70 to 80 % of the LRV DNA was hydrolysed within 4 h of incubation. Further evidence of linearity was provided by electron-microscopic examination of the

DNA using cytochrome *c* or diethylaminoethyl dextran (DEAE) monolayers. Only linear strands were observed, in contrast to øX 174 DNA, prepared in same manner, which showed 80 to 90 % circular configuration.

Crawford *et al.* (1969) proposed that if the DNA inside the particles of AAV-I was single-stranded, then it was possible that only negative or positive strains were enclosed by the protein coats. These complementary strands could then anneal after liberation to form double-stranded DNA. These suggestions were tested by Rose *et al.* (1969) using [^{3}H]-labelled 5-bromodeoxyuridine ([^{3}H]-BUDR) as a substitute for thymidine in AAV-III together with a similar preparation labelled with [^{14}C]. This system provided heavy ([^{3}H]) and light ([^{14}C]) populations of particles which could be easily separated on a gradient. Extraction of a mixture of heavy and light particles could result in the formation of a hybrid with about 50 % of the duplexes containing both heavy and light strands. Rose *et al.* (1969) found that when DNA was extracted from mixtures of heavy and light particles, a substantial quantity of hybrid DNA was formed. These results provided good evidence that the DNA of AAV III was single-stranded and separately encapsidated, and Mayor, Jordan & Ito (1969*a*) and Mayor *et al.* (1969*b*) provided further evidence of single strands within the nucleocapsid. Berns & Adler (1972) used AAV particles with bromodeoxyuridine-substituted DNA. The complementary strands of AAV DNA contain different amounts of thymidine (26 and 21 %), and if BUDR is substituted for thymidine then the complementary strands should show differences in density in CsCl equilibrium gradients. This was found to occur and Berns & Adler (1972) concluded that there are two types of particle of AAV, each of which contains only one type of complementary single strand. The AAV particle containing BUDR-substituted DNA was infectious and it should now be possible to establish the infectivity of the second type of particle and to investigate the role of each strand in virus development. Barwise & Walker (1970) and Kurstak *et al.* (1971) suggested that the DNA of *Galleria* DNV is also single-stranded and that complementary strands are present in different particles.

RELATIONSHIPS BETWEEN MEMBERS OF THE PARVOVIRUS GROUP

Serological evidence

Various parvoviruses have been compared by serum neutralization (SN), complement fixation (CF), immunofluorescence (FA) and immunodiffusion techniques. Little evidence of relationships has emerged from these studies, though by no means do they constitute an exhaustive study. The latent rat virus (LRV) is related to H-1 and HB viruses (Toolan, 1964, 1968; Payne, Beals & Preston, 1964; El Dadah *et al.* 1967). Hoggan (1971) found that MVM did not cross-react with H-1 or LRV using SN, but did so when examined by FA, and the bovine parvovirus (Haden) did not cross-react by SN, CF, or FA with LRV, H-1, MVM, *Galleria* DNV, or the four AAV types. Feline panleucopaenia virus and mink enteritis are very closely related (Burger, Gorham & Ott, 1963; Gorham *et al.* 1966; Johnson *et al.* 1971).

Hoggan (1971) reported that of the four AAV, only types II and III showed any cross-reactivity. The *Junonia* and *Galleria* viruses are very closely related, but differ widely both in host range and in histopathology in the host (Rivers & Longworth, 1973). Hoggan (1971) found that *Galleria* DNV was not related to AAV I–IV, LRV, H-1, MVM or bovine parvovirus.

Hallauer, Kronauer & Siegl (1971*a*), Hallauer, Novak & Kronauer (1971*b*) and Hallauer *et al.* (1972) examined 41 human cell strains derived from six different cell lines and two

stable cell strains of porcine and rat origin. Thirty-six cell strains proved to be infected with parvoviruses, including the pig and rat cells, and the extracted viruses could be classified into four distinct serotypes. It was thought that infections in the human cell strains were as a result of accidental contamination in the laboratory, whereas the infected rat cells most probably arose from explants being taken from an infected animal. Serotype 1 was the most frequent contaminant of the human cell strains and this was closely related to the porcine parvovirus. Bachmann (1969) and Cartwright, Lucas & Huck (1969) reported that porcine parvovirus occurred in most farm pigs as a latent infection and was present in practically every tissue. These findings prompted Hallauer *et al.* (1971*a*, *b*) to suggest that a probable source of this virus in the laboratory was in trypsin (derived from pig pancreas) and introduced into the cell cultures during the process of trypsinization.

Cell culture studies

Hallauer *et al.* (1972) have proposed a preliminary division of the parvoviruses into three groups based on a detailed study of their host ranges in cell cultures. Group I: the three serotypes found in human cell cultures and which replicate almost exclusively in permanent human cell lines, but fail to grow in either primary or continuous cell lines derived from rodents. Group II: the H-1 and H-3 (hamster osteolytic viruses), which replicate equally well in human and rodent permanent cell lines. Group III: the LRV, RTV (rat cell contaminant), X 14 and MVM, which will only replicate in rodent cells.

Unfortunately, the other parvoviruses have not been studied in cell systems in such detail and it would be of considerable interest to test the bovine, canine, cat and insect viruses in the same controlled manner.

The only records of a parvovirus of invertebrate origin infecting mammalian cells are those provided by Kurstak, Belloncik & Brailovsky (1969), who reported that the *Galleria* DNV could adapt to the mouse L cell (ATCC 929, C3H/AN) and that some cells supported replication of this virus. However, in the absence of other data, it is not known if this is an isolated phenomenon or not.

CONCLUSIONS

The parvoviruses provide considerable interest because of their small size and possession of single-stranded linear DNA. These viruses appear to have an affinity for tissue cell lines where they are usually present as inapparent infections. They require rapidly growing cells for maximum replication to occur. Present knowledge indicates that mammals and insects are the only hosts, but it would be worth while to look at cell systems derived from other vertebrates, e.g. fish and reptiles, and also in the rapidly growing number of invertebrate cell lines. In this connexion the technique of extracting the cell monolayers with an alkaline borate or glycine buffer at pH 9·0 (Hallauer & Kronauer, 1960) could be invaluable. This method allows the liberation of cell-associated viruses without damage to the extracted cells, and so they can be treated at frequent intervals during the growth cycle. This is particularly important with parvoviruses as they show alternating cycles of high and low concentration of virus (Hallauer & Kronauer, 1962).

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