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Characteristics of Feline Panleucopaenia Virus Strains Enabling Definitive Classification as Parvoviruses

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With 6 Figures

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Summary

The viruses causing feline panleucopaenia and mink enteritis are shown to have the morphology of parvoviruses, with a mean diameter between 21 and 24 nm. Buoyant density studies show that purified virus of both strains consists of particles which band as "complete" virus at a main density of 1.41 g/ml, with a minor population at a density of 1.44 g/ml. Other particle species with a lower content of DNA, or no detectable DNA, occurred at mean buoyant densities of 1.36 and 1.31 g/ml, respectively.

For both feline panleucopaenia and mink enteritis viruses, sedimentation coefficients of DNA from the three density levels which contain DNA, gave results conforming to those expected for single stranded DNA, whilst buoyant density studies showed the DNA of all three species to have a mean density of 1.722. The calculated molecular weight of the DNA of particles banding at 1.41 g/ml (1.7×10^6 daltons) represents 28.5 per cent of the total particle weight (5.9×10^6 daltons).

Viral protein can be separated into two major polypeptides with molecular weights of 60,300 and 73,100, amounting to 86 and about 10 per cent, respectively, of the total radioactive amino acids incorporated. A third minor component of molecular weight 39,600 daltons was present at concentrations between 3 and 6 per cent.

1. Introduction

The causal agents of feline panleucopaenia (FP) and mink enteritis (ME) are indistinguishable in their biological and antigenic characteristics (Burger *et al.*, 1963; Johnson, 1967), and on this basis the ME virus is referred to in the present report as a strain of FP virus. Both virus strains are classified by Wildy (1971) as possible members of parvovirus subgroup A, such tentative classification being

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based on their general physicochemical properties and *in vitro* behaviour (JOHNSON and CRUICKSHANK, 1966), which conform to those described for the parvovirus group. Definitive classification as parvoviruses requires further information on viral morphology and DNA characteristics as laid down by the International Committee on Virus Nomenclature (WILDY, 1971). The present report gives such data for both FP and ME viruses, and also includes information on the proteins of the virus strains.

2. Materials and Methods

2.1. Cell Culture

A stable line of feline kidney cells (Norden Laboratories, Nebraska) was used throughout the work reported. The medium used for these cells was based on Hanks' B.S.S. supplemented with 0.5 per cent lactalbumin hydrolysate, standard concentrations of BME amino acids and vitamins (Flow Laboratories, Scotland), bicarbonate buffer, and penicillin and streptomycin at concentrations of 200 I.U. and 0.2 mg/ml, respectively. Uninactivated calf serum at concentrations of 10 and 1 per cent was used for growth and maintenance of cells, respectively. Cell monolayers were passaged at 2×10^5 cells/ml by standard procedures.

2.2. Virus Strains

FP virus was represented by the 3rd *in vitro* passage of the leopard virus strain (JOHNSON, 1964), whilst ME virus was represented by the 3rd *in vitro* passage of a strain isolated from an outbreak of mink enteritis in the U.K. (JOHNSON and HEAD, unpublished information). Both strains had been stored at —20° C as culture fluids for over 8 years, and whilst both strains were shown to be viable, no attempt was made to determine virus titres, seed material being built up as described below. Both virus strains were characterized on seed virus samples as FP virus strains by *in vitro* behaviour (JOHNSON, 1965), physicochemical properties (JOHNSON and CRUICKSHANK, 1966), haemagglutination pattern (JOHNSON, 1971) and, using a specific FP antiserum, by haemagglutination-inhibition (JOHNSON, 1971) and immunofluorescence (KING and CROGHAN, 1965) tests.

2.3. Virus Production

For each virus strain a high titred seed material was produced. This was effected by selection for use in media of a calf serum with minimal levels of nonspecific inhibitors as measured by haemagglutination-inhibition (JOHNSON, 1971). Cells in suspension at a concentration of 2×10^5 cells/ml (adequate to form a complete monolayer in not less than 48 hours) were infected with stock virus and allowed to form monolayers. Such infected monolayers were passaged at 3—4 days intervals at 2×10^5 cells/ml until such time as obvious unstained cytopathic effect occurred, correlated with high levels of intranuclear inclusions in parallel stained coverslips (JOHNSON, 1965). Cell débris from such monolayers, after freezing and thawing, and centrifugation at $5000g$ for 15 minutes, was extracted with an alkaline glycine buffer (HALLAUER and KRONAUER, 1965), and the cell free extract was added to supernatant virus fluids. Seed material produced by this method contained up to 60,000 haemagglutinating units (HAU) of virus/ml.

2.4. Radioactive Labelling

Production of virus incorporating radioactive thymidine (^{3}H-TdR), or radioactive amino acids (^{14}C), was made by infection of cells in suspension as described, using an estimated 1 HAU of virus to every 500 cells. Eight hours after monolayers had been allowed to form, the medium was changed. For ^{3}H-TdR labelling an equivalent medium was used, containing in addition 4 μCi/ml of ^{3}H-TdR (Radiochemical Centre, Amersham. Specific activity 56 μCi/mM). For ^{14}C labelling, the change medium contained only 0.25 per cent of lactalbumin hydrolysate, no BME amino acids, and ^{14}C incorporated at 1 μCi/ml as a radioactive protein hydrolysate (Radiochemical Centre, Amersham.

Specific activity 57 mCi/mAtom of carbon). Development of cytopathic effect was followed by daily staining of coverslips infected in parallel. Virus was harvested at peak cytopathic effect or at 4 days following cell infection.

2.5. Virus Concentration and Purification

Virus was concentrated and purified as described by SIEGL *et al.* (1971) and SIEGL (1973). Briefly, crude concentrates were made by repeated freezing and thawing of infected cultures, and separating cell débris by low speed centrifugation. Cell débris, and crude virus pelleted from cell free fluids, were then treated with receptor-destroying enzyme, DNase, RNase, trypsin and deoxycholate. Radioactive virus was finally purified by differential centrifugation and repeated banding in CsCl gradients.

2.6. DNA and Viral Protein Studies

DNA was extracted from purified virus with sodium dodecyl sulphate (SDS) for 90 minutes at 72.5° C, sedimentation coefficients and buoyant densities being determined as described by SIEGL *et al.* (1971) and SIEGL (1973). Methods for studying the characteristics of the polypeptides of purified virus by polyacrylamide gel electrophoresis have been fully described by GAUTSCHI and SIEGL (1973).

3. Results

3.1. Morphology of the Virus Strains

Figure 1 depicts an electron micrograph of FP virus obtained from purified virus fractions at a density of 1.36 g/ml, similar results being obtained for both ME and FP viruses at all four density levels described below.

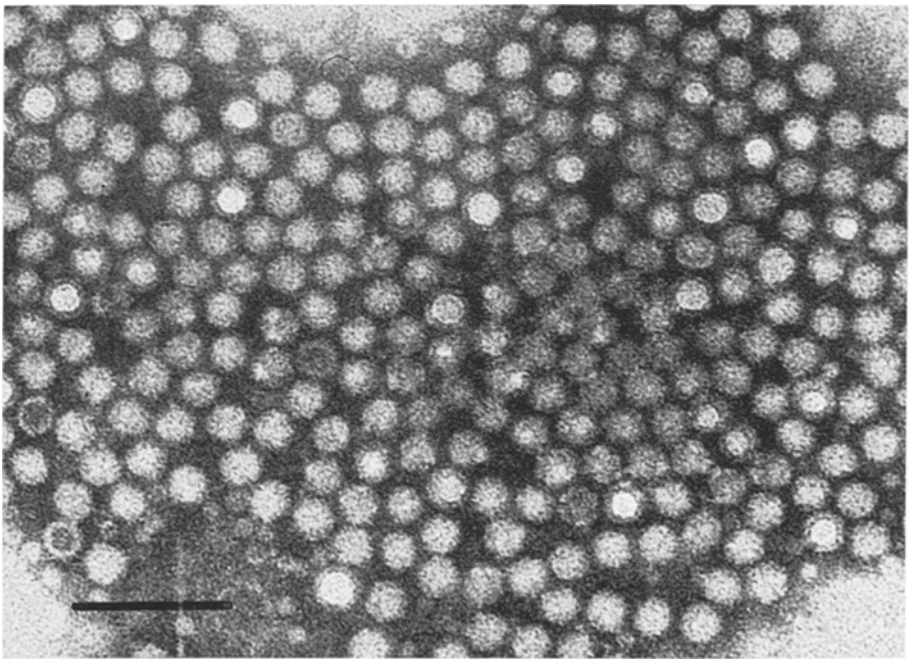

Fig. 1. Electron micrograph of feline panleucopaenia virus obtained from purified virus at a density of 1.36 g/ml
Negatively stained with potassium phosphotungstate. Bar represents 100 nm

Virus particles are seen as naked virions, hexagonal in outline, the majority of particles ranging between 21 and 24 nm in diameter (Fig. 2), compatible with the morphology of parvoviruses as outlined by WILDY (1971).

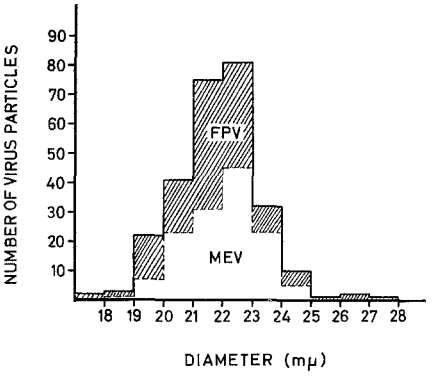

Fig. 2. Distribution of particle sizes in preparations of feline panleucopaenia and mink enteritis viruses
A total of 270 particles were measured in the case of FPV, whereas for MEV size distribution is representative for 140 individual particles

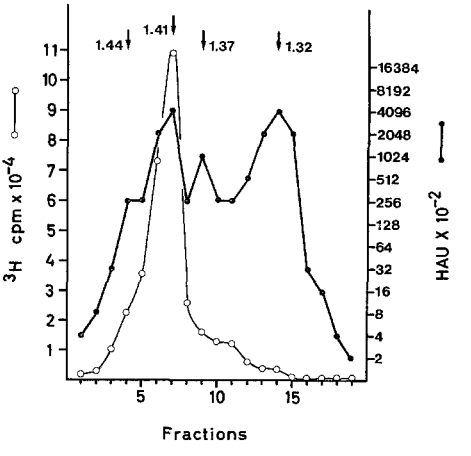

Fig. 3. Buoyant densities of feline panleucopaenia virus banded in CsCl gradients with a mean density of 1.35 g/ml

3.2. Buoyant Densities of the Viruses

Figures 3 and 4 show the results of primary and secondary purification runs of FP virus in caesium chloride gradients. Figure 3 also incorporates data on haemagglutination (HA) values at different density levels.

By means of radioactivity counts and HA titres, it could be shown that there were four main virus populations at different density levels of 1.44, 1.41, 1.36, and 1.31 g/ml. The 3 populations at the higher density levels showed coincident "peaks" of both radioactivity and HA, whilst the population at a density of 1.31 g/

ml was only detectable by HA or ^{14}C radioactivity, being apparently devoid of DNA. Further, the viral population occuring at a density of 1.36 g/ml appeared to contain less DNA than did the two populations at higher densities, as gauged from the HA/^{3}H-TdR ratios. The mean values for densities of the four viral populations is given in Table 1, where it may be seen that there is no significant difference in these values for either virus strain.

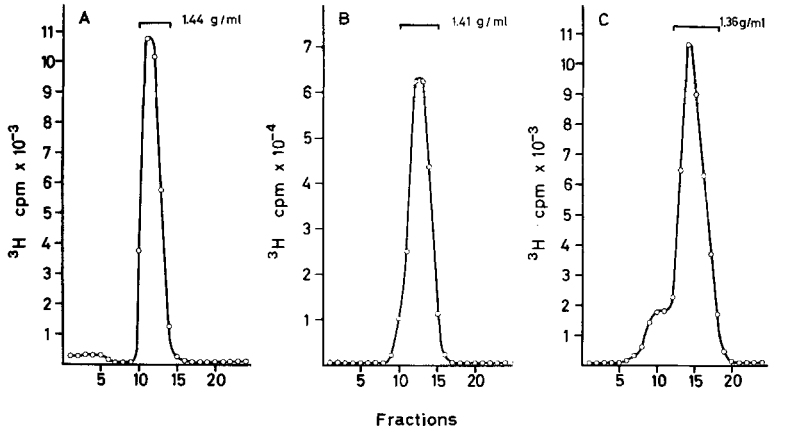

Fig. 4. The homogeneous buoyant densities of virus particles isolated from CsCl gradients (Fig. 3) at mean densities of 1.44, 1.41, 1.36 g/ml, and recentrifuged under identical conditions

3.3. Characteristics of Viral DNA

Sedimentation coefficients of DNA extracted from the 1.41 g/ml density fraction of ME virus are shown in Figure 4, whilst the mean values for ME and FP viruses is given in Table 1. ME DNA sedimented in neutral caesium chloride gradients as one major band at 23.3S, the sedimentation coefficient not altering

Table 1. *Physicochemical Characteristics of the Viruses and DNA of Feline Panleucopaenia and Mink Enteritis Viruses*

(Figures given are mean values obtained from 2—6 separate experiments)

Density in CsCl (g/ml)		Characteristics of the DNA								Molecular weight[a] (daltons)
		S value native DNA in neutral CsCl		S value denatured DNA in neutral CsCl		S value in alkaline CsCl		Density in neutral CsCl (g/ml)		
FP	ME	FP	ME	FP	ME	FP	ME	FP	ME	
1.44	1.44	23.6	23.4	22.8	23.0	15.8	15.8	1.7226/1.7240		1.7×10^6
1.41	1.41	23.3	23.3	23.4	24.0	16.6	16.0	1.7224/1.7229		1.7×10^6
1.36	1.36	12.8	12.1	12.7	12.0	12.0	11.4	1.7228/1.7230		7.8×10^5
1.315	1.31	—[b]	—	—	—	—	—	—	—	— —

[a] Calculated from S value in alkaline gradient.
[b] No DNA demonstrable.

significantly (24S) when DNA was denatured under alkaline conditions, neutralized and sedimented in neutral CsCl (Fig. 5A). When native DNA was centrifuged in alkaline gradients, the sedimentation coefficient gave a value of 16.6S. Small peaks of lower S values occurred in all gradients (Fig. 5B), and are assumed to be due to DNA breakdown products, or partial folding of DNA strands (SIEGL, 1973).

The DNA derived from viral populations at a density level of 1.44 g/ml showed a similar sedimentation pattern to that obtained from the population at 1.41 g/ml (Table 1). DNA obtained from the virions concentrated at a density of 1.36 g/ml

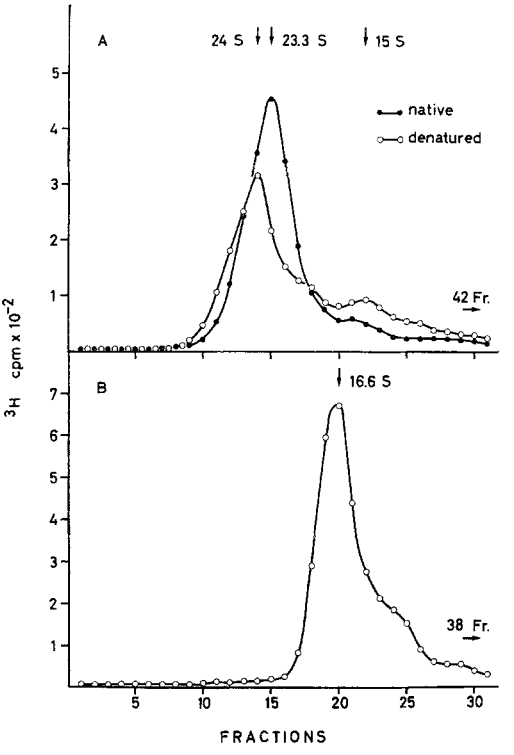

Fig. 5
A. The sedimentation profile of native and alkali-denatured DNA of ME virus banding at a density of 1.41 g/ml, and centrifuged in neutral CsCl
B. Sedimentation profile of native ME-DNA in alkaline CsCl

sedimented with lower S values as given in Table 1, which also incorporates data on the molecular weight of the DNA calculated from S values recorded during sedimentation in alkaline CsCl.

All three particle species of both FP and ME virus yielded DNA with a comparable high buoyant density of around 1.722 g/ml.

3.4. Characteristics of Viral Proteins

Virus particles of both the FP and ME strain labelled with ^{14}C-amino acids were purified following the experimental procedure referred to in section 2.5. As

indicated in Figure 6 A and B, particles recovered from different buoyant densities regularily contained two polypeptides with molecular weights of 60,300 and 73,100, amounting to 86 and 10 per cent of total radioactive amino acids incorporated, respectively. Such a polypeptide composition of FP and ME viruses would be in good accordance with the two polypeptides described for Lu III virus, the protein characteristics of which have been analysed using the same methodology (GAUTSCHI and SIEGL, 1973). However, there was evidence in all fractions of the viruses collected from CsCl gradients for a polypeptide of rather low (3 to 6 per cent) radioactivity and a molecular weight around 39,600. Whether the latter polypeptide has to be referred to as a constant constituent of the virion or whether its presence is due to insufficient purification of the particles awaits further experimental clarification.

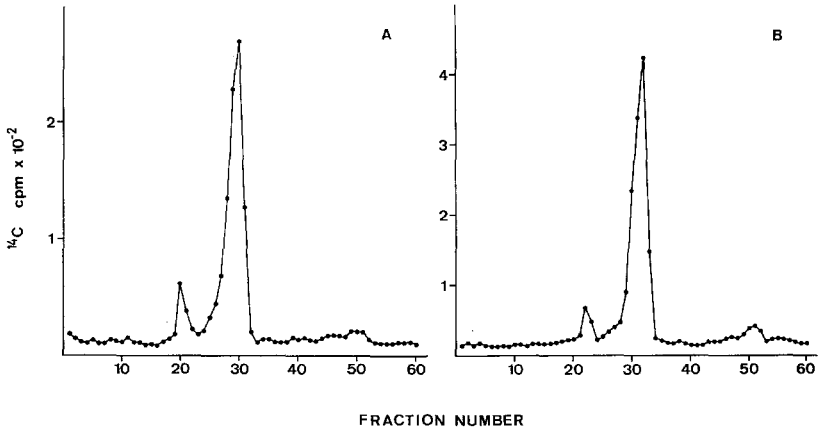

Fig. 6. Electrophoresis of ^{14}C amino acid labelled viral proteins on 7.5 per cent SDS-polyacrylamide gels
A. The polypeptide patterns of FP virions banding in CsCl at ϱ = 1.41 g/ml shows two major proteins and traces of a third protein around fraction 50
B. The third minor protein component is more pronounced in the electrophoretic pattern of ME virus particles isolated from gradient fractions with a mean density of 1.31 g/ml

4. Discussion

The morphology of FP and ME viruses is similar, and conforms to that described for the parvovirus group (WILDY, 1971). Previous reports on the size of the viruses are in general agreement with those presently reported; JOHNSON and CRUICKSHANK (1966) recording 20 nm for FP virus, KÄÄRIÄINEN *et al.* (1966) reporting 25 nm for ME virus, and STUDDERT and PETERSON (1973) a range from 20—24 nm for FP virus.

The buoyant densities of both FP and ME virus strains are comparable, and as with other parvoviruses (SIEGL *et al.*, 1971; SIEGL, 1973) viral populations banding at densities of around 1.44, 1.41, 1.36, and 1.31 g/ml can be identified by the use of radioactivity and/or HA techniques. It is apparent that the major population of virus with a full complement of DNA bands at a density around

1.41 g/ml, with a smaller but stable population banding at around 1.44 g/ml. SIEGL (1973) working with the Lu III parvovirus, suggests that virions concentrating at a density of 1.44 g/ml, whilst having the same DNA as virions at 1.41 g/ml, may have higher densities due to lower protein content, or loss of water during aggregate formation. Based on the buoyant density of complete and empty particles (1.41 and 1.315 g/ml) as well as on the molecular weight of the DNA isolated from viruses banding at 1.41 g/ml DNA should constitute about 28.5 per cent of the molecular weight of the infective virion (BREEDIS *et al.*, 1962). The total molecular weight of both FP and ME virus would then amount to about 5.9×10^6 daltons.

The population of virions banding at mean densities around 1.36 g/ml contain only a calculated 45 per cent of the DNA of virions at the higher density levels. The large population of haemagglutinating virus accumulating at a density around 1.31 g/ml is devoid of detectable DNA, and would correspond to "incomplete" or non-infectious virus particles. STUDDERT and PETERSON (1973) by titrating gradients of FP virus on infectivity, showed peaks of infectivity at density levels of 1.4 and 1.33 g/ml. Infectivity at 1.33 g/ml might in this instance be due to aggregates of complete and low density virions. STUDDERT and PETERSON (1973) suggest that the initial heterogeneity of densities might be due to impurities on the surface of the virion, but the present results show that this heterogeneity is correlated with varying concentrations of DNA in virions at different density levels. KÄÄRIÄINEN *et al.* (1966), using complement fixation techniques to detect ME virus at different densities, suggested a buoyant density of 1.3—1.33 g/ml, with a little complement activity detected at a density of 1.38 g/ml. In this instance, capsid protein rather than DNA was used as indicator of virus, and a relatively large incomplete virus population of low density was apparently present in the initial viral harvests. Dependent on the technique used for producing virus, the relative proportions of "complete" and "incomplete" virus may vary considerably (JOHNSON and SIEGL, unpublished observation).

The sedimentation coefficients of the DNA of both FP and ME virus strains are comparable, and conform to those reported for other parvoviruses (WILDY, 1971). The sedimentation coefficients of DNA extracted from "complete" virus at density levels of 1.44 and 1.41 g/ml show characteristics of single stranded DNA, similar coefficients being measured for native and denatured molecules. DNA from the lower density zone of 1.36 g/ml showed similar behaviour as single stranded DNA, but had lower sedimentation coefficients corresponding to lower molecular weight. The density of DNA extracted from all three particle species was similar (around 1.722 g/ml) and, hence, it may be assumed that the low molecular DNA obtained from viruses banding at 1.36 g/ml is also of viral origin.

A brief note has to be added concerning the molecular weight of viral DNA. The figure calculated according to STUDIER (1965) on the basis of the sedimentation behaviour of single stranded DNA in neutral solution is smaller than that obtained from the respective S value in an alkaline medium. To a certain extent this may be due to nonspecific base pairing or even a specific folding back of the single stranded molecule at neutral pH. Evidence for such a behaviour of the DNA of parvoviruses of subgroup A has been presented by TATTERSALL *et al.* (1973) and SIEGL (1973). Therefore, the true molecular weight can be derived from

sedimentation characteristics in alkaline media only, where base pairing is impossible.

Overall, the results show that the viruses causing FP and ME are indistinguishable on present data, and have the characteristics of parvoviruses. Virus produced *in vitro* contains a variable proportion of virions which either do not include the full complement of DNA or lack DNA completely. It would be logical to assume that preparations containing relatively high concentrations of such "incomplete" virus might not be effective in production of *in vitro* cytopathic effect or *in vivo* induction of disease. O'REILLY (1970) has suggested that interference between "incomplete" and "complete" virus may be the reason for the difficulty in reproducing clinical FP experimentally. It would be of interest to determine the effect *in vitro* and *in vivo* of purified virus obtained from density levels of 1.41 g/ml.

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