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THE NUCLEIC ACID AND PROTEINS OF EPIZOOTIC HAEMORRHAGIC DISEASE VIRUS

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ABSTRACT

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Purified epizootic haemorrhagic disease virus (EHDV) was shown to contain 10 double-stranded RNA segments and a double-layered protein capsid with 4 major and 4 minor polypeptides. The virus differed from bluetongue virus (BTV), the orbivirus prototype, in that EHDV had an additional minor polypeptide component. This component, together with the major polypeptides P2 and P5, formed the outer capsid layer of the virus. The extra polypeptide apparently stabilizes this layer since, unlike BTV, EHDV was quite stable on CsCl gradients at both pH 7,0 and 8,0. EHD virions were found to have a density of 1,36 g/mℓ, while particles without the outer capsid layer were isolated and had a density of 1,40 g/mℓ. Two non-capsid polypeptides, P5A and P6A, were identified in addition to the 8 capsid polypeptides. Polypeptide P5A was synthesized in excess of all the others.

There was little homology between the nucleic acids of EHDV and BTV with only 5–10% cross-hybridization. No hybrid double-stranded RNA segments were identified. We found by cross-immune precipitation that the major core polypeptides of the 2 viruses (P7 and P3) have common antigenic determinants.

Résumé

L'ACIDE NUCLÉIQUE ET LES PROTÉINES DU VIRUS DE LA MALADIE HÉMORRHAGIQUE ÉPIZOOTIQUE

Après purification, le virus de la maladie hémorrhagique épizootique (EHDV) s'est avéré contenir 10 segments d'ARN à filament double et une capside protéinique à deux couches avec 4 grands et 4 petits polypeptides. La différence entre l'EHDV et le virus de la fièvre catarrhale du mouton (BTV), prototype de l'orbivirus, consiste en la possession par le premier d'un petit polypeptide comme composant additionnel. Ce composant forme, avec les grands polypeptides P2 et P5, la couche externe de la capside du virus. Il semble contribuer à la stabilité de cette couche car, à la différence du BTV, l'EHDV est resté tout-à-fait stable sur des gradients CsCl tant au pH 7,0 qu'au pH 8,0. La densité des virions de l'EHD a été mesurée à 1,36 g/mℓ; des particules sans couche externe de la capside ont été isolées et on leur a trouvé une densité de 1,40 g/mℓ. Outre les 8 polypeptides de la capside, on en a identifié deux, P5A et P6A, qui n'en font pas partie. La synthèse du polypeptide P5A dépassait celle de tous les autres.

INTRODUCTION

Epizootic haemorrhagic disease virus (EHDV) is the causative agent of a highly fatal disease of white-tailed deer (Shope, MacNamara & Mangold, 1955; Shope, MacNamara & Mangold, 1960). The virus was classified as an orbivirus (Borden, Shope & Murphy, 1971), a subgroup of the Reoviridae family (Joklik, 1974), on the basis of its double-strandedness (Tsai & Karstad, 1973a) and typical orbivirus morphology (Thomas & Miller, 1971; Tsai & Karstad, 1973b).

The double-stranded RNA (dsRNA) genome of EHDV is segmented and has been separated (Kontor & Welch, 1976) into at least 5 different size groups. The exact number and molecular masses of the genome segments and the protein composition of the virus have not been reported.

Our interest in EHDV stems from the fact that a slight antigenic relationship with BTV can be demonstrated by complement fixation tests (Borden, *et al.*, 1971; Moore & Lee, 1972; B. J. Erasmus, unpublished results), agar gel precipitin tests (Moore, 1974; Jochim, 1976) and in the indirect fluorescent antibody test (Jochim, Barber & Bando, 1974). This raised the question as to what extent this serological relationship would be reflected in the nucleic acid and polypeptide components of BTV and EHDV.

In this paper we compare the RNA and polypeptide compositions of EHDV and BTV. An estimate of a possible relationship in RNA segments may be obtained by a comparison of the size of corresponding genome segments. A much better answer is obtainable, however, from hybridization studies. In this respect it has been shown that African horsesickness virus, an orbivirus antigenically unrelated to BTV, does not show any significant level of cross-hybridization with BTV (Verwoerd & Huismans, 1969). The antigenic relationship on the polypeptide level can be studied by cross-immune precipitations of soluble virus proteins and homologous and heterologous antiserum.

MATERIALS AND METHODS

Virus and cells

EHDV (New Jersey strain) serotype 1 (Barber & Jochim, 1975) was obtained from the Arthropod-borne Animal Diseases Research Laboratory, USDA, Science and Education Administration—Agricultural Research, Denver, Colorado, USA. The BTV strain used was the avirulent strain of serotype 10. BHK cells and L-strain mouse fibroblast cells were obtained from the American Type Culture Collection, USA. Cells were grown in Eagle's medium supplemented with 5% bovine serum.

Procedures for the propagation of the viruses in monolayer BHK cells, using a plaque-purified, low passage stock virus suspension have been described (Huismans, 1979).

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Virus purification

EHDV was purified according to a modification of the procedure described by Verwoerd (1969). Infected cells from at least 50 roller bottles were collected by centrifugation at 3 000 rpm for 30 min and resuspended in 2 mM Tris, pH 8,8 at a concentration of not more than 10^8 cells/mℓ. Heparin* was added to a final concentration of 250 units/mℓ and the cells were homogenized (Ultra Turrax homogenizer). Four successive fluorocarbon (Freon) extractions were carried out, using a half volume of Freon and 1/10th volume of 5% Sephadex G200. The first Freon phase was washed at least twice with half the original buffer solution and once with the successive Freon phases. The combined clear water phase was centrifuged at 24 000 rpm for 90 min in a SW 27 rotor through a 5 mℓ cushion of 40% sucrose in 2 mM Tris buffer. The pellets were resuspended in the Tris buffer and centrifuged on 10–30% sucrose gradients in Tris buffer at 24 000 rpm in a SW 27 rotor. A visible band could be seen halfway down the tube. A clearer band with little or no visible background material was obtained by repeating the sucrose gradient step with the resuspended pellet of the first gradient. The virus was pelleted by centrifugation at 24 000 rpm for 60 min, resuspended in 2 mM Tris and stored at 4 °C.

BTV was purified in the same way, except that no heparin was used. Yields of purified BTV were at least 5 times higher than yields of EHDV. If the heparin treatment was omitted in the EHDV purification, most of the virus was either aggregated or remained associated with cellular material and no bands were obtained on sucrose gradients.

CsCl density gradient centrifugation

Gradients were preformed with a density gradient former (Beckman) and starting solutions of 55% and 40% w/v CsCl solutions in 100 mM Tris at the specified pH. Approximately 150 μg purified virus per gradient was centrifuged in a SW 50.1 rotor for 16 h at 40 000 rpm and fractionated by drop collection from the bottom of the tube. The refractive index of every 2nd fraction was determined by a calibrated refractometer (Zeiss) and these values were used in calculating the density. Optical densities of the fractions were determined at 260 nm with a spectrophotometer (Beckman Acta CIII) after dilution to 1 mℓ.

Preparation of labelled subcellular fractions

Infected BHK monolayer cells were pulse-labelled 16–18 h post-infection with 5 mℓ amino acid-free Eagle's medium containing 1 μCi/mℓ ^{14}C-protein hydrolysate. Cytoplasmic extracts were prepared as described by Huismans (1979) and centrifuged for 2 h at 45 000 rpm, through a 40% sucrose cushion. The supernatant represented the soluble (S100) protein fraction and the pellet the insoluble (particulate) fraction of infected cells.

Labelling of cells before and during infection with EHDV

Before: Partially confluent uninfected BHK cells were labelled for a 24 h period with 0,5 μCi/mℓ ^{14}C-protein hydrolysate in Eagle's medium containing only 1/10th of the normal concentration of cold amino acids. After the labelling period the cells were rinsed to remove the isotope medium and used for infection with EHDV.

* Novo Industries, Pharmaceuticals

During: Confluent BHK monolayers were infected with EHDV and labelled 6–20 h post-infection at 37 °C, as described above.

Cross-immune precipitations

A volume of 0,2 mℓ of soluble ^{14}C-labelled BTV or EHDV protein extract was mixed with an equal volume of either BTV or EHDV guinea pig antiserum. After 16 h at 4 °C, suspensions were centrifuged for 15 min at 4 000 rpm. Precipitates were washed and analysed on polyacrylamide gels.

Purification and labelling of dsRNA

Procedures for labelling virus-infected BHK cells with ^{32}P-orthophosphate were described by Huismans & Verwoerd (1973). The dsRNA was purified from the infected cells, using the method of Verwoerd, Louw & Oellermann (1970). Purified RNA was dissolved in and then dialyzed against 0,01 M STE-PVS buffer (0,01 M NaCl, 0,01 M Tris HCl, 0,001 M EDTA, 0,05% polyvinyl sulphate, pH 7,4).

Purification and labelling of mRNA

The method described by Verwoerd & Huismans (1972) was used. Virus-infected L-cells were labelled in suspension with 5 μCi/mℓ ^{3}H-uridine between 10–12 h post-infection at 37 °C. Cytoplasmic extracts of the infected cells were deproteinized by SDS-phenol extraction at pH 5,0 and further purified by ethanol and LiCl precipitation. Purified single-stranded RNA (ssRNA) was dissolved in 0,01 M STE-PVS and freeze-dried for hybridization experiments.

Hybridization

The percentage hybridization was determined as follows: Constant amounts of ^{3}H-labelled ssRNA were mixed with increasing amounts of unlabelled dsRNA in 0,01 M STE-PVS buffer. The reaction mixture (0,20 mℓ) was heated for 5 min at 100 °C, after which the salt concentration was increased to 0,67 M by the addition of NaCl. The samples were incubated at 72 °C for 30 min, treated with RNase at a final concentration of 2 μg/mℓ and were further processed according to the hybridization procedure described by Verwoerd & Huismans (1969).

The procedure for the analysis of hybrids on polyacrylamide gels was described by Huismans & Howell (1973). The hybridizations were carried out with ^{3}H-labelled mRNA and ^{32}P-labelled dsRNA. The RNase treatment was omitted and all hybrids were purified on columns of methylated albumin kieselguhr before electrophoretic analysis on cylindrical polyacrylamide gels.

Electrophoresis

RNA: The comparison of EHDV and BTV dsRNA was done on cylindrical 5% polyacrylamide gels, 18 cm long. Gels were made as described by Schuerch & Joklik (1973), except that no SDS was added. Gels were run for 50 h at 53 volts and 2,4 mA/gel, stained in methylene blue (Verwoerd, Els, De Villiers & Huismans, 1972), and de-stained in distilled water.

Proteins: Freeze-dried samples, resuspended in 20 μℓ of a solution containing 0,8 M NaCl, 8 M urea, 1,5% SDS, 4% mercapto-ethanol, were analysed on polyacrylamide slab gels, using a SDS-phosphate buffer system in the presence of urea, as described by Stone, Smith & Joklik (1974). Electrophoresis was

for 16 h at 55 volts per gel. Gels were stained in Coomassie brilliant blue (Anderson, Cawston & Cheeseman, 1974) and de-stained in 4% acetic acid at 50 °C. They were then dried on filter-paper under vacuum on a heated gel-drying apparatus.

Autoradiography

Cronex No. 4 medical X-ray film was placed on top of the dried gel in an X-ray film holder. The films were developed after the exposure period and the bands scanned with a Vitatron densitometer.

Results

EHDV dsRNA

EHDV dsRNA was extracted from either purified virus or from virus-infected cells and the purified RNA characterized by electrophoresis on cylindrical polyacrylamide tube gels. The marker for the determination of the molecular mass of the RNA segments was purified BTV dsRNA. The result is shown in Fig. 1.

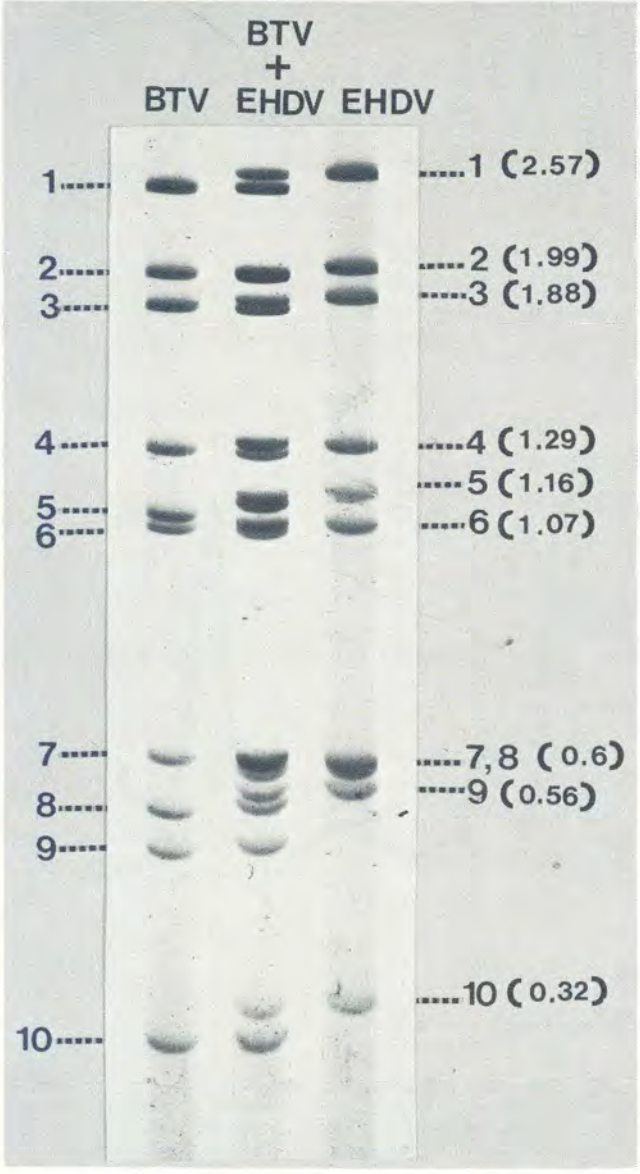

FIG. 1 Electrophoretogram of purified BTV and EHDV dsRNA. Calculated molecular masses of the EHDV dsRNA segments ($\times 10^{-6}$) are shown in brackets

Like BTV, EHDV contains 10 segments of dsRNA. The molecular masses of these segments were calculated from a plot of the mobility of BTV genome segments against the logarithm of their molecular masses (Verwoerd *et al.*, 1972).

EHDV polypeptides

EHDV was difficult to purify. The yield of virus, as compared to the yield of BTV, was low and the virus was strongly associated with material presumed to be of cellular origin. Purification was improved by the use of heparin, but, even with this procedure, virus obtained from sucrose gradients was not entirely free of cellular contaminants. Recentrifugation of the pellet obtained from the first sucrose gradient yielded the best preparations. Polypeptides from such purified EHDV preparations were fractionated on polyacrylamide gels. The result is shown in Fig. 2.

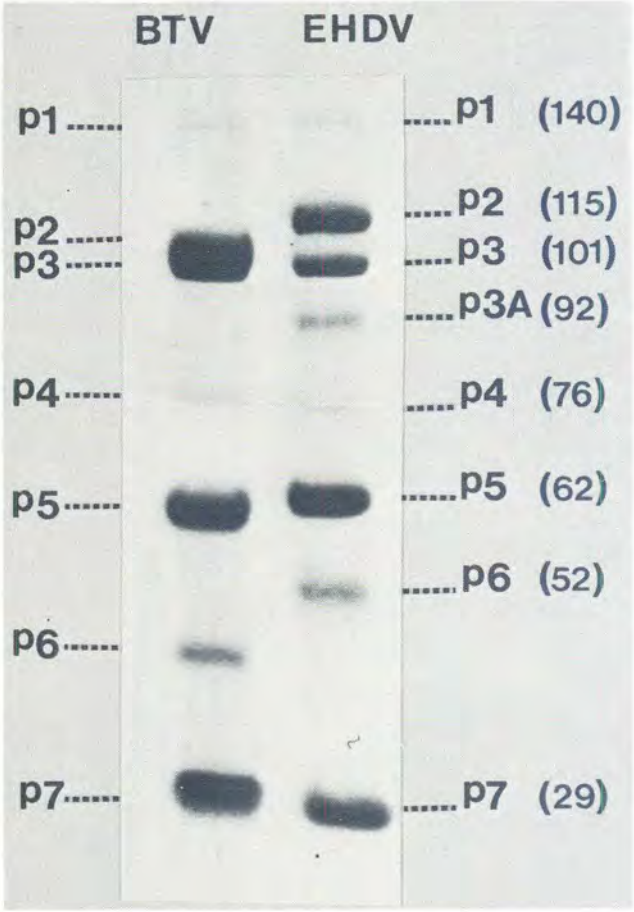

FIG. 2 Electrophoretogram of purified BTV and EHDV polypeptides. Calculated molecular masses of the EHDV polypeptides ($\times 10^{-3}$) are shown in brackets

Four major and 4 minor polypeptide components could be distinguished. Molecular masses were calculated, using the known values of the BTV polypeptides (Verwoerd *et al.*, 1972) as markers. There were small differences in size between the corresponding minor and major polypeptides of BTV and EHDV. The most important difference, however, was that EHDV contained an additional minor polypeptide. This polypeptide was numbered P3A rather than P4 in order to keep the numbers of corresponding BTV and EHDV polypeptides sequential.

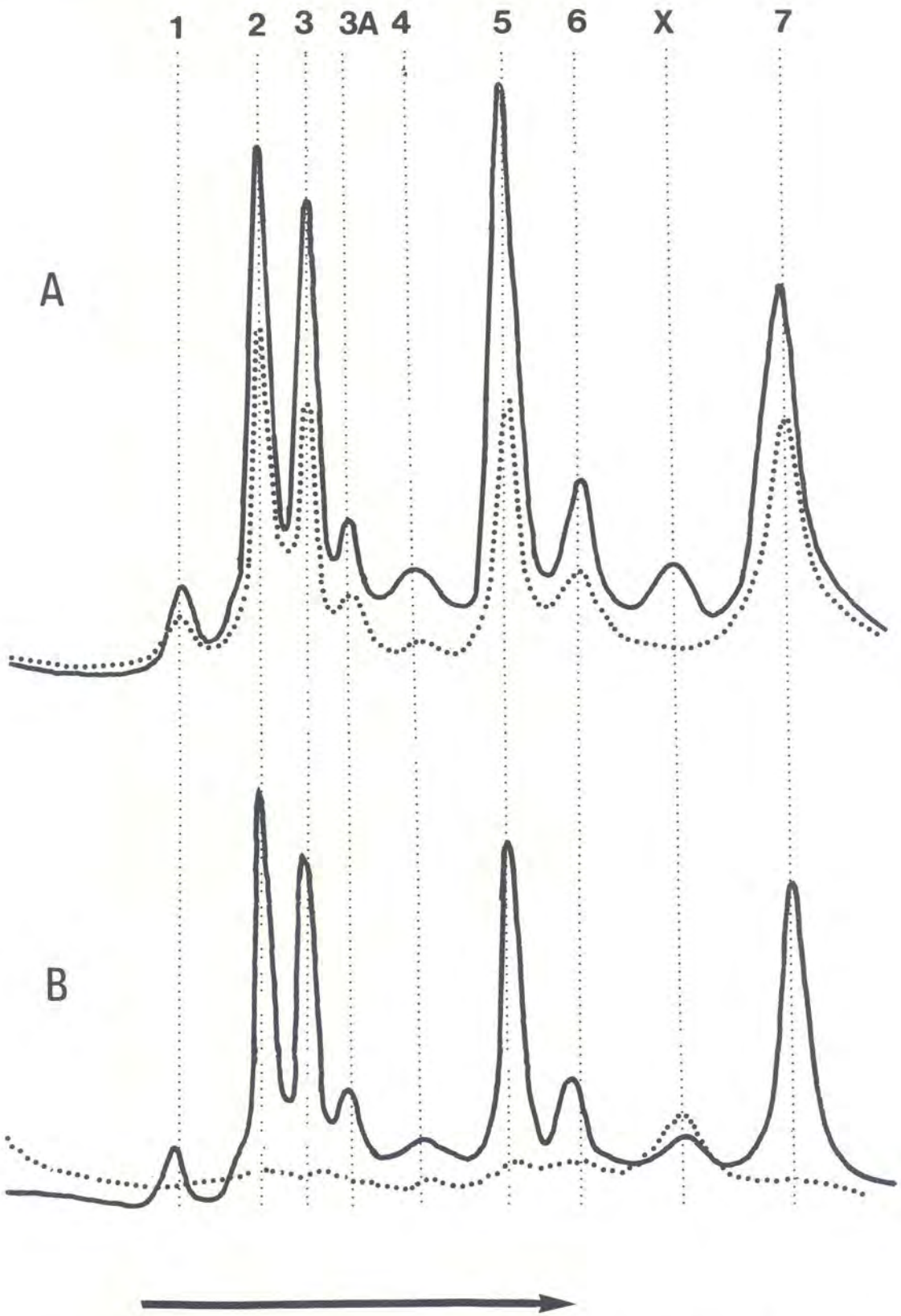

FIG. 3 An electrophoretic comparison of labelled and unlabelled EHDV-associated polypeptides. In panel A the virus was labelled during the infection cycle. In panel B the cells were labelled prior to infection. In both cases the virus was partially purified and the polypeptide components identified by electrophoresis. The solid line scans were obtained from the stained polypeptide bands. The dotted line scans are from the labelled polypeptide components in the autoradiograms made from the same gels

The possibility that some of the minor polypeptides were host cell-derived was investigated by labelling experiments. In the 1st experiment, EHDV was grown in cells that were labelled with ^{14}C-protein hydrolysate prior to infection, whereas in the 2nd experiment, the cells were only labelled during the infection with EHDV, as described under Materials and Methods. In both experiments virus was harvested 30 h post-infection and purified.

The partially purified virus preparations obtained from a single 1st sucrose gradient step in the 2 experiments were analysed on polyacrylamide gels. The gels were scanned and then dried on filter paper for autoradiography. The autoradiography plates were also scanned and a comparison of the superimposed scans of the stained and labelled components of the different virus preparations is shown in Fig. 3.

There were 4 major and 5 minor polypeptides in both partially purified virus preparations. In the labelled virus preparation (Fig. 3A), there was no radioactivity associated with minor polypeptide X. This component was labelled only in the control experiment in which the cells were labelled prior to infection (Fig. 3B). These results show that X is a cell-derived contaminant, a conclusion confirmed by further purification of the virus (Fig. 2 & 4). The labelling pattern of the other minor components all differ from X and they are presumed to be virus specific.

Isopycnic centrifugation of EHDV on CsCl gradients

EHDV was purified on sucrose gradients and analysed on each of 2 preformed CsCl gradients buffered with 100 mM Tris at pH 7,0 and 8,0 respectively.

On both gradients, EHDV banded at a density of 1,36 g/mℓ, and there was no breakdown to a particle of higher density at either pH as is the case with BTV (Verwoerd *et al.*, 1972).

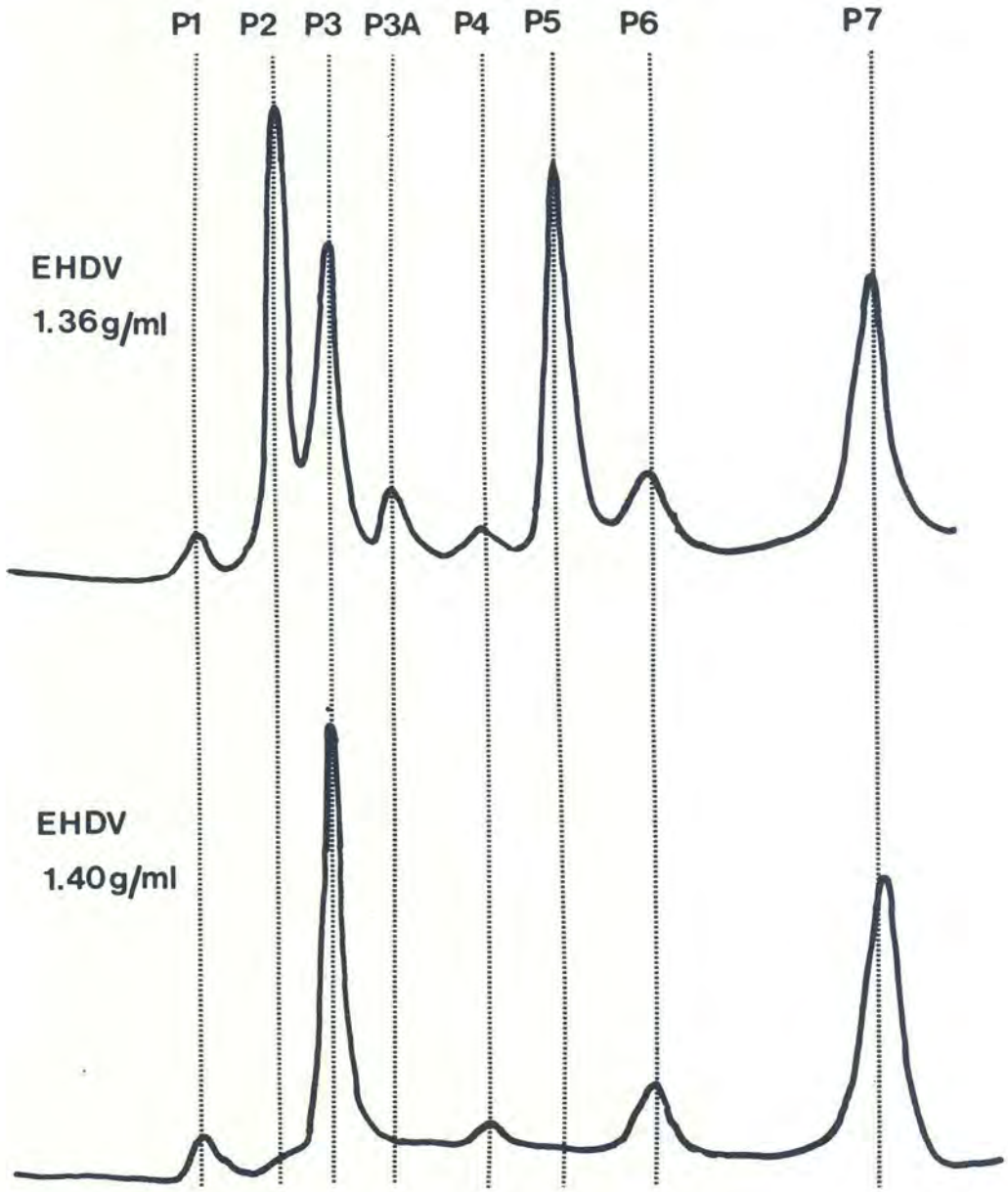

FIG. 4 A densitometer tracing of an electrophoretogram of the polypeptides in EHDV particles with densities of 1,36 and 1,40 g/mℓ on CsCl gradients respectively

When purified EHDV was kept at 4 °C for a period of 2 weeks or more, some breakdown of virus particles took place. When such virus was centrifuged on CsCl gradients, there was often a small band of particles at a density of 1,40 g/mℓ. Particles of the same density were also found in small amounts in the top region of sucrose gradients (above the virus band) during the purification step. This region, however, also contained empty particles and associated cellular proteins and there was no visible band. A comparison of polypeptides in the 1,40 and 1,36 g/mℓ density particles is shown in Fig. 4.

Particles at the 1,36 g/mℓ density consisted of 4 major and 4 minor polypeptides. The high density particles had lost 1 minor and 2 major polypeptides. Electron microscopy of this 1,40 g/mℓ fraction (result not shown) indicated that morphologically these EHDV particles were indistinguishable from BTV core particles (Verwoerd & Huismans, 1972).

EHDV polypeptides in the particulate fraction of infected cells

A characteristic feature of polypeptide synthesis in BTV-infected cells was the synthesis of 2 non-capsid polypeptides, 1 of which (P5A) was synthesized in very large amounts (Huismans, 1979). In the case of BTV, all of P5A and a large amount of the other non-capsid polypeptide were found in the particulate fraction of infected cells. An electrophoretic comparison of the virus polypeptides in these fractions of BTV- and EHDV-infected cells is shown in Fig. 5.

Apart from the major capsid polypeptides of EHDV, namely, P2, P3, P5 and P7, an additional polypeptide of the same size as the non-capsid polypeptide P5A in the BTV particulate fraction was identified. It was not found in uninfected cells (result not shown) and, as in BTV, it was numbered P5A. Fig. 5 shows it to be present in such large excess that its position overlaps that of minor capsid polypeptide P6. Co-electrophoresing a very small amount of the particulate fraction with purified EHDV indicates that polypeptide P6 is only slightly smaller than P5A (result not shown).

Polypeptide P6A is possibly another non-capsid component. Evidence for its virus specificity is mainly derived from immune precipitation experiments (Fig. 6).

Cross immune precipitation of BTV and EHDV

Cross-immune precipitation experiments were done to identify immunologically-related antigens of BTV and EHDV. Soluble ^{14}C-labelled BTV and EHDV protein extracts were immune precipitated with either BTV or EHDV guinea pig antiserum and the precipitates analysed on polyacrylamide gels. Scans of the autoradiograms are shown in Fig. 6.

The main polypeptides precipitated by homologous BTV immune precipitation were P2, P3, P4, P7 and the non-capsid polypeptide, P6A. EHDV immune precipitation is strikingly similar with precipitation of P2, P3, P4, P7 and P6A. The latter was tentatively described as a non-capsid polypeptide in the previous experiment (Fig. 5).

Heterologous precipitation of BTV soluble proteins with EHDV anti-serum resulted in the precipitation of small amounts of P3, some P6A and a proportionally large amount of polypeptide P7. In The reverse situation—EHDV soluble proteins plus BTV antiserum—the main precipitating polypeptides were P3 and P7.

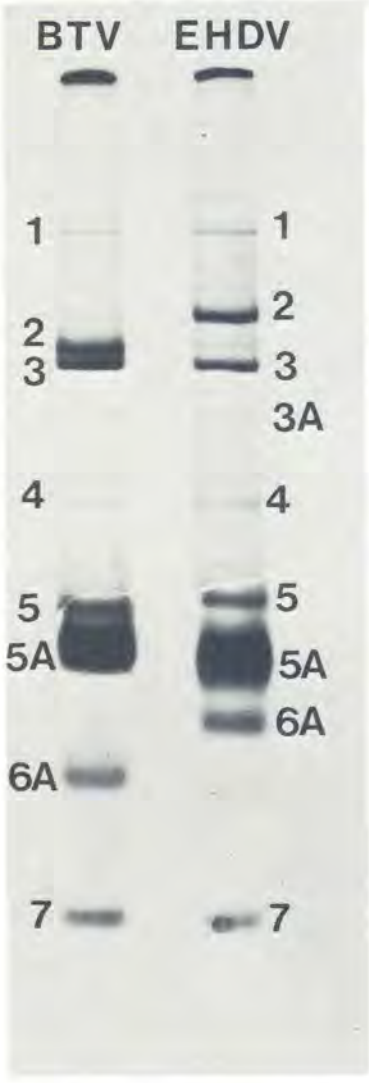

FIG. 5 Autoradiogram of the electrophoretic fractionation of the polypeptides in the particulate fractions of EHDV- and BTV-infected cells

Hybridization between the dsRNA genome of EHDV and BTV

Experiments to determine the percentage of cross-hybridization were carried out by hybridizing constant amounts of ^{3}H-labelled BTV and EHDV ssRNA with increasing amounts of either BTV or EHDV dsRNA. The percentage hybridization of BTV and EHDV ssRNA with homologous dsRNA were normalized to 100%. The actual percentage varied between 60% and 90% but was constant for each ssRNA preparation. The same normalization constant was used in calculating the percentage heterologous cross-hybridization. A typical result is shown in Fig. 7.

There was about 5–10% cross-hybridization between BTV and EHDV. The basic experiment was repeated several times, with the same result. An analysis of the different hybridization products on polyacrylamide gels was also carried out, but neither the hybridization of EHDV mRNA with BTV dsRNA nor the reverse type hybridization yielded any hybrid dsRNA segments within the size range of normal genome dsRNA. Analysis of 1 of the homologous hybridization products (EHDV mRNA plus EHDV dsRNA) is shown in Fig. 8.

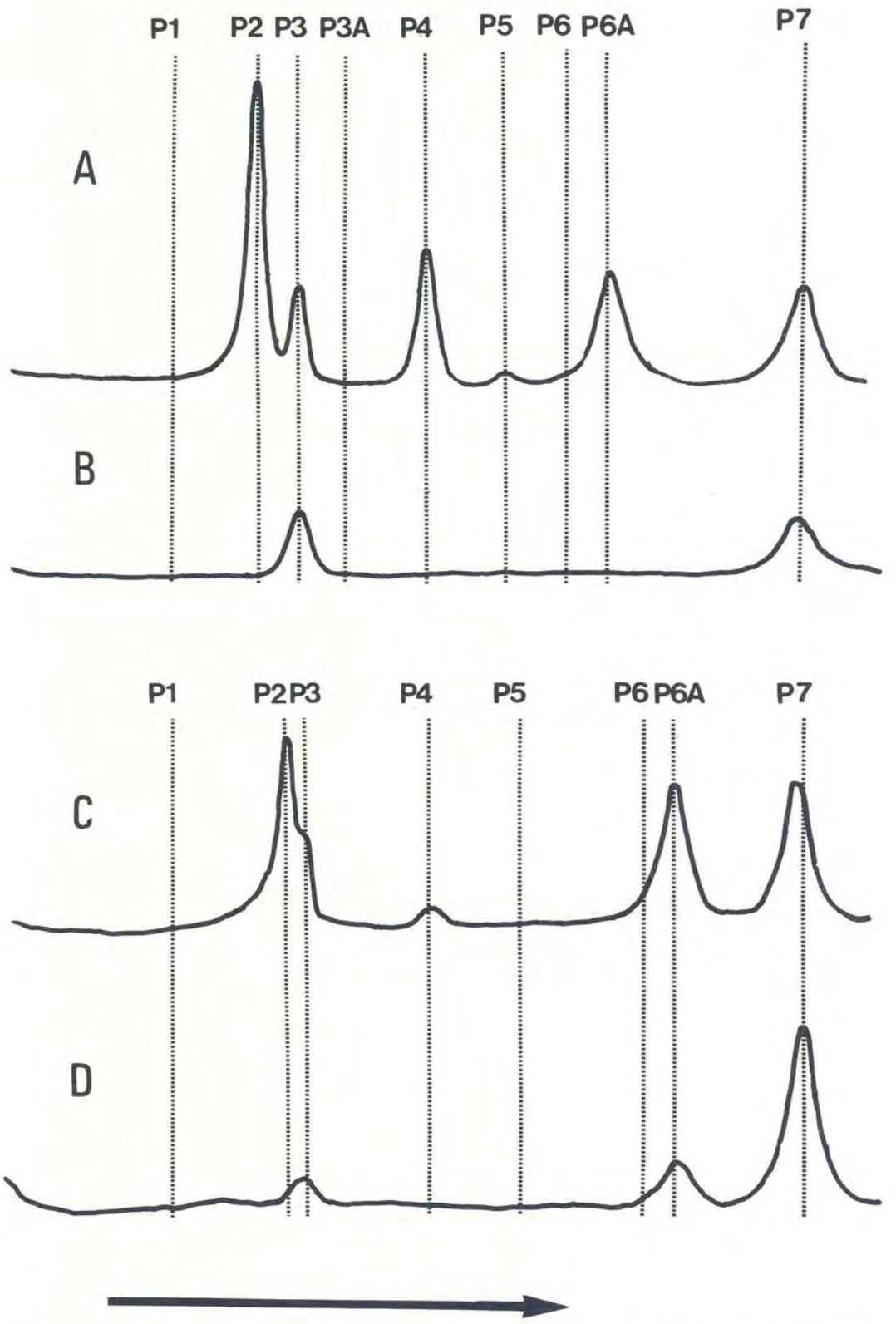

FIG. 6 Gelelectrophoretic analysis of homologous and heterologous immune precipitates of ^{14}C-labelled BTV and EHDV proteins with guinea pig antisera against these viruses. The autoradiogram scans of the immune precipitation of EHDV proteins with EHDV antiserum and with BTV antiserum are shown in (A) and (B) respectively and compared with the immune precipitation of BTV proteins with BTV antiserum (C) and with EHDV antiserum (D)

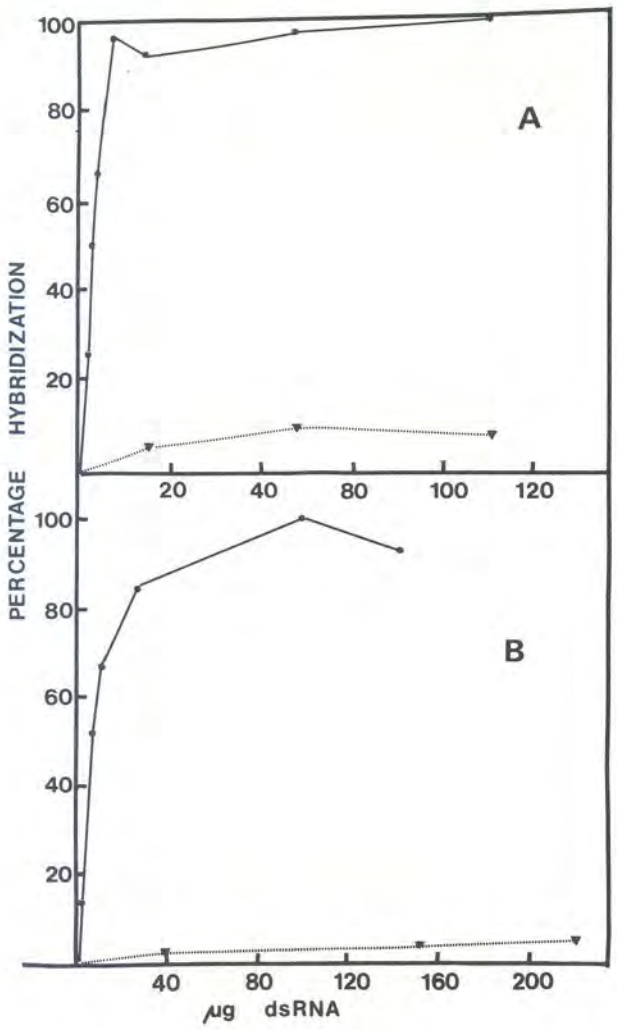

FIG. 7 A: Hybridization of EHDV ssRNA with increasing amounts of EHDV dsRNA (——●——) and BTV dsRNA (----------▼----------)

B: Hybridization of BTV ssRNA with increasing amounts of BTV dsRNA (——●——) and EHDV dsRNA (----------▼----------). Percentage hybridization was normalized to a 100% for the homologous hybridization

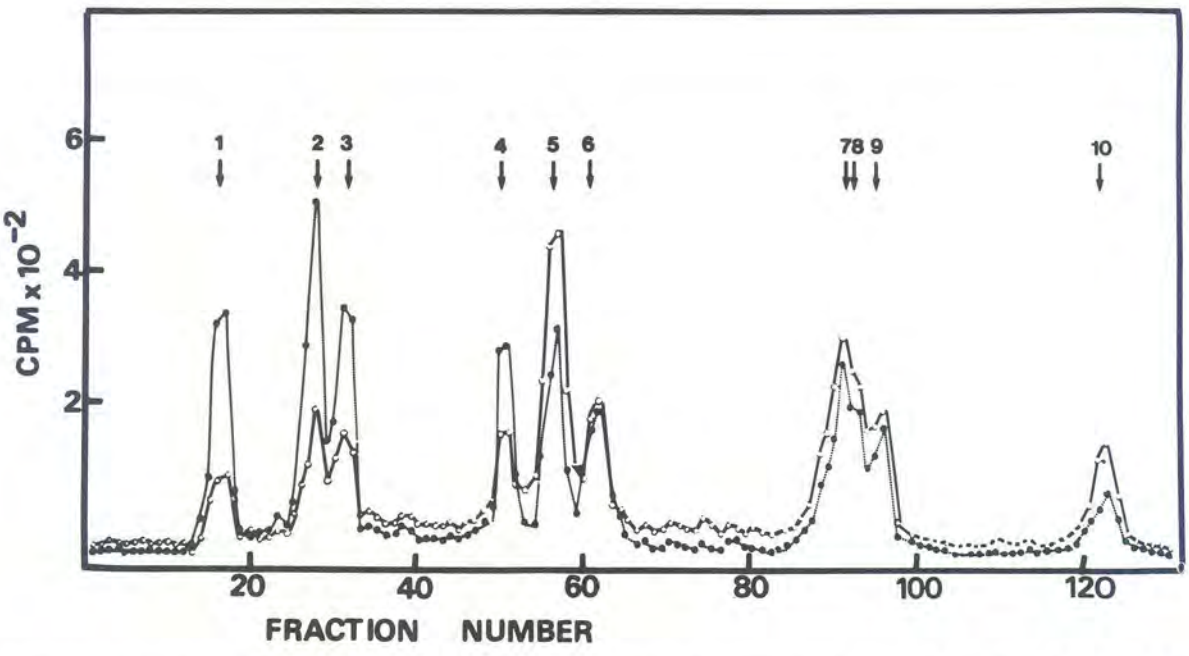

FIG. 8 Gelelectrophoretic analysis of the hybridization product of ^{3}H-labelled EHDV ssRNA and ^{32}P-labelled EHDV dsRNA. ^{3}H-label (——O——O——) ^{32}P label (---------●---------●---------)

The ^{3}H-labelled dsRNA hybrid was indistinguishable from normal EHDV dsRNA, indicating full transcription of all the EHDV dsRNA segments. However, since segments 8 and 7 were not fully resolved, a definitive conclusion as to the synthesis of these 2 mRNA species could not be made.

Discussion

In many respects EHDV resembles BTV. The 2 viruses are morphologically indistinguishable and both contain 10 segments of dsRNA with a very similar size distribution. The EHDV capsid consists of 4 major and 4 minor polypeptide components and differs in this respect from BTV, which has 1 less minor polypeptide.

The additional EHDV polypeptide, P3A, is part of the outer capsid layer of the virus. Virus particles with all 8 capsid polypeptides band on CsCl gradients at a density of 1,36 g/mℓ. EHDV core particles with a density of 1,40 g/mℓ lack the major capsid polypeptides P2 and P5 as well as the minor component P3A.

While EHDV is stable on CsCl gradients of pH 7,0, BTV loses its outer capsid layer under those conditions (Verwoerd *et al.,* 1972), a result that was confirmed recently under exactly the same conditions as the EHDV experiments were conducted under (Van Dijk & Huismans, unpublished observation). It is tempting to speculate that the greater stability of EHDV in CsCl is due to additional minor polypeptide P3A in the outer capsid layer.

The CsCl densities of the different EHDV particles differ from the published values for the corresponding BTV particles (Verwoerd & Huismans, 1972; Martin & Zweerink, 1972). The densities of the BTV particles were therefore re-investigated and found to correspond to those of EHDV (Van Dijk & Huismans, unpublished observation).

Although polypeptide P3A was always found to be associated with EHDV, except with the core particles, some variation in its relative amount in different virus preparations was observed. This might be explained by the possibility that it is more loosely associated with the particle than the other outer capsid polypeptides. The fact that P3A is not precipitated from soluble protein extracts of EHDV-infected cells by EHDV antiserum could indicate that this polypeptide has a low antigenicity, but this could also be explained if P3A is only present in soluble form in very small amounts. There is also no proof as yet that P3A is a primary genome product and it could well be a clevage product of one of the larger capsid polypeptides.

Two non-capsid polypeptides of EHDV were identified in infected cells. One of these, P5A, the main polypeptide component of the tubular structures associated with EHDV infection (Huismans & Els, 1979), is synthesized in excess of any of the other polypeptides. Little is known about the other non-capsid polypeptide, P6A. It is possible that it is a modified (for example, phosphorylated) form of 1 of the capsid polypeptides. Such a possibility is at present under investigation.

One of the aims of the investigation was to study the reported immunological relationship between BTV and EHDV and to determine if any of the corresponding capsid polypeptides of the 2 viruses have common antigenic determinants. It was found that an antiserum against BTV can precipitate EHDV polypeptides P3 and P7, while EHDV antiserum precipitates mainly BTV polypeptide P7, thus suggesting that the major core polypeptides of the 2 viruses (P7 and P3) have common antigenic determinants. This result could explain the observed reaction in serological tests and substantiates the finding (Huismans & Erasmus, unpublished observation) that P7 is the main complement fixing antigen of BTV.

There is, no evidence, however, that the genome segments of BTV and EHDV, which code for P7 or the other polypeptides, are homologous. The percentage homology between the dsRNA of the 2 viruses is low and varies between 5% and 10%. No dsRNA hybrids between the 2 viruses could be recovered.

The transcription pattern of EHDV (Fig. 8) is remarkably similar to that of BTV (Huismans & Verwoerd, 1973). The observation that genome segment 5 is transcribed at a higher rate than the other genome segments was of special interest. A similar finding with BTV led to the suggestion that segment 5 codes for non-capsid polypeptide P5A, which is also synthesized in large excess in BTV-infected cells (Huismans, 1979).

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References

ANDERSON, M., CAWSTON, T. & CHEESEMAN, G. C., 1974. Molecular-weight estimates of milk-fat-globule-membrane protein-sodium dodecyl sulphate complexes by electrophoresis in gradient acrylamide gels. *Biochemical Journal,* 139, 653–660.

BARBER, T. L. & JOCHIM, M. M., 1975. Serotyping bluetongue and epizootic hemorrhagic disease virus strains. 18th *Annual Proceedings of the American Veterinary Laboratory Diagnosticians,* 149–162.

BORDEN, E. C., SHOPE, R. E. & MURPHY, F. A., 1971. Physicochemical and morphological relationships of some arthropod-borne viruses to bluetongue virus—a new taxonomic group. Physicochemical and serological studies. *Journal of General Virology,* 13, 261–271.

HUISMANS, H., 1979. Protein synthesis in bluetongue virus-infected cells. *Virology,* 92, 385–396.

HUISMANS, H. & ELS, H. J., 1979. Characterization of the tubules associated with the replication of three different orbiviruses. *Virology,* 92, 397–406.

HUISMANS, H. & HOWELL, P. G., 1973. Molecular hybridization studies on the relationships between different serotypes of bluetongue virus and on the difference between the virulent and attenuated strains of the same serotype. *Onderstepoort Journal of Veterinary Research,* 40, 93–104.

HUISMANS, H. & VERWOERD, D. W., 1973. Control of transcription during the expression of the bluetongue virus genome. *Virology,* 52, 81–88.

JOCHIM, M. M., 1976. Improvement of the AGP test for bluetongue. 19th *Annual Proceedings of the American Association of Veterinary Laboratory Diagnosticians,* 361–376.

JOCHIM, M. M., BARBER, T. L. & BANDO, B. M., 1974. Identification of bluetongue and epizootic hemorrhagic disease viruses by the indirect fluorescent antibody procedure. 17th *Annual Proceedings of the American Association of Veterinary Laboratory Diagnosticians,* 91–103.

JOKLIK, W. K., 1974. Reproduction of Reoviridae. *In: Comprehensive Virology,* 2 (H. Fraenkel-Conrat & R. R. Wagner, ed.) pp. 231–334. New York: Plenum Press.

KONTOR, E. J. & WELCH, A. B., 1976. Characterization of an epizootic haemorrhagic disease virus. *Research in Veterinary Science,* 21, 190–196.

MARTIN, S. A. & ZWEERINK, H. J., 1972. Isolation and characterization of two types of bluetongue virus particles. *Virology,* 50, 495–506.

MOORE, D. L., 1974. Bluetongue and related viruses in Ibadan, Nigeria: Serologic comparison of bluetongue, epizootic hemorrhagic disease of deer, and Abadina (Palyam) viral isolates. *American Journal of Veterinary Research*, 35, 1109–1113.

MOORE, D. L. & LEE, V. H., 1972. Antigenic relationship between the virus of epizootic haemorrhagic disease of deer and bluetongue virus. *Archiv für die gesamte Virusforschung*, 37, 282–284.

SCHUERCH, A. R. & JOKLIK, W. K., 1973. Temperature-sensitive mutants of reovirus. IV. Evidence that anomalous electrophoretic migration behaviour of certain double-stranded RNA hybrid species is mutant group-specific. *Virology*, 56, 218–229.

SHOPE, R. E., MacNAMARA, L. G. & MANGOLD, R., 1955. Report on the deer mortality—epizootic hemorrhagic disease of deer. *New Jersey Outdoors*, 6, 17–21.

SHOPE, R. E., MacNAMARA, L. G. & MANGOLD, R., 1960. A virus-induced epizootic hemorrhagic disease of the Virginia white-tailed deer (*Odocoileus virginianus*). *Journal of Experimental Medicine*, 111, 155–170.

STONE, K. R., SMITH, R. E. & JOKLIK, W. K., 1974. Changes in membrane polypeptides that occur when chick embryo fibroblasts and NRK cells are transformed with avian sarcoma viruses. *Virology*, 58, 86–100.

THOMAS, F. C. & MILLER, J., 1971. A comparison of bluetongue virus and EHD virus: electronmicroscopy and serology. *Canadian Journal of Comparative Medicine*, 35, 22–27.

TSAI, K. S. & KARSTAD, L., 1973a. Ultrastructural characterization of genome of epizootic hemorrhagic disease virus. *Infection and Immunity*, 8, 463–474.

TSAI, K. S. & KARSTAD, L., 1973b. The pathogenesis of epizootic hemorrhagic disease of deer: an electron microscopic study. *American Journal of Pathology*, 70, 379–391.

VERWOERD, D. W., 1969. Purification and characterization of bluetongue virus. *Virology*, 38, 203–212.

VERWOERD, D. W. & HUISMANS, H., 1969. On the relationship between bluetongue, African horsesickness, and reoviruses: Hybridization studies. *Onderstepoort Journal of Veterinary Research*, 36, 175–180.

VERWOERD, D. W. & HUISMANS, H., 1972. Studies on the *in vitro* and the *in vivo* transcription of the bluetongue virus genome. *Onderstepoort Journal of Veterinary Research*, 39, 185–192.

VERWOERD, D. W., LOUW, H. & OELLERMANN, R. A., 1970. Characterization of bluetongue virus ribonucleic acid. *Journal of Virology*, 5, 1–7.

VERWOERD, D. W., ELS, H. J., DE VILLIERS, E. M. & HUISMANS, H., 1972. Structure of the bluetongue virus capsid. *Journal of Virology*, 10, 783–794.

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