

The Nucleic Acid of Infectious Bronchitis Virus

By

G. A. TANNOCK

Viral Products Section, National Biological Standards Laboratory,
Parkville, Australia

With 8 Figures

Received August 6, 1973

Summary

The nucleic acid of infectious bronchitis virus (IBV), like that of other enveloped viruses, consists of discontinuous single stranded RNA. However, unlike many other viruses, there is extreme heterogeneity in the sizes of the RNA fragments, as revealed by centrifugation in sucrose gradients or electrophoresis in polyacrylamide gels. Two principal classes of RNA fragments are present:

a) A larger class comprising 74.9—85.4 per cent of total RNA and consisting of fragments having molecular weights ranging from 0.5×10^6 to considerably greater than 3.0×10^6 daltons and,

b) A smaller class comprising 9.1—19.7 per cent of total RNA with the size approximately that of ribosomal 4S RNA.

All IBV RNA's were fully susceptible to ribonuclease and had a buoyant density in caesium sulphate identical to that of tobacco mosaic virus RNA. No difference in the RNA profile for IBV was observed from the use of different methods of virus purification. The single-stranded RNA's of poliovirus and tobacco mosaic virus remained undegraded after preparation in the presence of IBV.

1. Introduction

Infectious bronchitis virus (IBV), mouse hepatitis virus and certain human respiratory viruses have been classified as coronaviruses (ALMEIDA *et al.*, 1968). The particles of IBV are 80—120 nm in diameter and contain a thread-like internal component 7—8 nm in cross-section (APOSTOLOV *et al.*, 1970). They differ markedly from myxoviruses in having an envelope derived from the cytoplasmic reticulum instead of the cell membrane (BECKER *et al.*, 1967) and club-like projections 15—20 nm in length, projecting from the envelope, which give to the virion its characteristic halo- or corona-like appearance (BERRY *et al.*, 1964). No outer spiky layer containing haemagglutinin or neuraminidase subunits has been demonstrated for the IBV virion (APOSTOLOV *et al.*, 1970).

The nucleic acid of IBV has not been previously isolated but, from experiments with analogue inhibitors (CUNNINGHAM, 1963) is generally considered to be RNA. Other enveloped RNA viruses of similar size such as influenza (DUESBERG and ROBINSON, 1967) lymphocytic choriomeningitis (PEDERSON, 1970) and Rous sarcoma virus (BISHOP *et al.*, 1970), have been shown to contain several pieces of single-stranded RNA. The work reported here shows that the RNA of IBV is also fragmented, but the size distribution of the fragments differs from that of other groups.

2. Materials and Methods

2.1. Solutions and Reagents

The following buffers were used: NET 0.1 M NaCl, 0.01 M EDTA, 0.01 M Tris pH 7.4, Loening's electrophoresis buffer — 0.003 M NaH_2PO_4 , 0.001 M EDTA, 0.036 M Tris pH 7.8 (LOENING, 1969) containing 0.2 per cent sodium dodecyl sulphate (SDS; MATHESON, COLEMAN, and BELL), Phosphate Buffered Saline (DULBECCO and VOGT, 1954).

Other reagents included deoxyribonuclease, pancreatic ribonuclease-A, (5 \times crystallised, Sigma), Biogel P-30 (Calbiochem) and diethyl pyrocarbonate (Fluka). Ribonuclease-free sucrose (Sigma) was used in gradient preparations.

Acrylamide and bisacrylamide (Eastman Organic) were purified according to LOENING (1967), and prepared at 2.2 per cent and 0.15 per cent, respectively, in electrophoresis buffer. Polymerisation was accomplished with 0.2 per cent N-N'-N'-tetramethylethylenediamine and 1 per cent ammonium persulphate. Phenol (Merek reagent grade) was twice distilled for RNA extraction. Bentonite was prepared according to the method of FRAENKEL-CONRAT *et al.* (1961). Yeast carrier RNA (Sigma) was purified by two extractions with phenol/SDS at 37° C (see below) followed by the addition of sodium acetate to 0.15 M and two volumes of ethanol. After 1 hour at -15° C, the precipitate was collected by centrifugation, dissolved in NET, reprecipitated and adjusted to 1000 $\mu\text{g}/\text{ml}$ in NET for storage at -15° C.

Carrier-free ^{32}P orthophosphate was obtained from the Australian Atomic Energy Commission, Lucas Heights, Sydney and ^3H uridine (20—30 Ci/m Mole) from the Radiochemical Centre, Amersham U.K.

2.2. Preparation of Viral and RNA Markers

^3H tobacco mosaic virus, ^3H poliovirus and ^3H (BHK cell) ribosomal RNA were prepared by the method of TANNOCK *et al.* (1970), using ^3H uridine, unlabelled purified influenza strain BEL by that of TAYLOR *et al.* (1969). All preparations were equilibrated with NET buffer by passing through Biogel P-30 columns prepared in NET before use.

2.3. Acrylamide Gel Electrophoresis

This was carried out using 2.2 per cent polyacrylamide gels, whose preparation is described above. Fractionation was according to the method of TANNOCK *et al.* (1970).

2.4. Preparation of ^{32}P IBV

Thirty 8 day old chick embryos were each inoculated with 1 millicurie of ^{32}P orthophosphate and, after 24 hours, with 10^3 — 10^4 EID₅₀ of the Victorian S vaccine strain of IBV (35th embryo passage) by the allantoic route. The IBV preparation was obtained from Dr. W. A. Geering of Commonwealth Serum Laboratories, Melbourne. Incubation was continued for a further 48 hours at 35° C before the embryos were chilled and the allantoic fluid collected.

2.5. Virus Purification

This was accomplished by a modification of the method of BLAIR and DUESBERG (1970) which was used for purification of myxoviruses. Calf serum to 5 per cent was added to chilled allantoic fluid, followed by an equal volume of neutral saturated

ammonium sulphate. The mixture was stirred at 0° C for 30 minutes and the precipitate then collected by centrifugation at 2000*g* for 20 minutes and resuspended in approximately 25 mls of NET buffer. The virus was first concentrated by centrifuging onto a 2 ml 2.3 M sucrose cushion using a Spinco SW 25.1 rotor for 35 minutes at 22,000 r. p. m. It was then partially purified by further centrifuging onto a similar 0.5 ml cushion through a 1.5 ml 15 per cent sucrose interface using a Spinco SW 40 rotor at 22,000 r.p.m. for 35 minutes. Final purification was achieved by the use of isopycnic centrifugation. Material from the interface was centrifuged either (A) through a 26.6 ml 15—65 per cent sucrose gradient in NET at 22,000 r. p. m. for 16 hours using a Spinco SW 25.1 rotor or, (B) through an 11 ml 20—40 per cent potassium tartrate gradient for 2.5 hours at 35,000 r.p.m. using a Spinco SW 40 rotor.

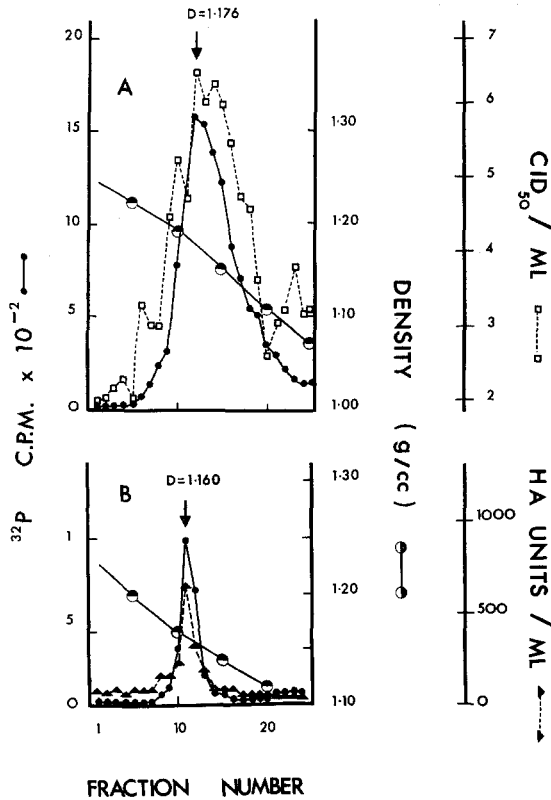


Fig. 1. Gradient purification of ³²P IBV

A. Concentrates of allantoic grown ³²P IBV, after partial purification were resuspended in NET and centrifuged through a 26.6 ml 15—65 per cent sucrose gradient for 16 hours at 22,000 r.p.m. using a Spinco SW 25.1 rotor. The distributions of acid insoluble radioactivity (—●—), infectivity (---□---) and density (—●—) are shown

B. Partially purified concentrates of ³²P IBV were mixed with 0.5 ml of unlabelled influenza strain BEL and 1.0 ml of NET and centrifuged through an 11 ml 20—40 per cent potassium tartrate gradient for 2.5 hours at 35,000 r.p.m. using a Spinco SW 40 rotor. The distributions of acid-insoluble radioactivity (—●—), haemagglutinin (---▲---) and density (—●—) are shown

Where sucrose gradients were used, fractions of 1.0 ml were collected from the bottom and the profiles for acid-insoluble radioactivity, density and infectivity are shown in Figure 1A. Fraction 12 representing the peak of infectivity and radioactivity had a density, determined from its refractive index, of 1.176 g/cc. Fractions 8—19, representing the common peak of radioactivity and infectivity were pooled for use in

RNA studies. Virus appeared as a flocculent band and sucrose was removed by passing the pooled fractions through a Biogel P-30 column equilibrated with NET. The identity of the virus material can be seen from the electron micrograph in Figure 2 showing a number of negatively-stained pleomorphic particles with characteristic club-like projections. The micrograph was prepared by Mr. J. E. Peterson, Division of Animal Health, C.S.I.R.O., Parkville, Victoria using an Hitachi 11B electron microscope.

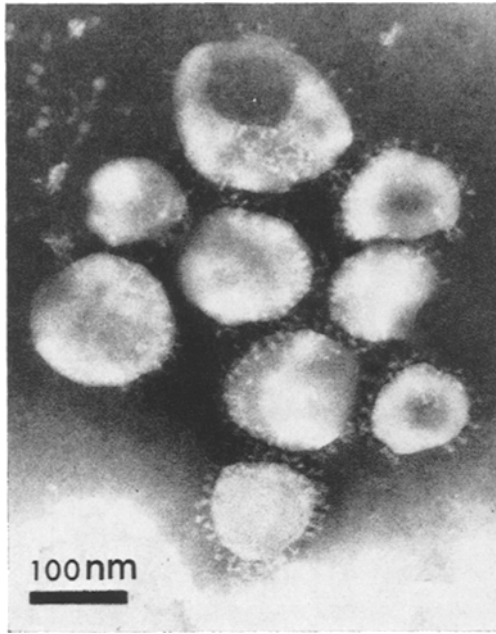


Fig. 2. Electron micrograph of gradient-purified IBV virions. Fractions 8—19 from the gradient in Figure 1A were pooled and negatively stained with potassium phosphotungstate. Magnification $\times 128,000$.

In an experiment to compare the buoyant densities of IBV and influenza virus strain BEL, 0.2 ml of partially purified IBV was mixed with 0.5 ml of unlabelled BEL virus and 1.0 ml of NET and centrifuged as described above in potassium tartrate gradients. Fractions of 0.5 ml were collected and the density of each determined from its refractive index. The profiles of radioactivity, haemagglutinin and density are shown in Figure 1B. As untreated IBV preparations have no haemagglutinin, the buoyant density of each virus is identical at 1.16 g/cc. Although a sharper resolution of virus was afforded than for sucrose gradients (Fig. 1), the use of potassium tartrate gradients for IBV purification usually resulted in a considerable loss in infectivity and, except where stated, virus purified in this manner was not used for RNA studies.

Several unsuccessful attempts were made to further purify IBV by velocity gradient centrifugation. Virus concentrates, either before or after isopycnic centrifugation, were centrifuged for 30 minutes at 12,000 r.p.m. through 26.6 ml 5—20 per cent sucrose gradients using a Spinco SW 25.1 rotor. The gradients were prepared in distilled water, NET or phosphate buffered saline, but on each occasion all virus in the suspension was deposited on the base of the tube as a fast sedimenting aggregate.

2.6. Infectivity Titration

Infectivity was determined either

a) in eggs according to the capacity of IBV to induce stunting, curling or death in infected 9—10 day old chick embryos (ANON., 1963) or,

b) from its capacity to inhibit ciliary activity (titres expressed as CID_{50}/ml) in chicken embryo tracheal organ cultures (CHERRY and TAYLOR-ROBINSON, 1970). The latter procedure, while more reliable, had a sensitivity approximately one tenth that of the egg titration.

2.7. RNA Extraction

The following procedure was used: Approximately 2–3 ml of purified ^{32}P IBV in NET buffer was shaken for 10 minutes at $37^{\circ}C$ with an equal volume of phenol containing 1 per cent SDS and 0.1 per cent bentonite. After centrifugation for 10 minutes at $2000g$, the upper aqueous phase was removed and the extraction procedure repeated. To the extract was added suitable marker or carrier RNA's followed by two volumes of ethanol and sodium acetate to 0.15 M. A precipitate was allowed to form at $-15^{\circ}C$ for 1 hour and was collected by centrifuging at 10,000 r.p.m. for 10 minutes and was finally resuspended in either NET or electrophoresis buffer.

An extract was obtained from a large batch of purified virus which had been purified in sucrose-density gradients from an initial volume of approximately 1 litre of infectious allantoic fluid. An ethanol precipitate was obtained from the extract, as described above, but without the addition of carrier or marker RNA. The precipitate was then collected and dissolved in 1 ml of NET buffer and its absorbance profile is shown in Figure 3. Values of $OD_{254/280}$ and $260/280$ of 1.20 and 1.80, respectively, were obtained, which clearly indicates that the extracted material contained RNA.

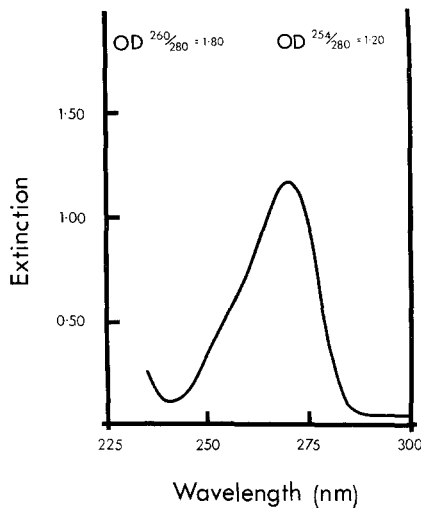


Fig. 3. UV adsorbance profile of IBV RNA

RNA was extracted from a large batch of purified unlabelled IBV and, after ethanol precipitation, was dissolved in 1.0 ml NET buffer. Adsorbance was determined in a Unicam SP.500 spectrophotometer, using NET for the blank determination

3. Results

3.1. Sucrose Gradient Analysis of IBV Genetic Material

This experiment was intended to confirm the genetic material of IBV as RNA and to characterize it. RNA was extracted from three IBV preparations purified in either sucrose (A and C) or potassium tartarate density gradients (B). In preparation A, the initial allantoic fluid was first treated with deoxyribonuclease and ribonuclease (each at $1 \mu g$ per ml) for 1 hour at $25^{\circ}C$ before purification. 3H ribosomal marker RNA ($0.2 ml$) and $100 \mu g$ of yeast carrier RNA were added and

total RNA's from each were precipitated, as described above, and resuspended in 0.5 ml NET buffer. Each preparation was then centrifuged through pre-formed 4.8 ml 5—20 per cent sucrose gradients (A, B and C) prepared in NET for 60 (C) or 90 minutes (A and B) at 65,000 r.p.m. using a Spinco SW 65 rotor. Fractions of 0.2 ml were collected, placed onto paper strips and the acid-insoluble radioactivity present was determined. The profile for each gradient is shown in Figure 4. As a

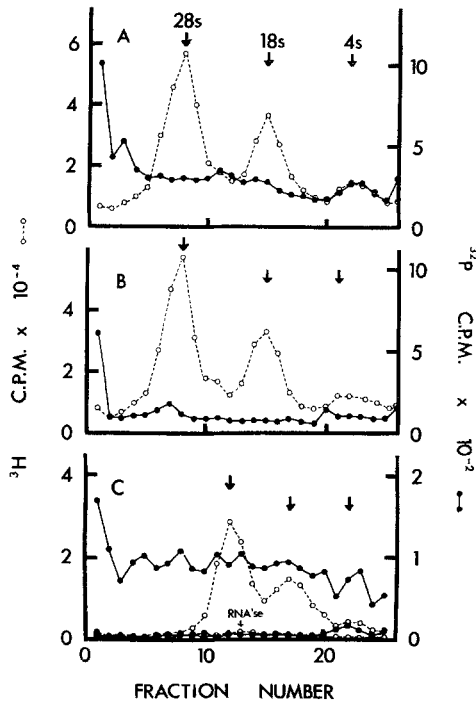


Fig. 4. Sucrose-gradient analysis of ^{32}P IBV and ^3H ribosomal RNA.

RNA was extracted from ^{32}P IBV, purified after centrifugation in either sucrose—(A and C) or potassium tartrate—(B) density gradients, as described in section 2.7. The initial allantoic fluid used to prepare sample A was treated for 1 hour at 25° C with RNA'se or DNA'se prior to purification. Yeast carrier RNA (100 μg) and ^3H ribosomal marker RNA (0.2 ml) were added and the total RNA in each mixture precipitated with ethanol and suspended in 0.5 ml NET. Each RNA preparation was centrifuged through 4.8 ml 5—20 per cent sucrose gradients in NET at 65,000 r.p.m. for 60 (C) or 90 minutes (A and B) using a Spinco SW 65 rotor at 7° C. The distributions of acid-insoluble IBV RNA (—●—) and ribosomal RNA (—○—) are shown. One half of each fraction from gradient C was first treated with 1 μg of ribonuclease for 30 minutes at 25° C.

test of identity for the viral nucleic acid, one half of each fraction from gradient C was treated with 0.1 ml of an aqueous solution containing 1 μg of ribonuclease for 30 minutes at 25° C and the acid-insoluble radioactivity remaining was then determined.

Irrespective of the purification procedure used, the results indicate that the nucleic acid of IBV is extremely heterogeneous, being comprised of a range of species spread throughout the gradients. Because of its sensitivity to ribonuclease (C) and UV absorbance profile (Fig. 3), the genetic material of IBV is undoubtedly RNA and further confirmation is provided by its buoyant density in caesium sulphate (see Fig. 8).

3.2. Analysis of IBV RNA by Polyacrylamide Gel Electrophoresis

The pattern obtained for IBV RNA in sucrose gradients was then compared with one obtained after electrophoresis in polyacrylamide gels. A mixture of ^{32}P IBV RNA, ^3H ribosomal RNA and 100 μg of yeast carrier RNA was prepared as described in the previous experiment. RNA was extracted from purified IBV and the final ethanol precipitate dissolved in 50 μl of Loening's buffer. Electrophoresis of the preparation was carried out in 2.2 per cent polyacrylamide gels at 70 volts and 6 mA per tube for 1.25 (A) and 2.5 hours (B).

The resulting electropherograms in Figure 5 again indicate considerable heterogeneity for IBV RNA. Two principal classes of RNA appear to be present:

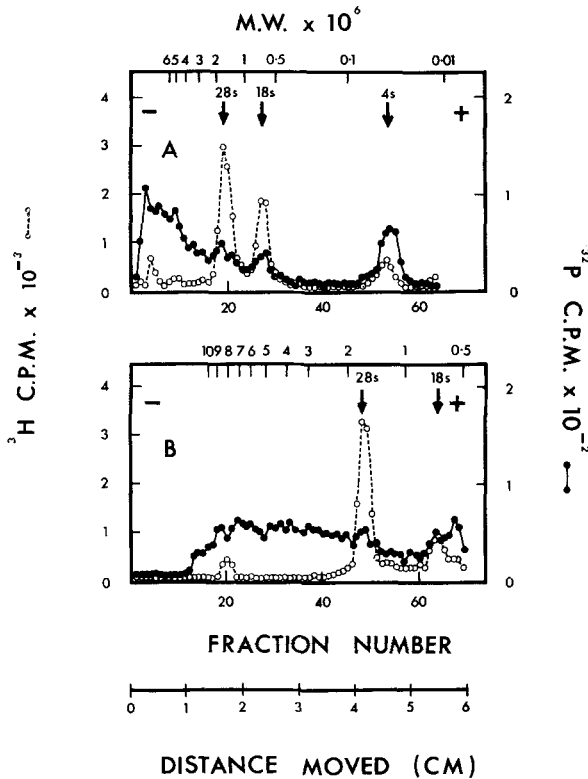


Fig. 5. Polyacrylamide gel electrophoresis of ^{32}P RNA and ^3H ribosomal RNA

RNA was extracted from ^{32}P IBV and mixed with 100 μg of yeast carrier and 0.2 ml of ^3H ribosomal RNA. Total RNA was precipitated with ethanol, resuspended in 50 μl of Loening's di-sodium phosphate buffer and submitted to electrophoresis for (A) 1.25 and (B) 2.5 hours at 70 volts and 6 mA per tube in 2.2 per cent polyacrylamide gels. The distributions of IBV (—●—) and ribosomal RNA (---○---) are shown

1. A much larger and more heterogeneous class comprising 74.9 per cent of total RNA (Fractions 1—35) whose fragments range in size from approximately 0.5×10^6 to well in excess of 3.0×10^6 daltons [the relationship between molecular weight and electrophoretic mobility is inexact for RNA molecular weights in excess of 3.0×10^6 (LOENING *et al.*, 1969)] and,

2. A smaller more homogeneous class comprising 19.7 per cent of total RNA (Fractions 47–62), having an electrophoretic mobility identical with that of 4S RNA.

The absence of two distinct RNA classes in Figure 4 can be accounted for by the relatively poor resolving properties of sucrose gradients compared with polyacrylamide gels. In an additional two electropherograms (C and D) a similar distribution of RNA fragment sizes was apparent and figures obtained for the RNA classes are as follows: C, high molecular weight RNA fragment 75.6 per cent and low molecular weight RNA 15.8 per cent and D high molecular weight 85.4 per cent and low molecular weight 9.1 per cent of total RNA.

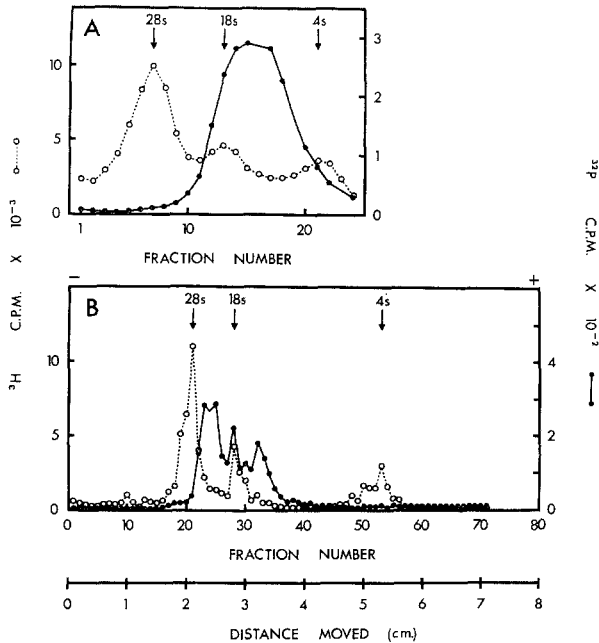


Fig. 6. Analysis of influenza strain PR8 RNA, prepared from purified virus under the same conditions as for IBV. PR8 virus was grown in eggs in the presence of ^{32}P and purified in the same manner as for IBV using potassium tartrate gradients. RNA was extracted from two samples of purified virus as described under Materials and Methods and mixed with 100 μg of yeast carrier and 0.2 ml of ^3H ribosomal RNA. Total RNA in the mixtures was precipitated with ethanol and resuspended in either 0.2 ml of NET (A) or 50 μl of Loening's electrophoresis buffer (B). Sample A was centrifuged through 4.8 ml 5–20 per cent sucrose gradients, prepared in NET, at 65,000 r.p.m. for 90 minutes using a Spinco SW 65 rotor at 7°C. Sample B was submitted to electrophoresis for 1.25 hours at 70 volts and 6 mA per tube in 2.2 per cent polyacrylamide gels. The distributions of PR8 (—●—) and ribosomal RNA (—○—) are shown.

As a further check on the experimental procedures used, a preparation of influenza strain PR8 was grown in eggs in the presence of radioactive orthophosphate and purified as described for IBV in section 2.5. RNA's present were extracted under similar conditions and were examined in the presence of ^3H uridine marker RNA by:

a) centrifugation through 5–20 per cent sucrose gradients prepared in NET for 1.5 hours at 65,000 r.p.m. using a Spinco SW65 rotor or

b) electrophoresis for 1.25 hours in 2.2 per cent polyacrylamide gels. The results in Figure 6 indicate a profile very similar to that obtained by DUESBERG (1968) for PR8 RNA.

3.3. Extraction of RNA from IBV in the Presence of Other Single-Stranded RNA Viruses

Discontinuity in the RNA of IBV may have been caused by the activity of nucleases released during extraction, or by the breakage of a large RNA molecule at specific weak points. In an examination of these possibilities, the RNA profiles of IBV and either poliovirus or tobacco mosaic virus (TMV) were compared after extraction together. Two mixtures consisting of 0.6 ml of ^{32}P IBV and 0.6 ml of purified

- a) ^3H poliovirus, or,
- b) ^3H TMV were diluted to 3.0 ml with NET buffer.

RNA was then extracted from each and precipitated with ethanol after the addition of 100 μg of yeast carrier RNA. Each RNA precipitate was suspended in 0.5 ml of NET and centrifuged through pre-formed 4.8 ml 5–20 per cent sucrose gradients, prepared in NET for 1 hour at 65,000 r. p. m. using a Spinco SW 65 rotor.

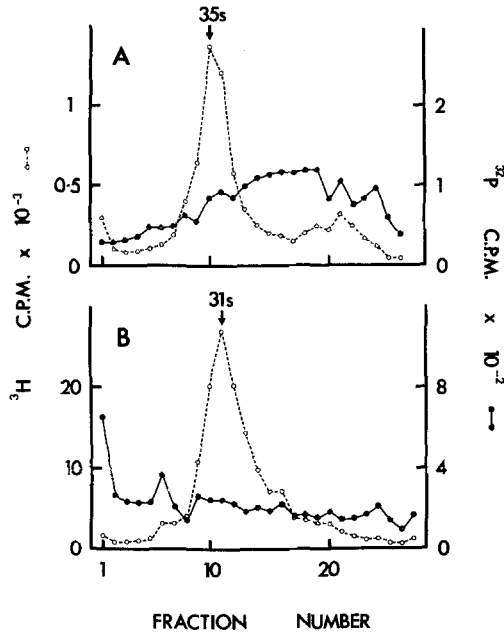


Fig. 7. Extraction of IBV RNA in the presence of poliovirus and TMV

RNA was extracted from mixtures of ^{32}P IBV and either (A) ^3H poliovirus or (B) ^3H TMV and precipitated with ethanol after the addition of yeast carrier RNA. Each RNA precipitate was resuspended in 0.5 ml of NET and centrifuged through 4.8 ml 5–20 per cent sucrose gradients in NET for 1 hour at 65,000 r. p. m. using a Spinco SW 65 rotor at 7° C (A) or 16° C (B). The distributions of acid-insoluble IBV (—●—) and either poliovirus or TMV RNA (---○---) are shown

Fractions of 0.2 ml were collected and profiles for acid-insoluble radioactivity are shown in Figure 7. They again suggest that IBV RNA is extremely heterogeneous being spread throughout the gradient as in Figure 4. Both poliovirus and TMV RNA appear to be present as a single undegraded species. This suggests that the extraction method used does not release virion nucleases nor degrade IBV RNA which, therefore, is presumably present within the virion as a discontinuous structure.

3.4. Equilibrium Centrifugation of IBV RNA in Caesium Sulphate

The buoyant density of IBV RNA was compared with that of TMV RNA. A mixture of both RNA's was prepared as described previously and, after ethanol precipitation, was suspended in 0.4 ml of NET buffer. The preparation was then layered onto 2.5 ml of a solution of caesium sulphate (density 1.64 g/cc) and centrifuged at 33,000 r.p.m. for 65 hours at 20° C using a Spinco SW39 rotor. Fractions of 0.1 ml were collected and the density of each determined from its refractive index. Each fraction was then diluted to 0.4 ml with NET and an 0.2 ml volume placed onto a paper strip for the determination of acid-insoluble radioactivity. The remaining 0.2 ml of each fraction was treated with 1 μ g of ribonuclease for 1 hour at 25° C and radioactivity remaining then estimated in the same manner. The results shown in Figure 8, indicate that IBV RNA has a distribution in the equilibrium gradient identical with that of TMV RNA, having a peak at 1.65 g/cc, which is characteristic of other single-stranded RNA molecules (ERIKSON and FRANKLIN, 1966). This peak is greatly reduced in the presence of ribonuclease.

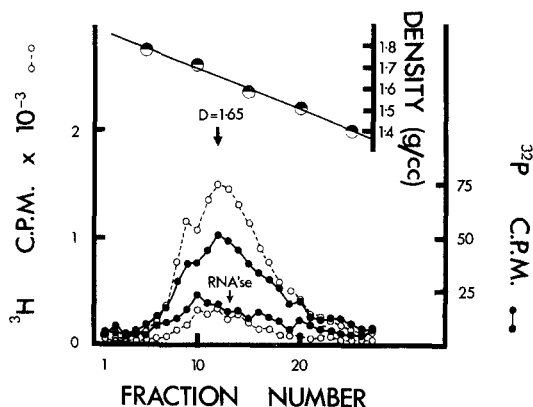


Fig. 8. Buoyant densities of IBV RNA and TMV RNA in caesium sulphate

A preparation of ^{32}P IBV RNA and ^3H TMV RNA was layered onto 2.5 ml of a solution of caesium sulphate (density 1.64 g/cc), and centrifuged for 65 hours at 33,000 r.p.m. at 20° C, using a Spinco SW39 rotor. Fractions of 0.1 ml were collected and diluted to 0.4 ml with NET, after determining their density. The distribution of acid-insoluble IBV RNA (—●—) and TMV RNA (—○—) is shown. One half of each diluted fraction from A was first treated with 1 μ g of ribonuclease for 30 minutes at 25° C

4. Discussion

Discontinuous single-stranded RNA is a feature of the genomes of influenza (DUESBERG and ROBINSON, 1967; PONS, 1967), lymphocytic choriomeningitis (PEDERSON, 1970), cowpea chlorotic mottle (BANCROFT *et al.*, 1968), alfalfa mosaic (HULL *et al.*, 1969) and cucumber mosaic (CIVEROLO *et al.*, 1969) viruses. With Rous sarcoma (RSV) (DUESBERG, 1968) and other leukoviruses, apparent continuity in the principal RNA components is maintained by regions of base pairing between RNA fragments, which are readily disrupted by gentle heating or the action of RNA denaturants. RSV contains, in addition, significant amounts of 4S RNA and smaller quantities of 7S, 18S and 28S RNA's (BISHOP *et al.*, 1970). A minor 4S RNA component has also been reported for Newcastle disease virus (NDV) RNA (DUESBERG and ROBINSON, 1965).

The RNA of IBV differs from all these viruses in that the great proportion of its RNA (74.9—85.4 per cent) consists of large, extremely heterogeneous fragments. Such an RNA profile has been obtained from all freshly prepared IBV preparations examined and from one prepared from virus after 72 hours of allantoic growth. With fowl plague RNA, prepared from virus purified after differing times of growth, differences in the sizes and amounts of various RNA classes have been described and a similar heterogeneity noted (BARRY *et al.*, 1970).

Heterogeneity in the high molecular weight RNA of IBV may reflect a degradative process, whereby a large molecule is broken down into smaller products, either mechanically at random weak points in the ribonucleoprotein component of the virion or through the action of nucleases. However, the following evidence is adduced against the observed distribution for IBV RNA being brought about in this manner:

a) The size of high molecular weight RNA fragments is such that a precursor molecule of size greater than 1×10^7 daltons would be required. Such a molecule would be larger than any known RNA and seems unlikely to be present within a virion of diameter 80—120 nm having a buoyant density of 1.16—1.17 g/cc.

b) No degradation was noted in the RNA's of poliovirus or TMV after preparation in the presence of IBV (Figure 6).

c) No diminution in the degree of fragmentation of RNA was observed when a freshly purified IBV preparation was shaken for 30 minutes with 3 per cent diethyl pyrocarbonate, a potent inhibitor of nuclease activity (SOLYMSY *et al.*, 1968) prior to RNA extraction.

The low molecular weight RNA of IBV has a molecular size comparable with that of cellular transfer RNA (Fig. 5A). Further knowledge of function would be provided by data on the base composition of each class IBV RNA and the degree of methylation of component bases.

The high molecular weight RNA's of IBV are comparable in size with the major 57S RNA of NDV and the 70S (non-covalently linked) RNA of RSV (DUESBERG, 1968) and AMV (ROBINSON and BALUDA, 1965), which are the largest known single-stranded RNA molecules. Messenger function for high molecular weight AMV RNA has been described by RIMAN *et al.* (1967), whereas that for NDV is known to be associated not with the major 57S RNA component, but with shorter complementary negative strands (BRATT and ROBINSON, 1967). Whether high molecular weight RNA or complementary strands act as the intracellular messengers for IBV cannot be determined from the present study.

It has been recently reported by HIERHOLZER *et al.* (1972) that at least 6 polypeptides are present within the virion of the related human coronavirus OC43, having molecular weights of 191,000, 104,000, 60,000, 47,000, 30,000 and 15,000 daltons. Occasionally, a seventh polypeptide with a molecular weight of 165,000 was also present. If values of 118.0 and 346.0 are assigned for the average molecular weights of individual amino acids and nucleotides, respectively, then an RNA molecule of molecular weight 3.92×10^6 daltons would be required to encode the genetic information for the first six polypeptides. If the seventh is also included, then the figure is 5.37×10^6 daltons. RNA fragments of this size range are clearly present within High Molecular Weight IBV RNA (Fig. 5).

The pattern of IBV RNA fragment sizes, as revealed by analysis in sucrose density gradients or polyacrylamide gels, suggests that considerable heterogeneity in the RNA content of individual virions must be present.

Were the particles homogeneous, representatives of each fragment size would have to be present and the total RNA complement for each virion would be well in excess of 20×10^6 daltons, which seems unlikely for a virion of the size and buoyant density of IBV.

Differences in the size of individual virions, which are apparent in Figure 2, are a characteristic of coronaviruses (ALMEIDA *et al.*, 1968). Such differences may reflect differences in the RNA content of individual particles whose buoyant densities may, in consequence, be relatively similar.

Heterogeneity in particle buoyant densities is apparent after purification in sucrose-density gradients (Fig. 1A). However, much better resolution was afforded by the use of potassium tartrate gradients (Fig. 1B) and similar differences have been noted by BISHOP *et al.* (1970) for the purification of RSV, whose RNA's are more uniform in size than those of RSV.

Acknowledgments

I thank Miss Beverly Smyth and Mrs. Glenyse Harmer for competent technical assistance.

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Authors' address: Dr. G. A. TANNOCK, Veterinary Research Section, Commonwealth Serum Laboratories, Parkville, Victoria 3052, Australia.