Ribonucleic Acid Components of BAI Strain A (Myeloblastosis) Avian Tumor Virus


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SUMMARY

Sucrose-density-gradient centrifugation of RNA extracted from BAI strain A virus with phenol revealed 4 distinct components in continuous-flow spectrophotometer diagrams. Two of these, not previously described, sedimented at about 16 S and 26 S in association with the components of about 60 S and 5 S reported by others. The 5 S material, like cell transfer RNA, exhibited the amino-acid-acceptor activity already observed with whole virus RNA. Experiments indicated that the RNA's were not derived from external contaminating particulate or low molecular weight materials but that all were intrinsic to the agent. Comparative sedimentation studies showed that the 16 S and 26 S components were similar to cell ribosome RNA. Evidence indicated that the 16 S, 26 S, and a part of the 5 S materials were present in the cell cytoplasm and included in the virus during budding. The remainder of the 5 S appeared to be degradation products of the 60 S specific virus RNA component. The possible origin and biologic significance of the components are discussed, particularly with respect to mechanisms of virus formation.

INTRODUCTION

The BAI strain A virus induces myeloblastic leukemia and other neoplasms in the chicken (4) and is synthesized and liberated by budding at the myeloblast cell membrane (45). RNA comprised 2.2% of the virus dry weight (10) corresponding to about 10^10 daltons/particle. Centrifugal fractionation of phenol-extracted virus RNA has revealed a 67 S fraction associated with the 60 S specific virus RNA component. The following abbreviations are used: sRNA, soluble ribonucleic acid; Tris, tris(hydroxymethyl)aminomethane; RNase, ribonuclease; EDTA, disodium ethylenediaminetetraacetate; tRNA, transfer ribonucleic acid; PTA, phosphotungstic acid.

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The BAI strain A virus was isolated from blood plasma of leukemic birds (18) or fluid from leukemia myeloblast cultures (31). Blood plasmas contained 3 x 10^{11.2} - 10^{12} virus particles/ml as estimated by adenosinetriphosphatase activity (5) or direct count by electron microscopy (41). Tissue culture fluids, about 80 ml/individual culture, contained 1 x 10^{11.2} - 10^{11} particles/ml.

Plasmas from leukemic chickens (18) were spun twice at 1000 x g for 15 min, shell-frozen individually in a Dry Ice-alcohol bath, and stored at -78°C until use. Thawed plasmas were pooled in volumes of 3-140 ml and centrifuged at 3200 x g for 10 min. Virus was sedimented from the supernate at 40,000 x g for 35 min, the walls of the tubes were wiped, and the pellets were suspended by pipetting in about 20% of the initial volume of 0.01 M Tris-HCl, 0.05 M NaCl of pH 7.4 to 7.6 measured at 2°C. In some preparations the medium also contained 0.01 M EDTA. Centrifugation of the suspension at 3200 x g for 10 min yielded very small, opaque pellets. The cycle of alternate high- and low-speed centrifugation was repeated 1-3 times. Rate zonal and equilibrium-density-gradient centrifugation procedures are described in Chart 3. All operations were at 2-4°C. Approximately 4 x 10^{14} virus particles, about 320 mg dry weight or 1.3 gm hydrated virus (10, 42), were obtained from a total of 1400 ml of plasma.

SUMMARY

Analyses of the phenol-extracted virus RNA in this laboratory showed again the 2 principal components of about 60 S and 5 S. In addition, 2 intermediate components, 16 S and 26 S, were consistently evident in optical density diagrams obtained with a continuously monitoring spectrophotometer. The sedimentation behavior of the phenol-derived, protein-free 16 S and 26 S RNA's corresponded closely to that of cell ribosome RNA. Moreover, the 5 S virus RNA resembled cell sRNA3 in this respect and likewise exhibited the capacity to attach amino acids. Numerous studies indicated that the 5 S, 16 S, and 26 S components, as well as the 60 S material, were intrinsic to the virus particle. This report describes properties of these virus RNA's and considers their possible origin and biologic significance.

MATERIALS AND METHODS

Virus. BAI strain A virus was isolated from blood plasma of leukemic birds (18) or fluid from leukemia myeloblast cultures (31). Blood plasmas contained 3 x 10^{11.2} - 10^{12} virus particles/ml as estimated by adenosinetriphosphatase activity (5) or direct count by electron microscopy (41). Tissue culture fluids, about 80 ml/individual culture, contained 1 x 10^{11.2} - 10^{11} particles/ml.

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Leukemia myeloblasts were cultured (31), by procedures specially designed to maintain the cells in good condition, in 100 ml of equal parts of M 199 and chicken serum with antibiotics and added glucose and folic acid (6, 8). Only fluids from cultures containing fewer than 10% dead cells as determined by staining with trypan blue (6) were used for virus isolation. Culture fluid harvested after 24-hr incubation at 37°C was centrifuged at 150 × g for 10 min and again at 3000 × g for 10 min. The supernate frozen rapidly in ampoules was stored at −78°C. Virus was recovered by the same procedures as those employed with blood plasma with a yield of about 6 × 10⁴ virus particles, about 480 mg dry weight or 1.9 gm hydrated virus (10, 42), from 6500 ml of culture fluid.

**Treatment of Virus with RNase.** Virus obtained by 1 centrifugation cycle was incubated for 15 min at 25°C with 5 μg of RNase/ml in 0.01 M EDTA, 0.01 M Tris-HCl, 0.05 M NaCl at pH 7.4, and then sedimented by centrifugation at 40,000 × g for 35 min.

**RNA Isolation.** Virus concentrates were mixed with 0.5% sodium dodecyl sulfate (40), and RNA was extracted at 0–4°C with equal volumes of redistilled phenol (20) saturated with 0.1 M Tris-HCl, pH 7.4. In some cases the phenol contained 0.1% 8-quinolinol (29). The pH of the extraction mixture was adjusted (33) to 7.0 with 1 N KOH. The aqueous and interface layers were re-extracted twice with phenol. Residual phenol was removed from the aqueous layer with ether, and RNA was precipitated with 0.1 volume of 20% potassium acetate, pH 5.4, and 2 volumes of 95% ethanol at −20°C, sedimented at 18,000 × g for 20 min, and dissolved for transfer to the density gradient.

**Cell Materials.** Myeloblasts in 50-ml cultures containing 10⁸ cells/ml were labeled with 2.5 μc/ml of uridine-3H (specific activity 8.1 c/mmole) for 24 hr. Cells sedimented at 400 × g for 10 min were washed with phosphate-buffered saline, resedimented, and then allowed to swell in a 10-fold volume of 0.1 M Tris-HCl, 0.01 M KCl, 0.005 M MgCl₂, pH 7.5, for 10 min at 0–4°C. The cells recovered by centrifugation were disrupted with a Dounce homogenizer in an equal volume of the same buffer until most of them were broken.

The homogenate was divided into 2 portions; 1 was centrifuged at 3200 × g for 10 min, and the supernate (Fraction M) was stored at −20°C. The 2nd part was centrifuged at 800 × g to remove nuclei and other cell debris. The supernate was then spun at 36,000 × g for 30 min to eliminate mitochondria and debris and again at 105,000 × g for 1 hr to sediment the ribosomes (Fraction R). The supernate from this procedure was further centrifuged at 105,000 × g for 2 hr, and the resulting supernate (Fraction S) was stored at −20°C. The pellet was discarded.

**Density Gradient Centrifugation of RNA.** RNA dissolved in 0.05- to 0.1-ml volume was layered on 4.8-ml linear gradients of 5–20% sucrose in 0.01 M Tris-HCl, 0.1 M NaCl, pH 7.0, containing 0.01% Brij 35, and centrifuged in a Spinco SW 50 rotor. The bottom of the tube was punctured, and the fluid led through a capillary flow cell (30) in a Cary 15 recording spectrophotometer to a fraction collector. Optical density was recorded at 260 μm. Sedimentation constant values were estimated (34) by comparison with yeast 28 S RNA (33). The quantity of RNA was estimated from the area under the optical density tracings (15).

**Electron Microscopy.** For determination of virus particle number (41), samples of preparations taken at various stages of purification were diluted with 1% glutaraldehyde in 0.1 M NaCl, 0.01 M sodium phosphate, pH 7, and stored at 4°C until used. Virus was sedimented onto an agar surface, and a pseudoreplica was prepared, chromium shadowed, and examined in the electron microscope. For examination by negative staining, samples of virus suspensions were mixed with 1 volume of 2% PTA, which had been adjusted to pH 7 with KOH, and 0.2 volume of 37% formaldehyde. After at least 30 min of fixation in the formaldehyde to help preserve any ribosomes which might be present (28), a carbon and Formvar-coated grid was rinsed in freshly distilled chloroform to improve wettability of the surface (14), dried, touched to a drop of the virus-PTA mixture, and then drained and dried.

**Amino Acyl tRNA Syntheticases.** Myeloblasts from leukemic birds were washed twice with tissue culture medium (31). To 10 ml of packed cells were added 20 ml of a solution containing 0.01 M Tris-HCl, 0.01 M KCl, 0.005 M MgCl₂, pH 7.5, and 10 ml of glass homogenizing beads. Cells were disrupted in a VirTis homogenizer, and the preparation was centrifuged at 2500 × g for 10 min. The supernate was spun at 10,000 × g for 10 min, filtered through glass wool, and finally centrifuged at 100,000 × g for 120 min. The supernatant fluid was adjusted to pH 5.15 with 1 N acetic acid, and the precipitate containing amino acyl tRNA synthetases was sedimented at 10,000 × g for 10 min. The supernate was discarded and the pellet suspended in 20 ml of 0.1 M Tris-HCl, pH 7.5, containing 0.05 M reduced glutathione. Precipitation was repeated three times as described. All operations were performed near 0°C. Protein concentration was determined by the method of Lowry et al. (32), and the enzymes were stored at −78°C in Tris-glutathione.

Myeloblast sRNA was obtained as described (7).

**Amino Acid Attachment.** 14C-labeled amino acids (NEC-445, New England Nuclear Corp., Boston, Mass.) at specific activity of 40 μc/milliatom of carbon were diluted 10-fold with the same proportions of unlabeled amino acids. The reaction mixture contained amino acids in high excess, and enzymes and ATP enough to saturate more than 200 μg of cell sRNA. Reaction was terminated by immersing the tubes in ice with addition of 1 ml of 10% NaCl, 200 μc carrier yeast RNA, and 2 volumes of absolute ethanol. After centrifugation at 12,000 × g for 20 min, the supernate was discarded, and the pellet was suspended in 1 ml of 0.1 M Tris-HCl, pH 7.5. One ml of 20% NaCl and 2 volumes of ethanol were added, and the preparation was centrifuged as before. This precipitation process was repeated twice. All operations were performed near 0°C. The pellets were dissolved in 1 ml of Hyamine (10X), and radioactivity was determined in a liquid scintillation spectrometer (Packard Instrument Corporation, Inc., LaGrange, Ill.). Residual radioactivity of 0-time samples varied between 40 and 70 cpm.

**RESULTS**

**RNA Components Extracted from BAI Strain A Virus Preparations.** The present work was begun as a part of a continuing program of studies on properties of the BAI strain A virus in this laboratory. It was the initial purpose to isolate RNA from the agent as described by other investigators (21, 23, 27, 39) with the expectation of further characterization of the derived components by use of relatively large amounts of ma-
CHART 1. Distribution of RNA components extracted with phenol from leukemic-chicken-plasma virus (A) and tissue-culture virus (B) separated by density gradient centrifugation. Solutions containing 52 µg (A) and 30 µg (B) of extracted RNA, respectively, were layered on 4.8 ml linear 5%-20% sucrose density gradients in 0.1 M NaCl, 0.01 M tris(hydroxymethyl)aminomethane-HCl, 0.01% Brij 35, pH 7.0, and centrifuged for 110 min at 40,000 rpm in a Spinco SW 50 rotor. The bottoms of the tubes were punctured and the fluids led through a capillary flow cell in a Cary 15 spectrophotometer recording optical density at 260 nm.

Material. Source material for such work was available as blood plasmas selected for high virus content, $3 \times 10^{10}$-2 $\times 10^{12}$ particles/ml, from chickens with myeloblastosis induced by the agent. Another source was the fluid from special myeloblast tissue cultures recently developed (31) for continuing production of virus in high concentration, approximately $1 \times 10^{10}$-2 $\times 10^{11}$ particles/ml, in which viable cells were maintained at 90-99%. Levels in 24-hr culture periods. With access to the large quantities of virus and extractable RNA, it was feasible to examine the results of density gradient RNA fractionation on a continuous-flow spectrophotometer without recourse to tracer procedures employing minute virus amounts and the necessity for analysis of the RNA in discrete fractions.

Results of experiments made under these conditions conformed with the previously reported (21, 23, 27, 39) observations in revealing RNA components of about 60 S and about 5 S. Chart 1 illustrates the characteristics of the smooth, continuous spectrophotometer curves corresponding to the respective peaks of these components. The diagram of Chart 1A was obtained with virus from plasma isolated by 2 cycles of centrifugal fractionation as described in the section on Materials and Methods. The isolated virus preparation contained $4 \times 10^9$ virus particles with an estimated (10) content of 65 µg of RNA. Chart 1B was the result of a similar analysis of RNA from agent recovered from tissue culture fluid by like centrifugal procedures. In this virus preparation, the RNA content was estimated as 72 µg. Measurements of the areas under the curves in Chart 1 yielded the values of 52 µg and 30 µg of total extracted RNA in the respective studies. The patterns obtained with the plasma virus and agent from tissue culture were closely similar in the characteristics of the peaks corresponding to the components sedimenting at rates of about 60 S and 5 S.

In addition to these components, the diagrams of Chart 1 likewise show 2 other distinct peaks corresponding to sedimentation rates of about 16 S and 26 S. The respective curves of these minor components were also closely similar in the diagrams observed with virus from both plasma and tissue culture sources. Components of these characteristics have not been described by other investigators working with the BAI strain A virus or other filterable agents. Further comparable studies consistently revealed the presence of the 16 S and 26 S components. Typical absorption spectra (Chart 2) of the separated fractions measured on 10 different preparations, yielding diagrams like those of Chart 1, were characteristic of RNA and showed no evidence of protein. The curves for all 4 fractions were similar with maxima at 257 nm and minima at 231 nm. The O.D.230/O.D.280 and O.D.250/O.D.260 ratios were about 0.5 and 0.49, respectively.

The consistent occurrence of the 16 S and 26 S components indicated a close association of the RNA’s with the virus particles either as intrinsic components of the agent or as contaminants regularly and rather uniformly present in the isolated virus preparations. That association of the RNA’s with the virus was more than casual was suggested by numerous previous studies of the BAI strain A virus. In plasma of $10^{10}$ virus particles/ml, the virus occurs naturally in very high homogeneity with respect to particle kind. The amount of virus in such plasmas is so great that other particulate components, estimated as about $10^9$ particles/ml (3), are negligible, about 0.1%, i.e., a ratio of 1000
virus particles to 1 nonvirus particle, by comparison. Although there is no criterion adequate for unequivocal estimate of homogeneity of purified BAI strain A virus preparations, various findings provide the basis for judgement with respect to possible contaminants. Plasma proteins and other soluble materials can be removed simply by repeated sedimentation and washing of the virus. Sedimentation diagrams obtained by examination of the agent directly in plasma showed (42) distinct single though somewhat diffuse boundaries. Like studies on virus purified by centrifugal fractionation as described yielded (42) single and sharper boundaries with no evidence of a second population of particles. With respect to possible nonviral RNA-containing material pertinent to the present work, ribosomes potentially derived by cell degradation in the diseased bird might conceivably be in the plasma. Since the sedimentation rate of ribosomes is about 80 S (37) and that of the virus is about 700 S (42), separation of particles of such disparate properties would be largely effected in the brief centrifugation period, 40,000 × g for 35 min, used here.

Another possible extraneous particulate material might be cytoplasmic membranes such as endoplasmic reticulum with attached ribosomes. Electron micrographs of virus purified from plasma as described do not show either these structures or ribosomes which would also be seen if present. The electron microscopic features of virus obtained by a single cycle of sedimentation for the experiment of Chart 3A are illustrated in the negatively stained preparation shown in Fig. 1. The micrograph reveals only virus particles and debris identifiable as virus particle degradation products (11, 12) without evidence of either cell membranes or ribosomes. Fig. 2 is an electron micrograph of a thin section of a virus pellet obtained by 4 cycles of sedimentation. The single structure at the lower right not clearly representative of virus could be a fragment of cytoplasmic membrane, but no ribosomes are evident. Electron micrographs of virus precipitated with specific chicken immune serum showed (18) the agent without significant extraneous material. Another criterion of homogeneity independent of particle size and density was applied in studies on virus migration in the Tiselius electrophoresis apparatus (18). The investigations were hampered by light scattering of the virus in high concentrations, but the migrating boundary was sharp and single.

**Velocity and Equilibrium Density Gradient Virus Sedimentation.** The observations recounted in the foregoing section demonstrated the efficacy of centrifugal fractionation procedures for obtaining BAI strain A virus preparations from the blood plasma of leukemic chickens free of contaminants detectable by the criteria applied. This in turn constituted presumptive evidence that the RNA components illustrated in Chart 1 were derivatives of the virus and not of hypothetical extraneous material which was not revealed by the methods cited. However,
other procedures much in use in recent years for isolation of viruses in general involve centrifugation of the agents on density gradients as a stage in the isolation process. Such methods have been avoided in studies on the BAI strain A virus in this laboratory, because the agent was more easily obtained in large amounts from virus-rich blood plasmas by fractional centrifugation. More important, exposure of the virus to high concentrations of low molecular weight solutes caused particle damage evident in electron micrographs (see Figs. 3 and 4) which was due presumably to adverse osmotic effects. This has been observed, also, by other investigators (27). Nevertheless, in order to determine whether the 16 S and 26 S components were present in RNA extracts of virus obtained by these methods, appropriate experiments were made by both velocity and equilibrium density gradient centrifugation.

In a study of velocity density gradient centrifugation, virus, $1.6 \times 10^{13}$ particles corresponding to 13 mg dry weight, in 18 ml of plasma was sedimented once, resuspended, sampled for electron microscopy (Fig. 1), then centrifuged on a linear 5–20% sucrose density gradient as described in the legend of Chart 3A. This resulted in a single dense band with a small amount of material at the top of the tube, probably consisting of residual plasma protein and pigment. There was also a small pellet of aggregated material. The character of the virus band was shown by the optical density analysis pictured in Chart 3A. Virus in those fractions corresponding to the sharp, symmetric peak between the arrows in Chart 3A was taken for RNA extraction and analysis (Chart 4A), and for electron microscopy (Fig. 3).

Virus for equilibrium density gradient centrifugation was obtained by 1 cycle of sedimentation in the same way as that for the velocity-density-gradient study from 30 ml of plasma containing $1.7 \times 10^{13}$ particles corresponding to about 14 mg dry weight of virus. The virus pellet was suspended in 3.200 g for 10 min and then at 40,000 x g for 35 min as described for A. The virus pellet was suspended in 2.0 ml of 0.01 M Tris, 0.05 M NaCl, 0.01 M EDTA, pH 7.6, and layered over a 30-ml linear 5–20% sucrose density gradient in 0.01 M Tris-HCl, 0.01 M EDTA, pH 7.6. After 23 min centrifugation at 25,000 rpm in a Spinco rotor SW 25.1, the bottom of the tube was punctured, and the fluid led through a flow cell in a Cary 15 spectrophotometer. The fractions corresponding to the arrows were sampled for electron microscopy (Fig. 3) and pooled for isolation of the RNA analyzed with the results given in Chart 4B.

CHART 3. Optical density analysis of bands obtained by velocity-density-gradient (A) and equilibrium-density-gradient (B) centrifugation of BAI strain A virus from the plasma of birds with myeloblastic leukemia.

A. Eighteen ml of plasma containing approximately $1.6 \times 10^{13}$ total particles (about 13 mg dry weight of virus) were centrifuged at 3,200 x g for 10 min, and the virus-containing supernate was then spun at 40,000 x g for 35 min. The virus pellet was resuspended in 2.0 ml of 0.01 M Tris-HCl, 0.05 M NaCl, 0.01 M EDTA, pH 7.6, and layered on a 30-ml linear 5–20% sucrose density gradient in 0.01 M Tris-HCl, 0.01 M EDTA, pH 7.6. After 23 min centrifugation at 25,000 rpm in Spinco rotor SW 25.1, the bottom of the tube was punctured, and the fluid led through a flow cell in a Cary 15 spectrophotometer. The fractions corresponding to the arrow were sampled for electron microscopy (Fig. 3) and pooled for isolation of the RNA analyzed with the results given in Chart 4A.

B. Approximately 30 ml of plasma containing about $1.7 \times 10^{13}$ total particles (approximately 14 mg dry weight of virus) were centrifuged at 3,200 x g for 10 min and then at 40,000 x g for 35 min as described for A. The virus pellet was suspended in 2.0 ml of 0.01 M Tris, 0.05 M NaCl, 0.01 M EDTA, pH 7.6, and layered over a 30-ml linear 2–40% potassium tartrate gradient containing 0.02% bovine serum albumin. After centrifugation for 180 min at 25,000 rpm in a Spinco rotor SW 25.1, the bottom of the tube was punctured, and the fluid led through the spectrophotometer. The fractions between the arrows were pooled for isolation of the RNA analyzed with the results given in Chart 4B.
RNA Components of Avian Tumor Virus

Chart 4. Distribution of RNA components extracted with phenol from the fractions obtained in the bands derived as described in the legend of Chart 3 by velocity-density-gradient (A) and equilibrium-density-gradient (B) centrifugation. The virus recovered by centrifugation from the virus-containing fractions from the respective density gradients was suspended in 0.05 M NaCl, 0.01 M Tris-HCl, 0.01 M EDTA, pH 7.6, and extracted with phenol containing 0.1% 8-quinolinol. Solutions containing about 37 μg (A) and 43 μg (B) of RNA were layered on 4.8 ml 5%-20% sucrose density gradients in 0.10 M NaCl, 0.01 M Tris-HCl, 0.01% Brij 35, pH 7.0, and centrifuged for 110 min at 40,000 rpm in a Spinco SW 50 rotor. The bottoms of the tubes were punctured and the fluids led through the capillary spectrophotometer flow cell recording optical density at 260 m/μ. Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate.

Serum albumin, and optical density analysis of the resulting band yielded the diagram in Chart 3B. Fractions corresponding to the region in the peak between the arrows in Chart 3B were pooled and sampled for electron microscopy (Fig. 4), and RNA was extracted.

Chart 4 shows the analyses of RNA from virus obtained by velocity- and equilibrium-density-gradient centrifugations, respectively. The patterns of virus RNA's yielded by both procedures were closely similar to each other and to those of RNA's derived from virus sedimented by centrifugal fractionation illustrated in Chart 1. The 16 S and 26 S components were distinct, and there were no significant differences with respect to the proportions of the 5 S, 16 S, 26 S, and 60 S components. It should be emphasized that virus for these RNA extractions was selected (Chart 3) from those regions of the bands containing particles most closely related in sedimentation properties. Thus, particles sedimenting more slowly or rapidly than the principal component were eliminated from the material subsequently analyzed.

Electron micrographs of the virus sedimented by a single centrifugation cycle for the experiment of Chart 3A (Fig. 1) showed essentially only virus particles and debris resulting from disruption of the particle membrane (11, 12) and escape of amorphous inner-particle substance with no resemblance to ribosomes. Structures not identifiable as of virus origin were rarely seen. The picture (Fig. 3) of the same virus population selected from the sedimentation band (Chart 3A) after velocity density gradient centrifugation in sucrose showed a slight increase in homogeneity similar to that observed after a second cycle of fractional centrifugation, reflecting chiefly the reduction of residual plasma protein. Nevertheless, evidence of particle damage and disruption was greater in this micrograph than in Fig. 1. A similar picture (Fig. 4) was obtained with virus after equilibrium-density-gradient centrifugation in potassium tartrate selected from the band as indicated in Chart 3B. As in Fig. 3, also, the virus was considerably damaged by the treatment. That such apparent damage had little effect on RNA components is illustrated by the similarities of the patterns in Chart 4 to those in Chart 1A.

RNA Components from Virus Preparations Treated with RNase. The observations, thus far, indicated clearly (a) that the characteristics of the 16 S and 26 S as well as the 5 S and 60 S RNA components were the same whether the virus preparations were obtained by conventional fractional centrifugation or with the density gradient procedures and (b) that hypothetical particulate contaminants were not detectable in electron micrographs of the negatively stained or sectioned virus source material. However, there was the possibility that trace amounts of contaminants such as ribosomes, free or attached to cell cytoplasmic structures, might have escaped detection by the methods used. In consequence, studies were made with virus preparations treated with RNase for the purpose of digesting such inapparent nonvirus RNA or RNA conceivably adsorbed to the virus particle surface but not seen in the electron micrographs (Fig. 1). This test was feasible, because RNA within intact virus was not digested by the enzyme (10).

Chart 5 gives the results of a typical experiment of a series of 12 separate studies. As noted in the legend of Chart 5, EDTA was included in the RNase digestion mixture. This was done to chelate Mg++ and cause breakdown of ribosomal structure
which greatly increases susceptibility of ribosomes to RNase action (37). Comparison of the diagram of Chart 5B obtained with RNA from a virus preparation exposed to RNase with that in Chart 5A from untreated virus shows that the enzyme did not affect the occurrence of the 16 S and 26 S components. Like results were observed in all experiments of this series. Some reduction of the 60 S peak and an increase in the 5 S peak occurred in some experiments, as seen in Chart 5. This may have been due to enzyme action on virus RNA of particles damaged during isolation.

**Efficacy of RNase to Degrade Cell-derived RNA.** Inasmuch as treatment of the virus with RNase did not influence the isolation of the 16 S and 26 S components, questions might be raised concerning the suitability of the conditions used (Chart 5) for degradation of suspected RNA contaminants. The sedimentation characteristics of the 16 S and 26 S components resembled those of cell ribosomal RNA's, and furthermore, ribosomes, free or attached to cell cytoplasmic membranes, might be regarded as most likely to contaminate the virus preparations. In consequence, tests of RNase activity were made with a crude extract of host-cell cytoplasm to fragments of less than 9 S. It was possible, however, that such RNA might be protected from RNase action in the presence of virus by adsorption to the particles or some other unrecognized interaction. This was tested by study of RNase influence on host-cell constituents mixed with virus as described in the legend of Chart 7.

**Action of RNase on RNA in Cell Constituents Added to Virus.** The preceding experiments showed that RNase degraded RNA in a crude extract of host-cell cytoplasm to fragments of less than 9 S. It was possible, however, that such RNA might be protected from RNase action in the presence of virus by adsorption to the particles or some other unrecognized interaction. This was tested by study of RNase influence on host-cell constituents mixed with virus as described in the legend of Chart 7.

Chart 7A shows the large distinct peak characteristic of the rapidly sedimenting 60 S virus RNA. The peaks in the 5 S, 16 S, and 26 S regions were somewhat complicated by cell RNA in the virus pellet sedimented by a single centrifugation cycle. Chart 7B shows, however, that cell RNA, even in the presence of virus, was degraded by the enzyme, and that, as seen also in Table 1, essentially none of the labeled cell RNA con-

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**Chart 5.** Distribution of RNA components extracted from untreated virus (A) and from virus treated with RNase (B). A virus preparation obtained by one centrifugation cycle was divided into 2 equal parts. One fraction was incubated for 15 min at 25°C with 5 μg of RNase/ml in 0.01 M EDTA, 0.01 M Tris-HCl, 0.05 M NaCl at pH 7.4, and the virus was sedimented at 40,000 × g for 35 min. The other fraction was kept at 4°C without RNase and centrifuged in the same manner. RNA was extracted from the respective pellets with phenol, precipitated, dissolved, transferred to a linear 5%–20% sucrose density gradient in 0.10 M NaCl, 0.01 M Tris-HCl, 0.01% Brij 35, pH 7.0, centrifuged, and analyzed as described for Chart 1. RNase, ribonuclease; EDTA, ethylenediaminetetraacetate; Tris, tris(hydroxymethyl)aminomethane.
Myeloblasts in cultures containing about 10^7 cells/ml were labeled. The tube was punctured, and fractions of about 0.2-ml volumes were disrupted, and 1.0 ml of the crude cytoplasmic homogenate, Fraction M, obtained as described in the section on Materials and Methods and containing about 0.7 mg of RNA, was incubated with RNase as described for similar treatment of virus in the legend of Chart 5. The mixture was centrifuged at 40,000 X g for 35 min, and RNA extracted from the supernate and that from the crude cytoplasmic fraction used in those studies. The results (Chart 10A, Table 1) revealed only traces of label in the pellet of virus not exposed to RNase. Most of this was eliminated (Chart 10, Table 1) by RNase treatment. These data indicated, also, that the material appearing in the 5 S region of Chart 9B was not related to an RNase-resistant sRNA.

RNA Components of Virus Sedimented from Mixtures Containing Added Cell sRNA. The foregoing studies indicated that a small portion of the RNA-containing cell cytoplasmic constituents, which were added in high concentration in mixtures with the virus, sedimented with the agent, but that components of 16 S and 26 S sedimentation rates were completely degraded by RNase (Charts 7, 9). There remained the possibility that sRNA might be adsorbed in some RNase-resistant combination with the virus particles and contribute to the 5 S component found with the virus. To test the point, experiments were made like those of Chart 7 except that uridine-3H labeled myeloblast cytoplasmic sRNA, fraction S, was substituted for the crude cytoplasmic fraction used in those studies. The results (Chart 10A, Table 1) revealed only traces of label in the pellet of virus not exposed to RNase. Most of this was eliminated (Chart 10, Table 1) by RNase treatment. These data indicated, also, that the material appearing in the 5 S region of Chart 9B was not related to an RNase-resistant sRNA.

Summary of Experiments on Addition of Cell RNA to Virus Preparations. These experiments with RNase gave unequivocal evidence of the efficacy of the enzyme to degrade cell RNA added even in large excess to virus preparations. Table 1 summarizes the results of studies on 16 S and 26 S (Chart 7) and 5 S (Chart 10) RNA components. In the experiment of Chart 7, 180 µg of labeled cell-derived 16 S plus 26 S components, 52,000 cpm, were added to the virus. Of this, 3100 cpm, corresponding to 11 µg, which was 6% of the original cell RNA added, were found in the pellet sedimented by the single centrifugation cycle applied to the preparation not treated with the enzyme. In contrast, only 63 cpm, 0.2 µg of cell 16 S and 26 S RNA, were recovered from the enzyme-treated preparation. Thus, about 99.9% of the added cell RNA were eliminated by the combined sedimentation procedure and degradation by RNase in the presence of the virus. The amount of 16 S and 26 S RNA's extracted from the sedimented RNase-treated virus pellet was 3.6 µg measured from the optical density analysis of Chart 7B. Therefore, at least 94% of the 16 S and 26 S components in this preparation must have been derived from the virus particles. This experiment was an exceedingly stringent test of RNase capacity to degrade cell RNA components, because electron micrographs have shown (Figs. 2-6 of Ref. 44) that preparations like Fraction M contain large and small cytoplasmic membrane fragments with attached ribosomes as well as free ribosomes. Furthermore, the amount of cell RNA added was far greater, about 53-fold (180 µg:3.4 µg) that of the analogous materials in the virus. Nevertheless, about 99.9% of the included RNA were degraded to components in the range of 5 S or lower sedimentation rate.

Even more definitive results were obtained in the study with cell sRNA (Chart 10B) cited in Table 1. In this case, about 99% of the virus-associated 5 S component could be identified as of virus origin.

Comparison of Virus and Cell-derived RNA's. As mentioned, the sedimentation behavior of the 16 S and 26 S virus-associated RNA constituents was that to be expected of cell ribosome RNA. Direct comparison of these respective materials was made by density gradient centrifugation of a mixture of
Fraction CHART 7. RNA components extracted from material sedimented from a mixture of virus and crude extract of labeled myeloblasts (A) and that sedimented from a sample of the same mixture after treatment with RNase (B). A large excess of labeled host cell RNA, 0.45 mg (150,000 cpm), in the form of crude myeloblast cytoplasmic Fraction M was added to a virus preparation, 8 X 10^9 particles estimated to contain 130 µg RNA, which had been isolated from plasma by one centrifugation cycle. One half of the mixture was incubated for 15 min at 25°C with 5 µg RNase and EDTA as in the experiment of Chart 5, and the other was kept at 2°C as control without RNase and EDTA. Both preparations were centrifuged for 35 min at 40,000 X g, and RNA was extracted from the respective pellets. The extracts were layered on sucrose (5%-20%) density gradients and centrifuged for 110 min at 40,000 rpm in a Spinco SW 50 rotor. Optical density of the preparations was determined with the spectrophotometer, and the collected fractions were analyzed for radioactivity. The continuous curve representing optical density was drawn through points taken from the spectrophotometer tracing corresponding to the fractions used for radioactivity measurements (dotted line). RNase, ribonuclease; EDTA, ethylenediaminetetraacetate.

TABLE 1

Distribution of RNA Components in Material Sedimented from Mixtures of Virus with a Large Excess of Uridine-3H-labeled Host-Cell RNA Not Exposed to RNase* and after Treatment with the Enzyme (See Charts 7 and 10)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Uridine-3H-labeled cell RNA added to each virus preparation</th>
<th>Uridine-3H-labeled RNA (16 S + 26 S or 5 S) recovered from the pellets</th>
<th>Virus RNA (16 S + 26 S or 5 S) extracted from the pellets (µg)</th>
<th>Ratio of virus RNA to residual cell RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine-3H-labeled RNA + 26 S or 5 S</td>
<td>Without RNase pretreatment</td>
<td>With RNase pretreatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>µg</td>
<td>cpm</td>
<td>µg</td>
<td>cpm</td>
</tr>
<tr>
<td>Chart 7</td>
<td>16 S + 26 S</td>
<td>52,000</td>
<td>180</td>
<td>3,100</td>
</tr>
<tr>
<td>Chart 10</td>
<td>5 S</td>
<td>22,000</td>
<td>35</td>
<td>140</td>
</tr>
</tbody>
</table>

* RNase, ribonuclease; sRNA, soluble ribonucleic acid.
† Estimated from areas under peaks in Charts 7B and 10A.
‡ In crude cytoplasmic fraction (M) of cell homogenate.
§ Fraction S.
∥ About 2 µg of this were estimated to be intraviral sRNA; the remainder was probably degraded 60 S RNA (see Table 4).
RNA Components of Avian Tumor Virus

Chart 8. RNA components from a mixture of virus with cell cytoplasmic constituents (A) and those from a part of the same mixture after treatment with ribonuclease (RNase) (B). Cell cytoplasmic material, Fraction M, containing 1.5 mg of RNA labeled with uridine-\(^{14}\)H (174,000 cpm) was mixed with virus obtained by 1 cycle of sedimentation. RNase was added to 1 part of the mixture and none to the other. The mixtures were processed as described in Chart 7 by centrifugation, extraction of RNA from the respective pellets, centrifugation of the extracts on sucrose density gradients for 110 min at 40,000 rpm in a Spinco SW 50 rotor, and analysis by optical density which yielded the diagrams of A and B. The diagram in A shows the distinct 60 S virus component together with components in high concentration in the 5 S, 16 S, and 26 S regions, whereas that in B was similar to the diagrams observed with RNA from virus alone (Chart 1), except for the high concentration of 5 S material due partly, at least, to degraded cell ribosomal RNA as shown in Chart 6. The fractions to the left of the arrows in both diagrams were pooled for further analysis (Chart 9).

labeled cell ribosomal RNA with a pool of 16 S and 26 S RNA's obtained from several virus preparations.

Chart 11A shows the characteristic and distinct 16 S and 26 S peaks observed with the pooled virus RNA fractions which contained also substantial amounts of 5 S component. The optical density and radioactivity diagrams of cell ribosome RNA (Chart 11B) revealed large peaks in the 16 S and 26 S regions with only a small amount of 5 S material. The diagram obtained with the mixture of virus and ribosome RNA's (Chart 11C) showed a close resemblance of the cell 16 S and 26 S radioactivity peaks to those of the optical density diagram representing the combined cell and virus 16 S and 26 S RNA's.

The sedimentation behavior of virus sRNA was compared in a similar manner with that of cell 5 S RNA, and the results showed that the respective virus and cell 5 S fractions sedimented together (Chart 12). The small amounts of 8 S–9 S virus RNA distinguished by the leading shoulder in the diagram in Chart 12 were seen also in several other preparations. This material has not been characterized further, but may represent degradation products of a larger molecule.

Amino Acid Attachment. A notable characteristic of BAI strain A virus RNA was the capacity to bind amino acids (7) in a manner similar to that displayed by cell tRNA, and tests for this activity were made with virus RNA isolated in the present work. RNA from virus treated with RNase during purification was separated into its components by density gradient centrifugation. Only 5 S and 60 S fractions were used for the study, because the quantities of 16 S and 26 S were too small for examination. Acceptor activity (Table 2) was exhibited consistently by the 5 S component, but the amino acids were not attached when they were added to the intact 60 S component. Tests with low-molecular-weight RNA of about 5 S derived by degradation of the 60 S component on storage at \(-20^\circ\)C likewise showed no attachment. The amount of amino acids attached was proportional to the quantity of sRNA in a given experiment but varied somewhat in different studies as was to be expected with...
Chart 9. RNA components of the fractions selected as indicated in Chart 8. These fractions were pooled, and RNA was reprecipitated, redissolved, and centrifuged on a sucrose density gradient for 240 min at 40,000 rpm. The RNA's were then analyzed by optical density (continuous line) and radioactivity (dotted line) measurements. The points of the optical density curve were taken from the spectrophotometer tracing corresponding to the fractions examined for radioactivity.

Chart 10. RNA components extracted from virus sedimented from a mixture with myeloblast soluble RNA (sRNA) labeled with uridine-3H (A) and from a like mixture treated with ribonuclease (B). Approximately 70 μg (44,000 cpm) of labeled cell sRNA, Fraction S (See Materials and Methods), were added to a virus preparation and one half of the mixture was incubated with RNase as described for the experiment of Chart 7B. After centrifugation of both preparations at 40,000 x g for 35 min, RNA was extracted from the respective pellets. The RNA was precipitated, redissolved, and transferred to sucrose density gradients for centrifugation for 110 min at 40,000 rpm (Spinco SW 50 rotor). Optical density and radioactivity assays yielded the diagrams A and B.
RNA Components of Avian Tumor Virus

**Chart 11.** Comparison of sedimentation behavior of virus 16 S and 26 S RNA's with that of myeloblast ribosomal RNA. A pool containing 40 μg of 16 S, 26 S, and 5 S components in selected fractions from several virus-RNA preparations was divided into 2 parts. Uridine-3H-labeled myeloblast ribosomes, Fraction R (see Materials and Methods), were extracted with phenol, and 40 μg of RNA were taken for study. To 1 sample of 20 μg of virus RNA were added 13.3 μg of the ribosomal RNA. The virus RNA preparation alone (20 μg) (A); the ribosomal RNA (26.6 μg) alone (B); and the mixture of virus RNA (20 μg) and ribosomal RNA (13.3 μg) (C) were centrifuged on 5%-20% sucrose density gradients for 4 hr at 40,000 rpm. The points of the continuous lines (optical density) were taken from the spectrophotometer tracing corresponding to the fractions measured for radioactivity (dotted lines).

The partially purified enzymes used. For this reason, the data presented in Table 2 were those observed in a single experiment with 1 virus preparation.

**Proportion of RNA Components Extracted from Virus Preparations.** In the present studies, examinations were made on 36 samples of RNA extracted with phenol from plasma virus and 27 specimens of the agent propagated in tissue culture. All of these consistently showed 5 S, 16 S, 26 S, and 60 S components qualitatively like those of Chart 1. Since the flow rate was constant in the analyses, the proportions of the respective RNA components could be estimated (15) by measuring the areas under the peaks yielding the data cited in Table 3. The proportions of 5 S and 60 S components varied substantially and inversely to each other, as illustrated for the plasma virus RNA's in Chart 13. RNA components observed with tissue-culture virus were of relatively low concentration, and the proportions were but little related to the 60 S and 5 S variations. As shown by the slightly larger points on the lines in Chart 13, the interrelationships of the different components (see also Chart 4) were the same with virus centrifuged on density gradients as those with agent isolated by conventional fractional centrifugation. It should be emphasized that neither the kinds nor proportions of the different components were dependent on the number of centrifugation cycles, 1-4, used for virus isolation. Furthermore, as already shown, the interrelationships were the same with extracts of virus treated with RNase, and the values from these determinations are included in Chart 13.

The basis for variations in the proportions of 5 S and 60 S components was not entirely clear. From the reciprocal relationships, however, it appeared that degradation of the 60 S component might give rise to 5 S RNA. Factors contributing to degradation of 60 S RNA have not been identified, but it seemed evident that the quality of the starting virus was important. Virus used in the density gradient experiments (Chart 4), for example, was from leukemia plasma collected and frozen with special care and stored only a short time. Higher proportions of 60 S RNA in these instances may also have been due partly to the use of EDTA and 8-quinolinol in the RNA extractions.

**DISCUSSION**

Studies on RNA extracted with phenol from BAI strain A virus obtained by centrifugation of plasma from chickens with
Comparison of sedimentation behavior of virus 5 S RNA with that of myeloblast cell soluble RNA (mRNA). A mixture containing 25 μg of virus 5 S RNA and 17 μg of uridine-3H-labeled cell mRNA extracted with phenol from Fraction S (see Materials and Methods) was transferred to a 5%–20% sucrose density gradient and centrifuged for 24 hr at 40,000 rpm. The continuous line shows the optical density data obtained with the same fractions measured for radioactivity (dotted line).

myeloblastic leukemia consistently revealed 4 distinct components with sedimentation rates of about 5 S, 16 S, 26 S, and 60 S. From earlier work by other investigators (21, 23, 27, 39), it has been the common interpretation that the 60 S component represented virus-specific RNA, and analogous components occurred in other avian tumor viruses (22, 40) and agents responsible for leukemia (19) and tumors (17) in the mouse. It has been supposed that the 5 S or 5 S–20 S materials were either degraded 60 S component (39) or environmental contaminants (23) or both. The occurrence of the 16 S and 26 S mRNA's was not reported by other workers.

There has been some basis for regarding the 60 S RNA as a specific and intrinsic component of the virus particle. RNA of these sedimentation properties was compatible with the value of about 10^7 daltons found earlier (10) for the mass of RNA/particle in BAI strain A virus in highly homogeneous preparations, and analogous components occurred in other avian tumor viruses (22, 40) and agents responsible for leukemia (19) and tumors (17) in the mouse. It has been supposed that the 5 S or 5 S–20 S materials were either degraded 60 S component (39) or environmental contaminants (23) or both. The occurrence of the 16 S and 26 S RNA's was not reported by other workers.

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1. As already mentioned there is no criterion for precise estimate of the homogeneity of purified BAI strain A virus preparations. The particles vary in size, and, consequently, sediment with a somewhat diffuse, though single boundary in the analytic ultracentrifuge (42). Quantitative studies by electrophoresis (18) show only a single boundary, but examination of material in concentrations sufficient for detection of a minor component is hampered by light scattering by the preparations. Electron microscopy, although not a reliable means for measuring homogeneity, nevertheless, does not reveal nonvirus particles (9) as illustrated by the micrographs of Figs. 1–4. A further basis for judgment of homogeneity of the preparations is afforded by consideration of the properties of the source material and the concentration of contaminants necessary to yield the 16 S and 26 S components found. It should be noted that only those contaminants containing RNA would be of concern in the present study. Blood plasmas containing 10^12 particles are already highly homogeneous with respect to particle kind (3). The only extravirus particles visible in electron micrographs are in concentrations of about 10^7/ml. Thus, the

**Table 2**

<table>
<thead>
<tr>
<th>Source</th>
<th>Total 14C recovered (cpm)</th>
<th>Amino acids-14C attached to RNA (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,407</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1,366</td>
<td></td>
</tr>
<tr>
<td>Myeloblast</td>
<td>6,437</td>
<td>5,051</td>
</tr>
<tr>
<td>Virus 5 S</td>
<td>6,197</td>
<td>4,811</td>
</tr>
<tr>
<td>Virus 60 S</td>
<td>11,951</td>
<td>10,565</td>
</tr>
<tr>
<td>Total</td>
<td>11,096</td>
<td>9,710</td>
</tr>
</tbody>
</table>

* The reaction mixtures of 0.5-ml volume contained 70 μmoles tris(hydroxymethyl)aminomethane buffer, pH 7.5; 5 μmoles KCl; 5 μmoles reduced glutathione; 10 μmoles adenosine triphosphate; 0.5 μmol cytidine triphosphate; 50 μmoles Mg**2; 25 μg amino acids-14C (specific activity 3 millieciuries/milliatom of carbon); and pH 5 enzymes containing 200 μg protein. The incubation time was 30 min at 37°C.

* Total 14C recovered at 0 reaction time was from 40–70 cpm in 12 determinations.

* The mean value of the duplicate controls was 1386 cpm.

**Table 3**

| Virus source | No. of experiments | % of total RNA recovered
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 S</td>
<td>16 S</td>
</tr>
<tr>
<td>Plasma</td>
<td>34</td>
<td>40 ± 16</td>
</tr>
<tr>
<td>Tissue culture</td>
<td>27</td>
<td>52 ± 13</td>
</tr>
</tbody>
</table>

* Mean ± standard deviation.
ratio of such particles to the virus in the plasma is about 1:1000. The combined 16 S and 26 S components comprised, on the average (Table 3) about 13% of the total virus-associated RNA. Thus, if the extraneous particles were ribosomes, for example, for which there is no evidence, the amount of the particles could contribute no more than about 0.1% of the 16 S and 26 S components observed. The results in the present work were observed with virus obtained by 1–4 cycles of sedimentation with a brief centrifugation period, 35 min at 40,000 × g, selected in accord with the requirements for sedimenting particles with the properties of the virus. By comparison of virus from leukemia plasma with nonvirus particles from control plasma, it was estimated (13) that about 99.97% of the RNA in preparations obtained with 4 centrifugation cycles were of virus origin. Moreover, RNA was not demonstrable in leukemia plasma after sedimentation of the virus (7). RNA obtained from virus isolated by centrifugal fractionation did not differ from that of agent obtained by density gradient centrifugation. In addition, the components from tissue-culture virus were the same as those with plasma virus, but comprehensive studies have not been made on the homogeneity of the virus isolated from culture fluids.

2. Although ribosomes were not directly demonstrable in the virus preparations, it seemed feasible to test for occult amounts of such structures by use of RNase. Treatment of virus with the enzyme did not eliminate either the 16 S and 26 S or 5 S and 60 S components. The significance of the finding was emphasized by the efficacy of the enzyme to degrade 16 S and 26 S components in crude cell cytoplasmic homogenates added in large amounts to the virus preparations. RNase also degraded cell sRNA introduced into the virus preparations.

In all of the experiments with virus isolated from plasma, the findings indicated that the 4 RNA components demonstrated were derived from the virus particles and not from contaminants. The similarity of tissue-culture virus data to those with plasma agent suggested further that RNA from the former was also from the virus particles. This conforms to the previous evidence that the 60 S component is intrinsic to the virus particle, and, in addition, provides the basis for regarding the 5 S, 16 S, and 26 S RNA’s, likewise, as normal complements of the total virus RNA. The state of the 5 S RNA in the particle is not clear, but the inverse relationship between the 5 S and 60 S components (Chart 13) strongly suggests that a portion of the 5 S material is derived from the 60 S and possibly in part, though not demonstrably so, from the 16 S and 26 S components. Nonetheless, some 5 S RNA must have another origin, since amino acids were attached by 5 S material but not by 60 S RNA or its degradation products.

Although there is no direct evidence of the mechanism of
inclusion of the tRNA and 16 S and 26 S components in the particle, there is substantial basis for interpretations. The diagram (38) (Fig. 5) illustrates the continuity of the interior of the budding virus particle with cell cytoplasm, some of which might well be incorporated in the finished particle. Thus, ribosome RNA of 16 S and 26 S and tRNA, which are normal cell components, could easily be included in the particle. It is notable that this virus contains other cell constituents: (a) chicken tissue and Forssman antigens (18); (b) adenosinetriphosphatase from the myeloblast cell membrane (45); (c) cartilage fibrils in virus budded from chondrocytes (25); and (d) probably collagen in particles formed at the surface of mesenchymal cells (25). Moreover, virus lipid (38) is not derived entirely from the cell membrane but must reach the particle in large part by way of the cytoplasm. Other evidence that the virus particles contain cell-derived material was obtained (2) by isolation of 30 S and 45 S components consisting of RNA associated with protein. Fibroblasts labeled with uridine-14C were infected and then treated with an excess of uridine-14C. Virus isolated from the cultures and disrupted with sodium dodecyl sulfate yielded a 62 S component labeled principally with uridine-14C, whereas the 30 S, 45 S, and 4 S–10 S materials, which contained both labels, were much more strongly labeled with uridine-14C than was the 62 S fraction. While the RNA in the 30 S and 45 S components was not isolated, the findings show that RNA of cellular origin and different from the 60 S–70 S RNA occurs in the virus particle. Further study will be required to determine whether the 16 S and 26 S RNA components may be related to the 30 S and 45 S nucleoprotein constituents.

With some assumptions, tentative calculations may be made of total virus RNA content. The sedimentation rate 60 S was comparable with the 62 S (2) and 67 S (23) data, but the probably more accurate value 71 S (39) was observed by analytic ultracentrifugation. Application of Spirin’s formula (43) yielded the value of 12 \( \times 10^4 \) for the molecular weight corresponding to 71 S. Similar calculations for the 16 S and 26 S materials gave values of 1.4 \( \times 10^4 \) and 5 \( \times 10^4 \), respectively. The average molecular weight of tRNA is about 2.5 \( \times 10^4 \) (16). The sRNA contained in the virus may have the same proportion of tRNA as that in the cell sRNA. In the experiment of Table 2, the 5 S component comprised 27% of the total virus RNA and exhibited specific amino-acid-binding capacity 25% of that of the host cell sRNA. It follows that the amount of cell sRNA incorporated in the agent would be about 7% of the total virus RNA, far higher than would be expected from contaminating sRNA from the medium (Table 1). A suggested virus RNA composition (Table 4) might be one 60 S–70 S, one 26 S, one 16 S, and forty 5 S strands. These values are in the right range of % composition for the 16 S and 26 S RNA's which might conceivably represent 1 ribosome/virus particle and likewise account for the 5 S fraction tRNA activity. The remainder of the 5 S and the lower proportion of 60 S–70 S RNA found in the actual extracts (Table 3, Chart 13) would be the result of degradation of some 60 S strands.

From these computations, the total virus RNA would be about 15 \( \times 10^6 \) daltons/particle, which is more than previously estimated (10) on the basis of 2.2% RNA and a particle mass of 7.5 \( \times 10^{-18} \) gm corresponding to particles of 100-\( \mu \) average diameter. This discrepancy could be related to several factors: (a) Spirin’s formula (43) may not be wholly applicable to the high molecular weight virus RNA; (b) particle mass is difficult to measure precisely on the basis of electron microscope particle count and ultramicro dry weight determination (10); and (c) some of the virus particles may be lacking in part or entirely in RNA. It is apparent that sufficient data are not yet available for precise determination of the virus RNA composition, but the values in Table 4 may represent reasonable molecular proportions.

There is no evidence to indicate whether the included transfer and ribosome RNA's are critical for virus integrity or functional in the infectious process. Incorporation in the particle may be incidental, but it may be speculated that even then the relationship is more than casual. The site of virus component synthesis is yet obscure, but electron micrographs show (24, 45) that the major events of avian tumor virus assembly, that is, deposition of nucleoid RNA’s formation of internal bounding structures and application of the external particle membrane, occur at or within the bud. It appears that the locus of the forming bud thus corresponds in part to the focal cytoplasmic sites of synthesis observed with some of the other RNA agents (36). Immunofluorescence has revealed virus antigen only at the cell membrane (46), and antigen can be detached from the cell by trypsin (1). Strands of 60 S RNA may be assembled, likewise, in the bud and, as suggested for influenza virus (26), coiled circumferentially to form the nucleoid shell (Structure B, Fig. 5). Moreover, condensation-like rearrangement of the nucleoid structure is seen by electron microscope comparison of immature buds with mature particles after detachment from the cell membrane (45). Both ribosome RNA and tRNA situated immediately in the bud might be involved in some yet undefined manner in these processes as well as in bud formation. Of potentially greater significance is the possibility that ribosome and transfer RNA’s present and acting simultaneously with the virus-specific genome are requisite for initiating the preliminary stages of infection. It may be noted that exposure of cells to the isolated 60 S component has not sufficed to induce infection (2); D. P. Bolognesi and A. J. Langlois, unpublished observations.

ACKNOWLEDGMENTS

The authors are indebted to Mrs. Pe-Hwa L. Hsiung for the electron microscopic counts of virus particles.
REFERENCES


FIG. 1. BAI strain A virus was sedimented once from plasma, resuspended, and centrifuged at 3200 × g for 10 min to remove any large debris and aggregates. The virus was a sample of that used for velocity-density-gradient centrifugation as described for Chart 3A. Without further purification, the suspension was treated with 4% formaldehyde and 1% phosphotungstic acid (PTA) which had been adjusted to pH 7 with KOH, and a drop was dried on a carbon and Formvar-coated grid. The preparation consists almost entirely of typical virus particles, a few of which show characteristic distortions (11, 12) such as tail-like protrusions or blebbing. Some particles were penetrated by the PTA, revealing aspects of the internal structure. Indistinct amorphous material represents internal particle substance from disrupted particles and residual plasma protein. X 64,000.

FIG. 2. Virus was separated from plasma by 4 cycles of centrifugation, fixed with OsO₄, embedded, and sectioned. The section was stained with lead subacetate. The field is filled with virus particles sectioned at various levels. A structure which appears to be a flattened sac at the lower right is probably a collapsed virus envelope. No ribosomes are apparent. X 100,000.
FIG. 3. Electron micrograph of negatively stained virus after velocity density gradient centrifugation. The virus preparation illustrated in Fig. 1 was centrifuged into a 5%–20% sucrose gradient. A sample of virus selected from the resulting sedimentation band, indicated by the arrows in Chart 3A, was fixed for at least 30 min with 4% formaldehyde and dried in phosphotungstic acid. The structures in the micrograph can be identified as virus particles or their degradation products (11, 12). Treatment on the gradient caused damage to many particles, but many others remained intact. Formation of bleblike protrusions, “tails,” and disintegration of particles are seen. × 64,000.

FIG. 4. Electron micrograph of negatively stained virus after equilibrium density centrifugation. A virus preparation like that of Fig. 1 was centrifuged to equilibrium on a 2%–40% potassium tartrate density gradient, and virus was selected from the sedimentation band (Chart 3B). The preparation was fixed with 4% formaldehyde for at least 30 min and dried in phosphotungstic acid. Virus in this micrograph is very similar to that shown in Fig. 3, indicating particle damage and lack of contamination of extraviral material. × 64,000.
Fig. 5. Diagrammatic interpretation (38) of steps in BAI strain A virus budding at the myeloblast cell membrane based on electron micrographs [(45), Figs. 10-16]. Structure (B) represents electron-dense shell enclosing less dense material (A) continuous with the cytoplasm. The shell lies in a less dense substance (C) also continuous with the cytoplasm and bounded by a thin, denser layer (D) forming an irregular cloak which terminates indefinitely in the cytoplasm. More superficial is a layer of low density (E) continuous with the cytoplasm and an outer structure (F) merging with the physically demonstrable cell membrane.
Ribonucleic Acid Components of BAl Strain A (Myeloblastosis) Avian Tumor Virus


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