Properties of Feline Leukemia Virus

III. Analysis of the RNA

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The kinetics of virus labeling was used to study the maturation of viral RNA in the Rickard strain of feline leukemia virus. Viral RNA labeled over differing intervals was characterized by gel electrophoresis and velocity sedimentation in sucrose gradients made up in aqueous buffer and 99% dimethyl sulfoxide. Labeled virus was found within 30 min after adding radioactive uridine to the cells and production of labeled virus reached a maximum at 4 to 5 h after pulse labeling. Native RNA from feline leukemia virus resolved into three size classes when analyzed by electrophoresis on 2.0% polyacrylamide-0.5% agarose gels: a 6.2 \times 10^6 to 7.1 \times 10^6 mol wt (50 to 60S) class, an 8.7 \times 10^4 mol wt (approximately 8S) class, and a 2.5 \times 10^4 mol wt (4 to 5S) class. From two experiments during which RNA degradation appeared minimal, these made up 57 to 76%, 2 to 5%, and 6 to 12%, respectively, of the total RNA. The 8S RNA in feline leukemia virus has not previously been reported. The 50 to 60S RNA from virus harvested after 4 h of labeling electrophoretically migrated faster and sedimented more slowly in sucrose gradients than did the same RNA species harvested after 20 h of labeling. This argues for an intravirion modification of the high-molecular-weight RNA. The large subunits of denatured viral RNA from both 4- and 20-h labeled-viral RNA electrophoretically migrated with an estimated molecular weight of 3.2 \times 10^6 but sedimented with 28S ribosomal RNA (1.8 \times 10^4 mol wt) when analyzed by velocity sedimentation through 99% dimethyl sulfoxide.

The RNA tumor viruses from all species studied to date possess in common a high-molecular-weight, single-stranded RNA which is an aggregate of smaller molecules thought to be held together by hydrogen bonding (3, 9, 19). This aggregate can be denatured into its subunits by heat, urea, formamide, formaldehyde, or ME\textsubscript{3}SO (3, 9), and subunits of two major sizes result: a large 28 to 35S subunit which possesses a sequence of polyadenylic acid \textit{[poly(A)]} (16, 18, 23, 29), and small tRNA-like subunits which can be aminoclaylated (13, 14). In addition to the aggregate there are smaller amounts of 36S, 28S, 18S, and 4 to 10S RNA present in the virion (5, 6, 19, 24) which, in immature virus, may represent unassembled subunits that eventually become part of the aggregate (5, 6).

When this work was initiated, characterization of feline leukemia virus (FeLV) RNA was limited to one report (19). Based primarily on velocity sedimentation studies, it was shown that FeLV possesses a uniquely large proportion of native 35S and 4 to 10S RNA molecules when compared to other oncornaviruses. This uniqueness made it desirable to further characterize FeLV RNA under conditions which minimize degradation and with an analytical system which gives superior resolution (gel electrophoresis). These experiments were undertaken (i) to measure the interval between the uptake of radioactive uridine into infected cells and the release of free radioactive virus particles, (ii) to assess the effect of virion age on the molecular weight and relative distribution of native viral RNA molecules, and (iii) to determine the molecular weight of the denatured high-molecular-weight subunit of FeLV RNA.

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MATERIALS AND METHODS

Source of cells and viruses. The permanently infected feline thymus tumor line (F-422) which produces the Rickard strain of FeLV (28) and grows in...

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suspension culture, was obtained from C. G. Rickard (Cornell University). Cells were propagated as previously described (17). The Kansas-Manhattan strain of Newcastle disease virus (NDV) was obtained from R. P. Hanson (University of Wisconsin).

Adaptation of F-422 cells for monolayer growth. Cells which adhere to a flask surface were selected by periodically replacing media on a stationary flask of cells. A monolayer of adhering thymocytes could be detached by vigorous shaking. Progeny cells readily formed monolayers in plastic flasks or glass roller bottles, or readily grew in spinner suspension culture.

Isotopic labeling of FeLV RNA. Cells were incubated in 36 to 200 ml of medium containing 10% fetal calf serum and either [H]uridine (40 Ci/mM), [3H]uridine (57 mCi/mM), or both as described below. New England Nuclear Corp., Boston, Mass. was the source for all isotopes.

Purification of FeLV. For studying kinetics of viral labeling with radioactive uridine, virus was purified as follows. Cells were pelleted at 1,000 rpm for 5 min in an International PR-6 centrifuge, and cellular debris was removed from culture medium at 10,000 rpm for 10 min in a Sorval GSA rotor. Virus was precipitated from clarified medium in 50% (wt/vol) ammonium sulfate or 5% (wt/vol) polyethylene glycol for 16 h at C, and the precipitate was pelleted at 10,000 rpm for 20 min in a Sorval GSA rotor and suspended in 0.5 ml of TNE-7.5 buffer [0.01 M Tris (pH 7.5), 0.1 M NaCl, and 0.001 M EDTA]. Virus was isopycnically centrifuged in 5.0-ml gradients of 15 to 40% (wt/wt) sucrose made up in TNE-7.5 for 2.5 h at 50,000 rpm in an SW50.1 rotor (Beckman) at C.

For studying viral RNA, FeLV was purified as follows. Clarified culture medium was layered over 8 ml of 20% (wt/wt) sucrose made up in TNE-7.5, and centrifuged at 25,000 rpm in an SW27 rotor (Beckman) for 2 h. The viral pellet was resuspended in 1 ml of TNE-7.5 by 15 s of sonic treatment in a 150-W Branson Ultrasonic cleaner (Branson Instruments, Co., Stamford, Conn.), layered onto 4.5 ml of 20% (wt/wt) sucrose in TNE-7.5, and pelleted at 45,000 rpm in an SW50.1 rotor for 1.5 h.

RNA extraction. The viral pellet was resuspended in 0.3 ml of TNE-9 buffer [0.1 M Tris (pH 9.0), 0.1 M NaCl, and 0.001 M EDTA] by 15 s of sonic treatment. The suspension was made 1.0% sodium dodecyl sulfate by adding 10% sodium dodecyl sulfate in TNE-9, and an equal volume of Pronase (Calbiochem) at 500 

\[ \mu \text{g/ml} \text{in TNE-9 (self digested 2 h at 37°C)} \] was added to make a final concentration of 250 

\[ \mu \text{g/ml} \]. The solution was incubated 5 min at 37°C before extracting three times with an equal volume of TNE-9 saturated phenol. When necessary, carrier RNA (5 absorbancy units [260 nm] per ml, Torula grade B RNA, Calbiochem) was added before precipitating in 67% ethanol at –20°C.

Preparation of cytoplasmic RNA. Cytoplasmic RNA was prepared from [H]uridine-labeled feline thymus tumor cells by the method of Erikson and Erikson (14). Rat cytoplasmic RNA was a gift from Ron Desrosiers (Michigan State Univ.).

Preparation of [3H]-labeled NDV RNA. Radioactive labeling, virus purification, and RNA extraction methods were modified procedures of Duesberg and Robinson (10) and Kingsbury (20). Labeled virus was pelleted from clarified allantoic fluid through 8 ml of 20% (wt/wt) sucrose made up in TNE-7.5 buffer at 25,000 rpm for 4 h in an SW27 rotor at C. The viral pellet was resuspended in TNE-7.5 buffer and isopycnically centrifuged on a 5.0-ml gradient of 65% (wt/wt) sucrose in D2O to 20% (wt/wt) sucrose in TNE-7.5 for 15 h at 35,000 rpm in an SW50.1 rotor at C. The virus was collected and pelleted at 45,000 rpm for 1 h in an SW50.1 rotor at C, and frozen at –76°C. RNA was extracted as described for FeLV RNA.

Polyacrylamide-agarose gel electrophoresis. The method of Peacock and Dingman (27), as described by Bunting (S. L. Bunting, submitted for publication), was modified for use in tubes to facilitate accurate fractionation in a Gilson gel fractionator. Ten tubes (18 × 0.5 cm ID) each containing 3.0 ml of 2.0% polyacrylamide-0.5% agarose were prepared as follows. A 0.16-g amount of agarose (Bio-Rad) was refixed 15 min with stirring in 22.6 ml of water and cooled to 48°C. Simultaneously, 3.2 ml of 20% acrylamide-bisacrylamide solution [20% (wt/vol) water solution of cyanogen 41, Fisher] was combined with 3.2 ml of Peacock’s 10-fold concentrated electrophoresis buffer (0.89 M Tris, 0.89 M boric acid, and 0.025 M EDTA in water) and warmed to 48°C. Within 1 min, (i) 1.0 ml of a 1.6% (wt/vol) ammonium persulfate solution was mixed into the agarose solution, (ii) 2.0 ml of a 6.4% (wt/vol) 3-dimethylaminopropionitrile solution was mixed into the acrylamide-bisacrylamide buffer solution, (iii) all solutions were mixed well together and dispensed into tubes that had been treated with Photoflo (Kodak). Gels were allowed to polymerize for 1 h at room temperature, capped, and stored at 4°C. Just before use, gels were slightly displaced with a Gilson gel piston and sliced transversely with a razor blade, forming a flat surface for the RNA sample, and retained for electrophoresis with perforated paraffin. Samples were electrophoresed at 150-V constant voltage at 4°C for approximately 3.0 h in Peacock’s electrophoresis buffer and gels were fractionated into 2-mm fragments.

Oligo(deoxythymidylic acid)-cellulose chromatography. Oligo(deoxythymidylic acid)-cellulose was prepared essentially as described by Gilham (15) and the chromatographic method was essentially that of Aviv and Leder (1, 7). RNA cosedimenting with 28S rRNA on ME$_2$SO gradients was precipitated in 67% ethanol after making the pooled fractions 0.1 M NaCl. The precipitate was dried in a nitrogen stream, redissolved in high salt buffer [0.01 M Tris (pH 7.4), 0.5 M NaCl, 0.001 M EDTA, and 0.2% sodium dodecyl sulfate] and applied to a column (0.5 × 6 cm) previously equilibrated with this buffer. Poly(A) (−) RNA was eluted by washing the column with high salt buffer. Poly(A) (+) RNA was eluted with low salt buffer [0.01 M Tris (pH 7.4), 0.001 M EDTA, and 0.2% SDS].

RESULTS

Kinetics of viral labeling. Continuous labeling experiments using F-422 feline thymus tumor cells (Fig. 1) demonstrate that radioac-
The virus was detected as early as 30 min after addition of the radioactive RNA precursor. No characterization was made of the viral RNA labeled within 30 min. Pulse-chase experiments during which 56% of the intracellular nucleotide precursor pool disappeared by 2 h (D. Brian, data not shown) demonstrate that production of labeled virus reached a maximum at 4 or 5 h after the beginning of the pulse (Fig. 2).

**Gel electrophoresis and velocity sedimentation of native viral RNA.** In an effort to resolve the entire RNA content of FeLV into size classes and to obtain an approximation of corresponding molecular weights and percentage composition, electrophoresis was done in combination gels of 2.0% polyacrylamide-0.5% agarose (27). Figure 3 illustrates the results obtained when virus was uridine labeled for 4 or 20 h and the RNA was extracted and electrophoresed. In each of six separate experiments, three molecular weight size classes were identified: a $6.2 \times 10^4$ to $7.1 \times 10^4$ mol wt class, an $8.4 \times 10^4$ mol wt class, and a $2.5 \times 10^4$ mol wt class. In two of the experiments, during which RNA
degradation appeared minimal, these size classes made up 57 to 76%, 2 to 5%, and 6 to 12% of the total RNA, respectively, with the remainder being heterogeneously dispersed throughout the gel. Molecular weights were determined by reference to $^{32}$P-labeled feline cytoplasmic 4S tRNA, 18S and 28S rRNA included in each gel with FeLV RNA, or to $^{32}$P-labeled 50S NDV RNA (20) included in plasmic RNA run in parallel gels under identical conditions. Under these electrophoretic conditions a straight line relationship existed for migration distances versus molecular weights for all marker molecules (see insert, Fig. 3). The electron microscopically determined molecular weight of $5.2 \times 10^4$ to $5.6 \times 10^4$ mol wt (22) was used for NDV. To use this graph for extrapolating the molecular weight of 50 to 60S viral RNA, it was assumed that a straight line could be projected for up to three fractions beyond NDV (insert, Fig. 3).

Velocity sedimentation in sucrose gradients (Fig. 4) resolved FeLV RNA into a broadly sedimenting 50 to 60S class and a group of unresolved molecules sedimenting between 4 and 10S. The sedimentation coefficient of 50 to 60S was determined by the method of Martin and Ames (26) in reference to $^{32}$P-labeled 50S NDV RNA included in the gradient. The sedimentation coefficient of the 8S class was not measured. It is tentatively called 8S because its electrophoretic mobility appeared similar to the 8S species reported in the murine sarcoma virus (13, 24). Trichloroacetic acid-precipitable RNA from the 4 to 10S region of sucrose gradients repeatedly represented 25 to 50% of the total viral RNA (Fig. 4), contrasting with the 9 to 26% recovered from gels (Fig. 3). This high percentage is similar to the findings of Jarrett et al. in which phenol-extracted FeLV RNA is analyzed on sucrose gradients (19). The occurrence of small-molecular-weight RNA in the gradients was not decreased by making the gradient sucrose solutions 0.1% sodium dodecyl sulfate.

Effects of labeling time on native viral RNA. In six experiments in which virus was doubly labeled, 4 h with $[^3H]$uridine and 20 h with $[^{14}C]$uridine, the 50 to 60S RNA of 20-h RNA electrophoretically migrated 0.5 to 1.0 fraction slower than the 50 to 60S RNA from 4-h virus, giving an average difference of 0.7 fractions. This represents an apparent differ--
ence in molecular weights of 0.5 to 10⁴ mol wt in this region of the gel (insert, Fig. 3). By comparison to the reference RNA molecules, mean molecular weight estimates of 6.4 x 10⁴ and 7.1 x 10⁴ mol wt were derived for the 4- and 20-h high-molecular-weight molecules, respectively (Table 1).

The apparent size difference in the 50 to 60S RNA between 4- and 20-h labeled virus was confirmed by subjecting the RNA from one experiment to velocity sedimentation in an aqueous sucrose gradient (Fig. 4). Sedimentation coefficients of 54S and 60S were measured for 4- and 20-h viral RNA, respectively.

These results confirm the findings by East et al. (12) in which FeLV RNA collected after 2 h of labeling had a sedimentation coefficient of 50S, whereas after 20 h of labeling it had a coefficient of 58S. The explanation offered by East et al. (11) is that an intravirion structural modification of the RNA, either by a joining of subunits or an alteration in the secondary structure of an already assembled molecule, gives the older form of the viral RNA (20 h) a higher sedimentation coefficient. If this explanation is correct, then one should see a more slowly sedimenting 50 to 60S molecule in virus particles released over any 2- to 12-h period, not just the first period after addition of isotope. To test this, cells which had undergone 20 h of labeling with [³H]uridine 4 h with [⁴C]uridine, were suspended in fresh medium (with no isotopes) for an additional 4-h period. Under these conditions, [¹⁴C]uridine-labeled viral RNA becomes incorporated into the virion during the 20- to 24-h interval postlabeling from an intracellular pool of [¹⁴C]-labeled viral RNA. Likewise the [³H]-labeled RNA becomes incorporated during the 4- to 8-h interval postlabeling.

The 50 to 60S RNA incorporated during the 4- to 8-h and 20- to 24-h postlabeling periods electrophoretically comigrate (Fig. 5). Averages from three experiments show them respectively migrating with apparent molecular weights of 6.2 x 10⁴ and 6.3 x 10⁴ (Table 1).

Recent studies with Rous sarcoma virus (RSV) demonstrate the existence of unassembled 35S subunits in newly formed virus particles, particles 3 min to 1 h in age (5, 6). Such subunits were sought in 1-h FeLV. F-422 cells were adapted for adherence to flasks were used to facilitate rapid medium changes. Adapted cells were grown to concentrations of 10⁹/ml in spinner culture and then placed in a roller bottle to which most of the cells adhered forming a near-monolayer in 8 h. Unattached cells were removed by pipetting, and adhered cells were incubated with [³H]uridine for 16 h, after which collections of culture medium were made at 1-h intervals. Figure 6 demonstrates the electrophoresis of [³H]-labeled 1-h viral RNA, prepared from monolayer cells with [¹²⁵I]-labeled NDV RNA. No 35S subunits were found in the 1-h virus (Fig. 6). The 4S, 8S, and 50 to 60S RNA species were present in the amounts of 21, 3, and 58%, respectively.

**Gel electrophoresis and velocity sedimen-**

![Graph](image-url)
Table 1. Electrophoretic molecular weight estimates of FeLV RNA species

<table>
<thead>
<tr>
<th>RNA species</th>
<th>Interval of virus labeling (h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0→20</td>
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<tr>
<td>50-60S RNA</td>
<td>7.1 ± 0.5 x 10^9 (a)</td>
</tr>
<tr>
<td>High-mol-wt subunits in ME₃SO-treated RNA</td>
<td>3.2 ± 0.4 x 10^9 (c)</td>
</tr>
<tr>
<td>8S RNA</td>
<td>8.7 ± 0.5 x 10^9 (d)</td>
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* Average of six experiments; three using NDV marker RNA, three using rRNA markers.
* Average of three experiments, each using NDV marker RNA.
* Average of three experiments, each using rRNA markers.
* Average of five experiments.

Fig. 5. Electrophoresis of native 4→8-h and 20→24-h FeLV RNA. Cells from the experiment described in Fig. 3 were resuspended in fresh unlabeled medium for 4 h. All other steps were carried out as described in Fig. 3 except that FeLV RNA was ethanol precipitated with carrier before dissolving in electrophoresis buffer along with \(^{32}P\)-labeled NDV RNA.

**tation of denatured viral RNA.** The high-molecular-weight subunits of denatured viral RNA were studied for the purpose of making a careful estimate of their molecular weight and determining whether differences in size (length) or integrity of these subunits might explain the observed differences in migration rates between the 4- and 20-h 50 to 5S molecules. Figure 7 illustrates the results obtained when viral RNA was labeled for 20 h with \([^{14}C]\)uridine and 4 h with \([^{3}H]\)uridine, extracted, treated with 90% ME₃SO, and electrophoresed with rRNA markers included in the gel. From three separate experiments the average molecular weight of the high-molecular-weight subunit was estimated to be 3.2 x 10^9 for both 20- and 4-h viral RNA (Table 1). When RNA prepared the same way was analyzed in one experiment by the method of velocity sedimentation in sucrose gradients made up in 99% ME₃SO (Fig. 8), the high-molecular-weight subunits for both 20- and 4-h RNA cosedimented or nearly cosedimented with 28S rRNA, giving a molecular weight estimate of 1.8 x 10^9 (25).

By both methods of analysis and in every experiment, high-molecular-weight subunits from RNA labeled over the 20-h interval electrophoretically migrated or sedimented with a broader, more heterogeneous pattern (Fig. 7 and 8). Such a heterogeneous pattern was not ob-
Fig. 6. Electrophoresis of native 1-h FeLV RNA. FeLV labeled 1 h with \([^{1}H]\)uridine was prepared by incubating \(4 \times 10^{7}\) F-422 cells in monolayer with 200 \(\mu\)Ci of uridine for 16 h, after which a series of eight 1-h collections were made. Harvested culture medium was kept at 0 C until all collections were made and virus was purified immediately after the last 1-h harvest period. Virus purification and RNA extraction was as described in text. Addition of \(^{32}P\)-labeled NDV RNA and electrophoresis was as described in Fig. 3.

Fig. 7. Electrophoresis of denatured FeLV. Virus was labeled as described in Fig. 3. Extracted RNA was ethanol precipitated with carrier RNA, solubilized in water containing 0.001 M EDTA, made 90% with 99% ME\(_{2}\)SO and incubated 5 min at 56 C. RNA was ethanol precipitated again, solubilized in electrophoresis buffer, made 10% sucrose and 0.006% bromophenol blue, and electrophoresed with \(^{32}P\)-labeled feline thymus tumor cell cytoplasmic RNA. Electrophoresis and gel fractionation were described in Fig. 3.
served for native viral RNA (Fig. 3). This suggested that the 50 to 60S aggregate in the 20-h virus possessed discontinuities, perhaps endonuclease nicks, which became apparent only after complete denaturation.

**DISCUSSION**

The minimum interval for labeling FeLV in F-422 cells with an RNA precursor (uridine) was

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**Fig. 8.** Velocity sedimentation of FeLV RNA in ME$_3$SO gradient. $^{14}$C-labeled 20-h FeLV RNA and $^3$H-labeled 4-h FeLV RNA were prepared as described in Fig. 3 and ethanol precipitated with carrier. Pelleted precipitate was dried in a nitrogen stream, solubilized in water with 0.001 M EDTA and 0.1% sodium dodecyl sulfate, made 90% ME$_3$SO, incubated 5 min at 56 C, and layered onto a 5.0-ml linear gradient of 5 to 20% (wt/wt) sucrose (ribonuclease-free) made up in 99% ME$_3$SO [reagent grade 99.2% ME$_3$SO (Fisher), and 0.001 M EDTA. Centrifugation was for 15 h at 45,000 rpm in an SW50.1 rotor at 25 C. $^3$H]uridine-labeled rat cytoplasmic RNA was centrifuged on a parallel gradient. Fractionated gradients were assayed as described (17).

**Fig. 9.** Oligo(deoxythymidylic)-celullose chromatography. F-422 cells in suspension at $3 \times 10^6$/ml were incubated 16 h with 4 $\mu$Ci of $^3$H]uridine per ml. Virus was purified and RNA was extracted as described in text. RNA was ethanol precipitated with carrier, dissolved in water with 0.001 M EDTA and 0.1% sodium dodecyl sulfate, made 90% with 99% ME$_3$SO, heat dissociated at 56 C for 5 min, then sedimented through 99% ME$_3$SO as described in Fig. 8. The 28S peak (profile was very similar to Fig. 3, data not shown) was collected, made 0.1 M NaCl, and ethanol precipitated with carrier. The precipitate was dried in a nitrogen stream, dissolved in high salt buffer and chromatographed on oligo(deoxythymidylic acid)-cellulose as described in text. Elution with low salt buffer started at fraction 16.
30 min. This short interval of virus production is similar to the Sohner-Dmochowski murine sarcoma virus described by East et al. (11), but shorter than the 80-min interval for Rauscher murine leukemia virus described by Bader (2) and for the 1.5- and 2-h interval for avian myeloblastosis virus and Rous sarcoma virus—Rous associated virus (RSV-RAV) described by Baluda and Nayak (4). While no characterization was made of the RNA labeled within 30 min, it is unlikely that cell vesicles are giving this result since rRNA appears absent in virus particles purified by less stringent means (Fig. 3 and 6). It remains to be determined whether the shorter interval for FeLV labeling is a reflection of a higher rate of virus production or more efficient uridine incorporation. Pulse-chase experiments demonstrated what production of labeled FeLV reached a maximum at 4 to 5 h after the beginning of the pulse (Fig. 2). This interval is similar to the 5-h interval seen for RSV-RAV, and murine leukemia virus (2) and the 3- to 6-h interval seen for avian myeloblastosis virus (4), and was used as the earliest interval, following addition of the isotope, over which virus was collected for examining RNA.

Analysis of FeLV RNA by gel electrophoresis yielded three size classes of RNA: (i) a 6.2 x 10^4 to 7.1 x 10^4 mol wt class sedimenting with a 50 to 60S sedimentation coefficient and comprising 57 to 76% of the total viral RNA; (ii) an 8.7 x 10^4 mol wt class not yet reported in feline leukemia or sarcoma viral RNA corresponding to the 8S RNA reported in murine sarcoma virus RNA (13, 24), comprising 2 to 5% of the total RNA; and (iii) a 2.5 x 10^4 molecular weight class sedimenting with 4S cellular tRNA and comprising 6 to 12% of the total viral RNA. Assuming that the 50- to 60S molecule exists at the rate of 1/virion, then the 8S molecule was present to the extent of 2 to 7/virion, and the 4 to 5S molecule was present to the extent of 20 to 60/virion. RNA species sedimenting as 18S, 28S, or 34S were not detectable, and in this respect the Rickard strain of FeLV appears to differ from that used by Jarrett et al. (19).

The molecular weight estimate of 6.2 x 10^4 to 7.1 x 10^4 for the 50 to 60S RNA was based on the demonstration by Peacock and Dingman that a straight line relationship exists between the electrophoretic migration distance of a single-stranded RNA molecule and the log of its molecular weight (27). The molecular weight of the 50 to 60S RNA was extrapolated under conditions in which a straight line could be plotted among the 5.2 x 10^4 to 5.6 x 10^4 mol wt NDV RNA, 1.8 x 10^4 mol wt 28S feline rRNA, 0.7 x 10^4 mol wt 18S feline rRNA, and 0.25 x 10^4 mol wt tRNA. To make this extrapolation we assumed that a straight line can be projected for up to three fractions beyond the NDV marker RNA (insert, Fig. 3). The molecular weight estimate of 6.2 x 10^4 to 7.1 x 10^4 is lower than the 9.7 x 10^4 to 11.7 x 10^4 obtained when one applies Spirin's equation (30): M = 1550 x s^2 to a 50- to 60S molecule. The molecular weight derived for a large aggregate molecule by either of these methods is tentative, however, since single-stranded RNA molecules of 0.3 to 2.1 x 10^4 mol wt in size were used to demonstrate the validity of these methods (27, 30).

The measured sedimentation coefficient of 50 to 60S agrees closely with 50 to 58S for the Rickard strain of feline leukemia virus measured by East et al. (12). A direct comparison under identical conditions is necessary to resolve what appears to be a significant difference between the high-molecular-weight RNA of Rickard strain FeLV and other (A, B, and C) strains for which a sedimentation coefficient of 74S is obtained (19, 33).

The electrophoretic mobility of the 50 to 60S molecule was variable depending upon the length of time over which the virus was labeled. High-molecular-weight RNA from virus labeled for 20 h has an apparent molecular weight of 7.1 x 10^4, whereas RNA from virus labeled for 4 h has an apparent molecular weight of 6.4 x 10^4. These data confirm and extend findings by East et al. in which the sedimentation coefficients for the Rickard strain of FeLV were measured to be 58S and 50S, respectively, for 20- and 2-h viral RNA (12). Attempts to explain this observed difference have hinged on a maturation hypothesis, i.e., that the modification giving rise to a faster sedimenting molecule occurs within the virion after budding (5, 6, 11, 12). Immature (3 to 60 min) RSV contains subunits from 15 to 60S which apparently assemble into the larger aggregate of 68S (5, 6). Assembly of a 58S molecule in murine sarcoma virus (11) and FeLV (12) is suggested to arise from two or more 50S molecules. While no RNA species ranging from 8- to 50S were identified in FeLV harvested at 1-h intervals (Fig. 6) or after 4 h of labeling (Fig. 3), any assembly process occurring between 4 and 20 h giving rise to a slower electrophoretically migrating (faster sedimenting) 60S molecule would seem to occur between two or more 50S species or a 50S molecule and smaller (4 to 8S) molecules. For technical reasons, harvest intervals of less than 1 h were not studied. Our data do not rule out the possibilities of an assembly process, but rather open a third alternative explanation. The denatured high-molecular-weight subunits from
20-h RNA are shown to electrophorese and sediment with a more heterogeneous pattern than the same RNA species from 4-h viruses (Fig. 7 and 8). It is conceivable, therefore, that nicks harbored within the loops of an aggregate molecule (20 h) might allow secondary structural changes to occur (perhaps the ends of the loops becoming more tightly folded) thereby creating a slower electrophoretically migrating (faster sedimenting) molecule compared to the unnicked aggregate (4 h). If this is true, then what we observe as a difference in electrophoretic migration and sedimentation rates between 4- to 20-h 50 to 60S RNA is not the result of a true difference in molecular weight, but only structural rearrangement of the molecule. It has been shown that 60 to 70S molecules of RSV apparently retain their aggregate structure while possessing nicks which are revealed only after the aggregate has been denatured (2).

The molecular weight of the large viral RNA subunit after ME$_3$SO denaturation is estimated to be 3.2 \( \times \) 10$^6$ by gel electrophoresis with reference to feline RNA (Fig. 7, Table 1). The same subunits sediment with 28S RNA on a ME$_3$SO gradient, however, (Fig. 8) giving them an estimated molecular weight of 1.8 \( \times \) 10$^6$. This discrepancy is remarkably similar to the phenomenon observed for Sendai viral RNA by Kolakofsky and Bruschi (21) and can be explained if viral (subunit) RNA possesses a greater degree of secondary structure than 28S RNA in the undenatured state or behaves anomalously because of its poly(A) content. The molecular weight estimate of 3.2 \( \times \) 10$^6$ is somewhat larger than the electrophoretically determined 2.2 \( \times \) 10$^6$ mol wt (33) or the electron microscopically determined 2.5 \( \times \) 10$^6$ mol wt estimated for the subunit of other FeLV strains. This again may reflect a difference between the Rickard strain and other strains used. From our data, the Rickard strain of FeLV 50 to 60S (6.2 \( \times \) 10$^4$ to 7.1 \( \times \) 10$^4$ mol wt) RNA is composed of as few as two 3.2 \( \times \) 10$^4$ mol wt subunits, or as many as four 1.8 \( \times \) 10$^4$ mol wt subunits.

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LITERATURE CITED