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Physicochemical Studies on L-Cell Virions

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The L-cell virion (LCV) has been purified from supernatant fluids of mouse L cells grown in suspension culture. The virion is similar to other RNA tumor viruses by several criteria: (i) the density of the virion is 1.16 g/cm^3; (ii) the virion appears as a rounded membranous particle with an outer diameter of 146.7 $\pm$ 11.8 nm, and contains knobs (7-nm diameter) over its surface; (iii) 15 polypeptides (ranging in molecular weight from 7,000 to 110,000) are detectable after electrophoresis of virion protein in sodium dodecyl sulfate-polyacrylamide gels; (iv) three species of RNA can be isolated—high molecular weight (80 to 88*s*) (50%), 7*s* (35%) and 4*s* (15%); (v) heat denaturation of the high-molecular-weight RNA yields a heterogeneous population of molecules (20 to 35*s*) as well as a 7*s* and 4*s* species. Despite the general similarity to infectious RNA tumor viruses, LCV is apparently defective as evidenced by the fact that it does not induce tumors in animals or transform normal mouse cells in vitro (Kindig and Kirsten [17]). The defective nature of the LCV might be related to the fact that assays for DNA polymerase in the virion showed only a negligible activity when compared to Rous sarcoma virus.

The presence of a C-type virus particle in established lines of mouse L cells was first reported by Dales and Howatson (7). Subsequent studies have shown that this L-cell virion (LCV) banded at 1.16 to 1.17 g/cm^3 in sucrose gradients, was unable to induce tumors in mice or rats, and did not replicate in other cell lines (Kindig and Kirsten [17]; Kindig et al. [18]). Faras and Erikson (13) have shown in a preliminary characterization of LCV RNA that there is both a high-molecular-weight component (82*s*) and a low-molecular-weight RNA component (6*s*). Further, they found that the high-molecular-weight RNA dissociates upon heat denaturation into a homogenous RNA species with a sedimentation coefficient of approximately 33*s*. In this paper we present a more detailed report on the physicochemical properties of the LCV RNA, as well as additional studies on the morphology, polypeptide constituents, and DNA polymerase activity of the virion.

These studies were undertaken as an initial effort toward determining the defective nature of the LCV.

MATERIALS AND METHODS

Virus purification. Mouse L cells were propagated in Eagle minimal essential medium (MEM) (Joklik's Modification, Grand Island Biological Co.) containing 5% fetal calf serum. Cultures to be used for the isolation of virions were allowed to grow for 24 h after the cell suspension had reached a concentration of 10^6 cells per ml.

For isolation of the virions, the cells were removed from the medium by centrifugation at 10,500 $\times$ *g* for 10 min. All subsequent procedures were carried out at 4 to 10 C. Ammonium sulfate (360 g/liter) was added to the supernatant fluid; a pH of approximately 7.0 was maintained by dropwise addition of 10 M NaOH. The solution was allowed to stand overnight. The precipitate was removed by centrifugation at 10,500 $\times$ *g* for 10 min and suspended by the addition of 0.05 M tris-(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.2), 10^{-3} M ethylenediaminetetraacetic acid (EDTA), and 0.15 M NaCl. The crude suspension was centrifuged at 12,000 $\times$ *g* for 10 min to remove any remaining cellular debris or insoluble protein. The virions were then sedimented to the interface of a discontinuous gradient containing 1 ml of 60% sucrose and 2 ml of 38% sucrose (solutions were made in 0.05 M Tris-hydrochloride [pH 7.2], 10^{-3} M EDTA, 0.15 M NaCl). Centrifugation was for 2 h at 30,000 rpm in an SW41 rotor. Virions were collected from the top of the 60% sucrose shelf and dialyzed against 0.05 M Tris-hydrochloride (pH 7.2), 10^{-3} M EDTA, and 0.15 M NaCl overnight.

The virion suspension was next subjected to centrifugation through linear 30 to 60% sucrose density gradients (in STE; 0.01 M Tris-hydrochloride [pH 7.2], 10^{-3} M EDTA, 0.15 M NaCl) for 3 h at 30,000 rpm in an SW41 rotor. The virion band, which sedimented about halfway through the gradient, was collected, dialyzed against STE

overnight, and further purified by centrifugation through linear 10% sucrose to 30% Ficoll gradients for 1 h at 30,000 rpm in an SW41 rotor. The collected bands were diluted fivefold in STE and pelleted by centrifugation in an SW50 rotos for 1 h at 40,000 rpm.

Electron microscopy. The method of depositing carbon films on 400-mesh copper grids (Ladd) as well as adhering viral specimens to the support film has been described previously (21). The virus droplet was allowed to adhere to the support film for 2 min, and the grid was then floated face down on a 5-ml solution of 1.5% glutaraldehyde (Ladd) in BUM (phosphate buffer containing 0.7 g of Na_2HPO_4, 0.3 g of KH_2PO_4, 0.4 g of NaCl, 2.4 g of $MgSO_4$ in 1 liter of water, pH 6.7) for 6 min. The grid was then stained with 2% uranyl acetate (pH 4.2) for 2 to 4 min.

Specimens were examined in a Philips EM300 electron microscope. Catalase crystals were used for calibration (20). Counting of particles was performed at a magnification of ×17,000, as previously described (21).

Preparation of radioactive virion RNA. ^{32}P-LCV was prepared by suspending L cells in phosphate-free MEM containing 5% fetal calf serum at a concentration of 2×10^6 cells per ml. After 8 h, carrier free ^{32}P-orthophosphate (Schwarz/Mann) was added to a final concentration of 5 μCi/ml. Twelve hours later, the cells were removed by centrifugation (300 × *g* for 10 min) and resuspended in fresh MEM which contained 10 μCi of ^{32}P-orthophosphate per ml. After 24 h the culture was diluted with an equal volume of phosphate-free MEM containing 10 μCi of ^{32}P-orthophosphate per ml. The cell suspension was maintained at 37 C for an additional 24 h at which time the virions were isolated and purified. The specific activity of RNA isolated from the virions was 30 to 50,000 counts per min per μg.

For the incorporation of ^3H-adenosine into LCV RNA, cells were suspended in Eagle MEM containing 10% fetal calf serum, at a concentration of 2×10^6 cells per ml. The medium contained 4 μCi of ^3H-adenosine per ml (New England Nuclear Corp.; 14 Ci/mmol), and incorporation was allowed to proceed for 24 h before the virions were isolated and purified. The specific activity of RNA isolated from the virions was 1,500 to 2,000 counts per min per μg.

Isolation of virion RNA. Suspensions of virions in STE were made 0.1% in sodium dodecyl sulfate (SDS) by the addition of a 10% SDS solution and immediately shaken with an equal volume of water-saturated phenol. The phenol phase was reextracted with buffer, and the RNA in the combined aqueous phases was precipitated with three volumes of 95% ethanol at −20 C overnight. The RNA precipitate was removed by centrifugation at 32,000 × *g* for 30 min and washed twice with cold 95% ethanol.

Sucrose density gradient fractionation of virion RNA. Ethanol-precipitated RNA was briefly placed under a vacuum to remove any remaining ethanol and then dissolved in 200 μliters of STE. The solution was layered on top of 5 to 20% linear sucrose gradients (in STE) and centrifuged for 45 min at 43,000 rpm in an SW50 rotor. Twenty-drop fractions were collected from the bottom of the tube.

Denaturation of high-molecular-weight RNA. High-molecular-weight RNA dissolved in STE was heated at 70 C for 3 min and then cooled rapidly in an ice bucket (13).

Polyacrylamide gel electrophoresis: RNA. RNA was subjected to electrophoresis either on cylindrical (120 mm in length and 7 mm diameter) or slab (250 mm by 10 mm by 2 mm) 10% polyacrylamide gels (1, 25) at 10 C. RNA samples in a volume of 50 to 100 μliters, containing a final concentration of 20% sucrose and 0.02% bromophenol blue, were layered on the top of the gel, and electrophoresis was carried out at a current of 5 mamp per tube in the case of the cylindrical gels or 40 mamp in the case of the slab gels until the marker dye approached the bottom of the gel. The gel was then fractionated into 2-mm slices and each slice was treated with 1 ml of Protosol (New England Nuclear Corp.) at 37 C overnight prior to the addition of 20 ml of toluene-based scintillation fluid. The samples were counted in a Beckman scintillation spectrometer.

Polyacrylamide gel electrophoresis: proteins. Solutions of virion proteins (50 to 150 μg) in a volume of 100 μliters (in STE) were combined with 100 μliters of 10 M urea containing 2% SDS and 2% β-mercaptoethanol. These solutions were placed in a boiling-water bath for 2 min and, after cooling, 50 μliters of a 60% sucrose solution containing 0.1% bromophenol blue was added to each. The samples were subjected to electrophoresis on 7.6% or 10% polyacrylamide gels (ratio of acrylamide to bis-acrylamide was 37.5:1) which were 0.1 M in sodium phosphate (pH 7.2), 5.8 M urea, and contained 0.1% SDS. Electrophoresis was carried out at room temperature at a current of 4 mamp per tube for 16 to 20 h. The electrophoresis buffer consisted of 0.1 M sodium phosphate (pH 7.2), 0.02 M EDTA, and 0.1% SDS.

The gels were stained for 2 h with a 0.2% solution of Coomassie brilliant blue in acetic acid-methanol-water (1:5:4) and then destained in 7% acetic acid for 48 h. Spectrophotometric scans of the stained proteins in the gels were obtained using a Quick-Scan apparatus (Helena Laboratories).

Molecular weight estimates of virion proteins. Estimates of the virion protein molecular weights using SDS-polyacrylamide gels (Shapiro et al. [32]) were obtained using the following proteins as standards: myosin (220,000), phosphorylase-a (93,000), bovine serum albumin (68,000), pepsin (35,500), trypsin (23,300), cytochrome c (12,380), and reovirus proteins λ1 (155,000), λ2 (140,000), μ1 (80,000), μ2 (72,000), σ2 (38,000), and σ3 (34,000) (33). The proteins were purchased from Sigma Chemical Co. with the exception of trypsin which was obtained from Worthington Chemical

Corp. Myosin was kindly provided by M. Adelman and reovirus by W. K. Joklik.

Protein and RNA determinations. Protein determinations were by the method of Lowry et al. (19) using bovine serum albumin as standard; RNA determinations were by the method of Mejbaum (22) using wheat embryo ribosomal RNA as standard (Calbiochem: $E^{1\%}_{260}$ = 203).

DNA polymerase activity. DNA polymerase was assayed by a method essentially similar to that described by Garapin et al. (14). The stock reaction mixture contained: 80 pmol of [*methyl*-^{3}*H*]deoxythymidine 5′ triphosphate (dTTP) (9,875 counts per min per pmol; Schwarz/Mann), 200 pmol of dTTP, 20 nmol of dATP, 20 nmol of dCTP, 20 nmol of deoxyguanosine 5′ triphosphate, 2 μmol of $MgCl_2$, β-mercaptoethanol (4%), Triton X-100 (0.2%), and 20 μmol of Tris-hydrochloride (pH 8.3). Equal volumes (0.05 ml) of the stock reaction mixture and virus suspension were mixed and incubated for 30 min at 38 C. The reaction mixtures were then chilled, and an equal volume of 0.1 M sodium pyrophosphate was added, followed by one drop of 0.25% bovine serum albumin and 2.5 ml of 5% trichloroacetic acid. The precipitates were collected on membrane filters (type HA, Millipore Corp.) and counted in a toluene-based scintillation fluid.

RESULTS

Purification of the LCV. Most purification procedures for RNA tumor viruses have included both sedimentation velocity and equilibrium banding steps (8, 9, 13, 28). We have employed such procedures for isolating the LCV after an initial concentration step with ammonium sulfate. During the isolation and purification procedures (described in Materials and Methods), LCV always behaved as a single homogeneous population with a density of 1.16 g/cm^3 (average of three experiments). This is illustrated in Fig. 1, where it can be seen that the peak of absorbancy (260 nm) of the virions following sucrose density gradient centrifugation is coincident with the numbers of particles observed by electron microscopy. As a further indication of purity, the amount of protein and RNA in the virion preparations was determined. Approximately 100 to 150 μg of purified LCV was obtained from one liter of culture medium. Electron microscope examination of purified preparations indicated that at least 90% of the particles appeared as virion structures. The RNA-to-protein ratio in these preparations, estimated on a weight basis, was 1:36. This ratio is in agreement with the results of Quigley et al. (27), which show 1.9% RNA content for avian tumor viruses. The LCV also contain phospholipids (K. Quade and J. Nichols, unpublished observations) but their contribution to the total mass of the particle is not known.

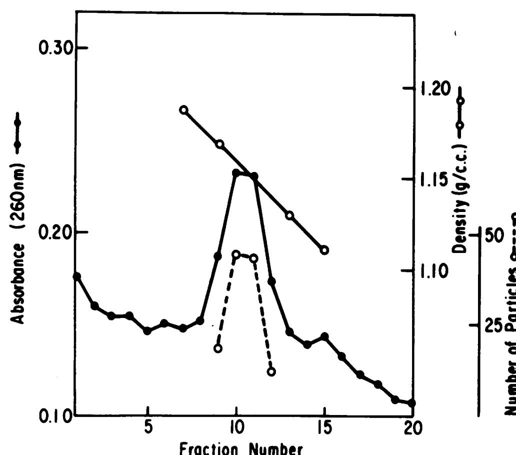

FIG. 1. *Density profile of LCV after sucrose density gradient equilibrium centrifugation. The 30 to 60% sucrose (in STE) gradient was centrifuged for 25 h at 35,000/rpm in an SW50 rotor. Twenty-drop fractions were collected, and absorbancy, refractive index, and virion particle count determinations were made on each fraction. The viral particles observed in each of the fractions represent an average over 10 fields at an electron microscope magnification of ×17 000; only those fractions which showed >2 particles per field are included.*

Virion polypeptides. A comparison of SDS-polyacrylamide gel electrophoresis of the polypeptides comprising the LCV with those of a murine leukemia virus showed that the LCV polypeptide pattern exhibits certain similarities with the pattern for Friend leukemia virus (kindly provided by D. Bolognesi) (Fig. 2). Specifically, the lower-molecular-weight components (Fig. 2, arrow, and below) are almost identical, and further both virions exhibit a multiple number of higher-molecular-weight components. The similarity is especially of interest for the most densely staining protein (Fig. 2, arrow) which corresponds to the group-specific antigens IV (species) and V (interspecies) as described by Schäfer et al. (31). Fourteen other major polypeptide components were detected in the LCV by Coomassie blue staining of the gels after electrophoresis. A spectrophotometric scan of the electrophoretically resolved polypeptides present in purified virions is shown in Fig. 3. Polypeptides designated 1 through 15 in Fig. 3 were always present in quantitatively similar amounts in many different virion preparations. The major polypeptide constituent (no. 12, Fig. 3) accounts for approximately 35% of the virion protein. The polypeptide pattern was the same if either intact virus or phenol-isolated proteins of the virus were used as starting material for treatment with SDS, urea, and β-mercaptoethanol prior to

electrophoresis. Polypeptide 11 frequently occurred as a leading shoulder of the major polypeptide 12 (Fig. 3) but could not be completely resolved from the major component using different concentrations of acrylamide in the gels. For this reason, it cannot be stated with certainty that it is a unique polypeptide. The molecular weights of the polypeptides range from 110,000 to 7,000 (Table 1) and were estimated by comparing their rates of migration with those of marker proteins of known molecular weights (see Materials and Methods).

Virion RNA. Radioactive RNA extracted from LCV and centrifuged in sucrose density gradients showed a characteristic heterogeneous high-molecular-weight component (80 to 88*s*) as well as a low-molecular-weight component (Fig. 4). Approximately 50% of the total radioactivity in the RNA preparations was present in the fast-sedimenting component. Electrophoresis of the low-molecular-weight component on polyacrylamide gels demonstrated the presence of two distinct species of RNA, a 7*s* and a 4*s* component. (Although an accurate *s* value has not been determined for this polynucleotide chain, it will be identified here as 7*s* RNA, since Erikson and Erikson [Abstr. Annu. Meet. Amer. Soc. Microbiol., 1972] have shown that it is closely related in both composition and chain length to 7*s* RNA molecules in other RNA tumor viruses.) Approximately 75% of the radioactivity in the low-molecular-weight RNA was present in the 7*s* species. Figure 5 shows the polyacrylamide gel resolution obtained when ^{3}H-labeled 4*s* and 7*s* virion RNAs were subjected to electrophoresis with unfractionated ^{32}P-RNA isolated from L cells. It can be seen that virion 4*s* RNA coelectrophoresed with L-cell 4*s* RNA. No other species of RNA, apart from the high-molecular-weight RNA and the 7*s* and 4*s* RNAs, could be detected in the virion.

Heat denaturation of the (80 to 88*s*) RNA, resulted in release of RNA with a sedimentation value of approximately 35*s*. This is a characteristic feature of the high-molecular-weight RNA of the RNA tumor viruses (8, 12) and has already been described in detail for the LCV (13). However, it should be pointed out that we have observed some variability in different preparations: in some cases a homogeneous peak of 35*s* RNA resulted from denaturation, in other cases the RNA was heterogeneous and ranged from 35*s* to about 20*s* as estimated by the distribution of radioactivity in velocity sucrose density gradients. This may be a reflection of some variability in viral harvesting that we have not yet determined.

When denatured fast-sedimenting RNA was subjected to electrophoresis on polyacrylamide

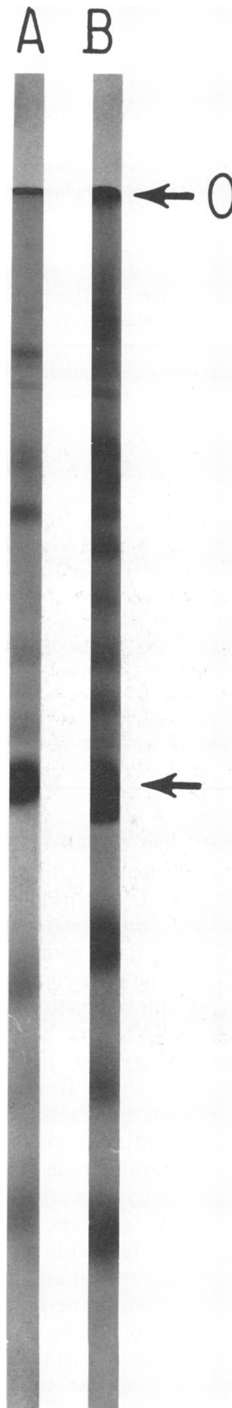

FIG. 2. *SDS-polyacrylamide gel electrophoretic separation of the polypeptides of LCV (A) and Friend leukemia virus (B). The arrow indicates the major protein, and the letter O indicates the origin. The concentration of acrylamide in the gels was 10%. The direction of electrophoresis was from top to bottom in relation to the photograph.*

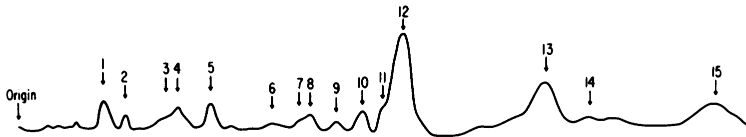

FIG. 3. *Spectrophotometric scan of Coomassie blue-stained LCV polypeptides after electrophoretic separation in a 7.6% SDS-polyacrylamide gel. The direction of electrophoresis was from left to right in relation to the photograph.*

TABLE 1. *Molecular weight estimates of LCV polypeptides after polyacrylamide gel electrophoresis*

Polypeptide number	Apparent molecular weight
1	110,000
2	97,000
3	85,000
4	76,000
5	65,000
6	49,000
7	42,000
8	40,000
9	36,000
10	33,000
11	28,000
12	27,000
13	14,000
14	11,500
15	7,000

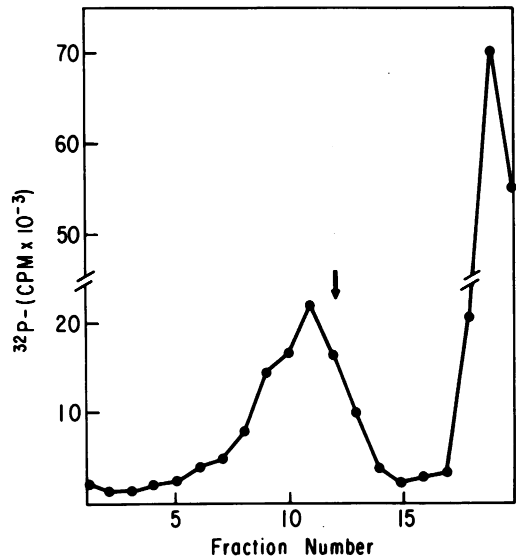

FIG. 4. *Separation of fast- and slow-sedimenting RNA components by centrifugation in a 5 to 20% sucrose density gradient. Bacteriophage f2 (kindly provided by R. E. Webster) sedimented slightly behind the peak of the high-molecular-weight RNA (arrow), indicating an s value of 80 to 88s for this fast-sedimenting RNA species. Samples (25 μliters) of each fraction were counted in Bray solution (6); the figures plotted represent the total radioactivity present in each fraction.*

gels, two distinct size classes of low-molecular-weight RNA were released. One of these (R-7*s*) moved slightly ahead of virion 7*s* RNA, the other (R-4*s*), coelectrophoresed with virion 4*s* RNA (Fig. 6). The R-4*s* component accounts for about 2.5%, and the R-7*s* component accounts for about 5.0% of the radioactivity in the high-molecular-weight RNA.

Virion morphology. In previous electron microscope studies with Rauscher leukemia viruses (21), the most satisfactory procedure for the preparation of viral specimens was found to be fixation with glutaraldehyde (1.5%), followed by staining with uranyl acetate (2%). By this procedure the LCV appears morphologically identical to Rauscher leukemia virus, with over 70% of the particles remaining intact and appearing as round, membrane-bound structures (Fig. 7A-E). A few of these particles exhibit 7-mm knobs over their surface (Fig. 7B–E). These knobs do not appear in large numbers over most of the particles (Fig. 7A) and are probably fragile in some degree as a result of the isolation procedure. Such loosely bound knob structures have recently been observed in studies with Friend leukemia virus (24).

A quantitative measurement of the outer diameter for the virion was obtained using the catalase crystal internal marker technique (20). The distribution of sizes for intact virions was gaussian (Fig. 8), which is representative of a homogeneous population of particles. The average value of 146.7 nm for the virion diameter is almost identical with the 147-nm value obtained for Rauscher leukemia virus (21). This value is larger than the 106-nm diameter reported by Nermut et al. (24), and it may be argued that the increased size is perhaps due to flattening of the virion. However, preliminary studies (R. Luftig and K. Culbreth, unpublished observations) suggest that the smaller diameter seen in freeze-dried preparations (24) may be due to shrinkage of the virions during preparation. The recent report (29) that Soule mouse

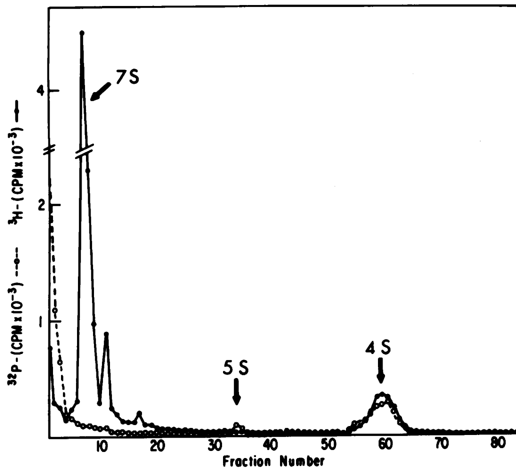

FIG. 5. *Coelectrophoresis of unfractionated ^{32}P-labeled L-cell RNA and ^{3}H-virion RNA (slow-sedimenting component) on a 10% polyacrylamide slab gel. The direction of electrophoresis was from left to right in relation to the photograph.*

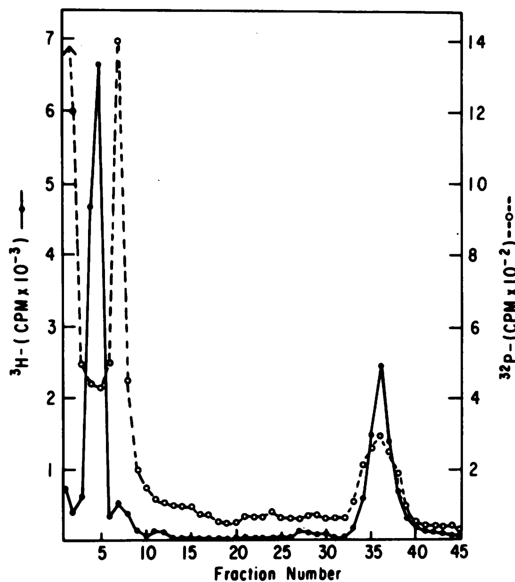

FIG. 6. *Coelectrophoresis of virion 4s and 7s ^{3}H-RNA with heat-denatured, high-molecular-weight ^{32}P-RNA on a 10% polyacrylamide cylindrical gel. The direction of electrophoresis was from left to right in relation to the photograph.*

leukemia virus has a diameter of 136 nm as determined by the independent technique of laser beat frequency spectroscopy, is more in line with the value given in Fig. 8 and suggests that only a minimal flattening, if any, may occur by the glutaraldehyde-uranyl acetate staining procedure.

Assay for reverse transcriptase activity. When reaction conditions suitable for the demonstration of DNA polymerase activity in purified Rous sarcoma virus (RSV) (Prague) were used with LCV, only a very slight (2 to 3%) relative incorporation of deoxythymidine-5'-monophosphate could be demonstrated (Table 2). Under the same conditions, RSV showed extensive incorporation of radioactive precursor. The addition of calf thymus DNA to the reaction mixtures did not significantly stimulate synthesis by LCV in comparison with RSV. Furthermore, when LCV and RSV were present in the same reaction mixture, essentially the full activity of RSV was recovered, indicating that the diminished LCV polymerase activity was probably not due to the presence of an inhibitor in the virion preparation.

DISCUSSION

The results presented in this paper, together with the studies by other workers (7, 13, 15, 17, 18), clearly demonstrate a close relationship between LCV and other RNA tumor viruses. The relative complexity of the polypeptide pattern, the presence of fast- and slow-sedimenting RNA species, the size, morphology, and density of the virion, together with the fact that there is a low RNA-to-protein ratio in the virion, are general characteristics of oncornaviruses (2, 3, 23, 24, 26, 31). On this basis, LCV appears to be an excellent model system for studying how latent tumor virions are propagated in mammalian cells.

In addition to the defective nature of the virion, there are some characteristics of LCV which distinguish it from other RNA tumor viruses. First, the proportion of 7*s* RNA relative to the high-molecular-weight RNA is much higher than in viruses such as RSV (4, 5). Also, denaturation of the fast-sedimenting RNA from LCV releases both a 4*s* and 7*s* component, whereas only a 4*s* species is released from denatured high-molecular-weight RNA from avian-myeloblast virus, Schmidt-Ruppin virus or mixtures of mouse sarcoma-mouse leukemia viruses (11). Preliminary studies (J. L. Nichols, unpublished data) indicate that LCV 7*s* and R-7*s* molecules are identical species of RNA and show slightly different electrophoretic mobilities because of different conformational states.

Secondly, assays for reverse transcriptase with LCV showed a negligible level of activity (<3%), relative to that of an infectious virus, RSV (Prague). It should be noted, however, that a slight stimulation of activity was apparent when calf thymus DNA was added to the reaction.

FIG. 7. *A, Typical field of L-cell virions seen after zone centrifugation on a 30 to 60% sucrose gradient. Magnification is ×60,000. B-E, Selected particles showing knobs on the virion surface (arrow). Magnification is ×135,000.*

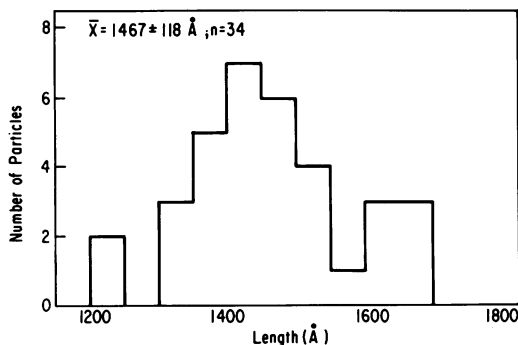

FIG. 8. *Histogram of LCV particle diameters calibrated by the catalase crystal internal marker technique. The average diameter ($\bar{x}$), standard deviation, and number of particles counted (n) are indicated.*

For this reason, it is possible that there is a reduced amount of the enzyme present in the virion, or, the LCV enzyme is sufficiently different from that of other tumor viruses so that it was not fully active with the assay conditions employed.

In view of the defective nature of LCV it is interesting to note the recent results of Hanafusa et al. (16), in which an absence of polymerase proteins has been found in virions of RSV-alpha, a noninfectious mutant derived from RSV.

The recent findings of Schäfer et al. (30) indicate one further way in which LCV differs from other murine leukemia viruses. In a study of six different murine viruses it was shown by Ouchterlony and complement fixation tests that LCV does not possess either FMR or G host range

TABLE 2. *DNA polymerase activity of LCV compared with RSV (Prague)*[a]

Virus	Amount added (µg)	Calf thymus DNA[b]	[*methyl*-^3H]dTMP incorporated[c] (pmol)
LCV	28	−	0.015
RSV (Prague)	36	−	0.608
LCV	28	+	0.129
RSV (Prague)	36	+	18.40
LCV plus RSV (Prague)	28 µg LCV; 36 µg RSV (Prague)	−	0.476

[a] The values listed for incorporated radioactivity represent an average over at least four different experiments, with a standard error of 20% among duplicate assays. Reaction conditions are described in Materials and Methods.

[b] A 25-µg amount of calf thymus DNA was added to the reaction mixtures where indicated.

[c] A background equivalent to 0.0276 pmol was found for incubated control samples, and this value was subtracted from each experimental sample.

serotypes. However, knobs which are thought to correspond to virus surface glycoproteins (10), can be found on the LCV (Fig. 1B-E). Since type-specific antigens such as FMR or G are thought to correspond to these surface glycoproteins (30), the inability of LCV to cross-react most probably indicates a different antigenicity for the surface knob polypeptide.

Studies are in progress to fully characterize the constituent polypeptides and ribonucleate chains of LCV and to elucidate its morphogenesis within L cells.

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