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# Chromatographic Separation and Antigenic Analysis of Proteins of the Oncornaviruses

## II. Mammalian Leukemia-Sarcoma Viruses

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Proteins of leukemia and sarcoma viruses from the chicken, mouse, hamster, and cat were analyzed by gel filtration in guanidine hydrochloride. The mammalian viruses were found to contain six major proteins, whereas the avian viruses contained seven proteins. Proteins of viruses from different mammalian species had the same molecular weights which also closely resembled the molecular weights of the six equivalent avian viral proteins. These results defined a basic similarity in protein composition for the C-type viruses of avian and mammalian origin. The two glycoproteins of murine leukemia virus were identified immunologically as constituents of the viral membrane. Antisera prepared against other proteins distinguished individual internal viral antigens in immunodiffusion tests and also reacted in immunofluorescence with cytoplasmic components of infected cells. Antisera which reacted with the major internal viral proteins did not contain antibodies inhibitory to the viral reverse transcriptase.

Analysis of the oncornaviruses [oncogenic ribonucleic acid (RNA)-containing viruses] by polyacrylamide gel electrophoresis (PAGE) shows that these viruses possess between five and seven major structural proteins. Since leukemia-sarcoma viruses from the chicken, mouse, hamster, and cat have similar morphology and morphogenesis, it would not be unexpected to find common structural elements in these agents. In fact, studies by Oroszlan et al. (16) and Schafer et al. (19) of proteins from mouse, hamster, and cat leukemia viruses show similarities between these viruses. Similar studies of avian viral proteins by Duesberg et al. (4), Bolognesi and Bauer (3), and Hung et al. (10), however, suggest differences between the avian and mammalian leukemia-sarcoma viruses.

In a previous report (5), the proteins of viruses from the avian group were examined by gel filtration of viral proteins in guanidine hydrochloride (GuHCl) and by PAGE. Seven distinct viral proteins were identified, two of them glycoproteins; antigenic analysis revealed (i) that the two glycoproteins were constituents of the viral membrane and (ii) that four of these proteins were internal group-specific (gs) antigens. The location of the seventh protein was undetermined. With this report, we extend the analysis by comparing the avian leukemia-sarcoma viruses with leukemia-sarcoma viruses from the mouse, hamster, and cat. Marked similarities were observed between C-type viruses from avian and mammalian sources, indicating analogous protein composition for viruses of this morphological type.

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## MATERIALS AND METHODS

**Source of viruses.** (i) Avian leukosis virus (AvLV), strain MC-29, was obtained from J. W. Beard. The virus was propagated in primary chick embryo cell cultures (5). (ii) Murine leukemia virus (MuLV) was obtained from the tissue culture line RRTC, an epitheloid cell line derived from a rat leukemia induced by Rauscher leukemia virus. (iii) Hamster sarcoma virus (HaSV) was isolated from the B34 cell line (clone 8), established by Bassin et al. (2) from a sarcoma induced in a neonate hamster by inoculating MuSV-infected mouse cells. (iv) Feline leukemia virus (FeLV) was obtained from a FeLV-infected feline embryo line that is continually producing virus (11).

**Radiolabeling of virus.** Oncornaviruses were radiolabeled by the incorporation of radioactive precursors into the medium of virus-producing cells in vitro. Cells were incubated for 24 hr with labeled precursors before harvest of virus-containing fluids. The quantities of radioactive precursors used were: 2 $\mu$Ci of $^{14}$C-L-amino acid mixture per ml, 5 $\mu$Ci of $^{3}$H-L-amino acid mixture per ml, and 2 $\mu$Ci of $^{3}$H-glucosamine per

ml. Radioactive materials were purchased from New England Nuclear Corp.

**Isolation of virus.** Tissue culture fluids were first prespun at 5,000 rev/min for 10 min in a centrifuge (Ivan Sorvall, Inc.) to remove cells and cell debris, and then the virus was precipitated by the addition of crystalline ammonium sulfate to a final concentration of 50% saturation. Virus pellets were suspended in tris(hydroxymethyl)aminomethane (Tris)-NaCl (0.1 Tris-hydrochloride, 0.1 M NaCl, *p*H 8.0) buffer and were layered upon 15 to 60% sucrose density gradients. After centrifugation for 3 hr at 119,000 × *g* (SW27 rotor), the banded virus at a density of 1.16 to 1.18 g/ml was recovered, diluted with Tris-NaCl buffer, and repelleted by centrifugation for 1 hr at 119,000 × *g*.

In some instances, the virus was further purified by sucrose velocity sedimentation. For this purpose, the virus isolated by sucrose density gradient centrifugation was suspended in 3 ml of Tris-NaCl buffer and was layered upon a 38-ml 15 to 30% sucrose density gradient in the SW27 rotor; these gradients were then centrifuged for 55 min, at which time most oncornaviruses had sedimented approximately one-half to two-thirds the length of the gradient. Fractions were collected by puncturing the bottom of the tube. Virus preparations, isolated by density gradient centrifugation or velocity sedimentation, or both, were examined in the electron microscope and were found to contain predominantly virions. Contaminating vesicles rarely exceeded 10 to 15% of the banded material.

**Gel filtration of viral proteins in GuHCl.** Viral pellets were suspended in 1 ml of saturated GuHCl, 2% mercaptoethanol, and 10% sucrose, *p*H 8.6. Samples were heated at 100 C for 3 min and were then layered upon an agarose (BioGel A-5m, 200 to 400 mesh) column (15 by 100 cm). Column buffer was 6 M GuHCl with 0.01 M dithiothreitol, *p*H 6.5. Columns were run at room temperature at a flow rate of 1.5 ml/hr. For radioactive determinations, 0.5-ml samples in GuHCl were mixed with 1 ml of distilled water in scintillation vials and brought up to 16.5 ml with Triton X-100 (Packard Instrument Co.) liquid scintillation cocktail.

**Reference typing antisera.** (i) For AvLV and avian sarcoma virus (AvSV), antisera were obtained from hamsters bearing transplants of AvSV-induced sarcomas (9). (ii) For MuLV and murine sarcoma virus (MuSV), antisera with precipitating activity against antigens of Gross leukemia virus were obtained from (W/Fu × BN)F hybrid rats inoculated with leukemia W/Fu C58(NT)D of the inbred W/Fu strain (7).

**Antisera against isolated viral proteins.** Antisera against isolated viral proteins were prepared in (W/Fu × BN)$F_1$ hybrid rats. Antigens for immunization were prepared by preparative gel filtration in GuHCl of proteins from MuLV. Peak fractions were dialyzed free from GuHCl and dithiothreitol and were lyophilized. The initial immunizing material was antigen (0.3 mg of protein) emulsified with an equal volume of complete Freund's adjuvant and was injected in multiple subcutaneous sites. Incomplete Freund's adjuvant was used for the subsequent inoculations (each containing 0.3 mg of protein); these were given at triweekly intervals, also subcutaneously at multiple sites. Precipitating antisera were obtained after the third immunization. For immunodiffusion studies, antisera prepared against proteins from tissue culture-derived viruses did not require absorption.

**Immunological methods.** Double-diffusion (Ouchterlony) tests were performed on slides in 2% Noble agar (immunoplate pattern C, Hyland Laboratories). The slides were left at room temperature in a humidified chamber. Optimal precipitation occurred within 24 hr.

The following immunological tests were kindly performed by collaborators: the cytotoxic test by E. Stockert, the immunofluorescence test by J. Hilgers, and enzyme inhibition assays by J. Schlom and K. Watson (Columbia University).

The indirect immunofluorescence test on fixed cells was used for the localization of intracellular viral antigens (14). Monolayers of various tissue culture lines were grown for 48 hr in individual wells on glass microscope slides and fixed by immersion of the slides in acetone at room temperature for 15 min. The fluoresceinated goat anti-rat globulin (Hyland Laboratories) and viral antiserum were both used at 1:40 dilution.

For the cytotoxic test, equal volumes (0.05 ml) of cells (5 to 7 × $10^6$/ml), diluted antiserum, and pooled guinea pig serum diluted 1:3 (source of complement) were incubated for 45 min at 37 C. Viability percentage was determined microscopically after the addition of freshly prepared trypan blue solution. The diluent was medium 199. In each test, controls were included in which cells were incubated with either guinea pig serum or a 1:4 dilution of antiserum alone. These controls contained no more than 10% stained cells and are omitted from the tables (15).

In enzyme inhibition studies, 0.5 μg of purified AvLV RNA-directed deoxyribonucleic acid (DNA) polymerase was incubated with 30μg of antiserum in a total volume of 60 μliters for 1 hr at 4 C; this mixture included 20% glycerin and 0.15 M KCl at *p*H 8.0 to stabilize the enzyme. The reaction mixture was then brought to 100 μliters by the addition of triphosphates and template and was permitted to run for 10 min (12). Reaction conditions and purification of enzyme were described in detail in reference 12.

## RESULTS

**Chromatography of viral proteins.** MuLV was grown in the presence of radiolabeled $^{14}$C-amino acids and $^{3}$H-glucosamine and was isolated by density gradient centrifugation. Labeled virus was then disrupted in 6 M GuHCl and 1% mercaptoethanol and was fractionated by molecular sieving on an agarose column. Figure 1 shows that six proteins were observed in MuLV. The two largest proteins contain glucosamine and are thus considered glycoproteins. In keeping with the nomenclature of the previous report (5), we have designated the two largest proteins as m1 and m2 and the remaining pro-

teins as p1 to p4 in order of decreasing molecular weight.

Next, a comparison was made between MuLV and oncornaviruses from the chicken, hamster, and cat. As before, the viruses were grown in radiolabeled amino acids, and the proteins were chromatographically separated in the presence of guanidine and reducing agents. Figure 2 shows a double-label experiment with MuLV (labeled with $^{3}$H-amino acids) and HaSV (labeled with $^{14}$C-amino acids). Six proteins were observed in both viruses; the elution positions of the proteins of these viruses were strikingly similar, indicating a close coincidence in their molecular weights. Four of the six proteins moved as coincident peaks in the two preparations, whereas the other two differed only to a minor degree. A similar protein profile was observed with FeLV (Fig. 2, bottom), indicating a chromatographic pattern that is common to the mammalian leukemia-sarcoma viruses. For the three mammalian viruses analyzed here, the relative amounts of proteins p2, p3, and p4 differed. It has been shown in the case of the avian oncornaviruses that nonglycoproteins may appear in aggregated form at the elution position of m1 (5). This phenomenon would account for the considerable radioactivity associated with the m1 species in Fig. 2, as well as some variation in peak heights later in the elution pattern.

Figure 3 shows the results of a double-label

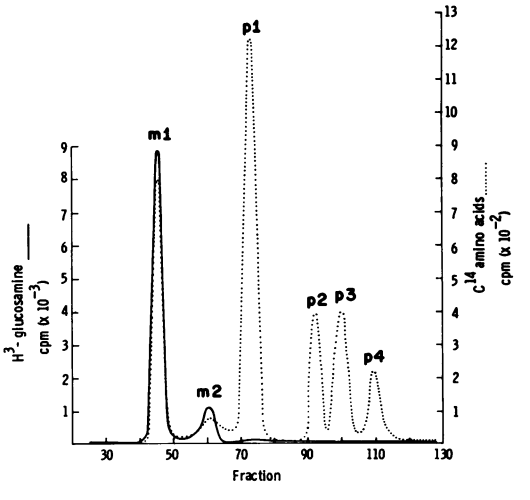


FIG. 1. *Chromatographic separation of murine leukemia virus (MuLV) proteins radiolabeled with $^{14}$C-amino acids and $^{3}$H-glucosamine. Six viral proteins are identified. The two largest of these are glycoproteins. The glycoproteins are designated m1 and m2 (m = membrane); other viral proteins are designated p1 to p4, in order of decreasing molecular weight.*

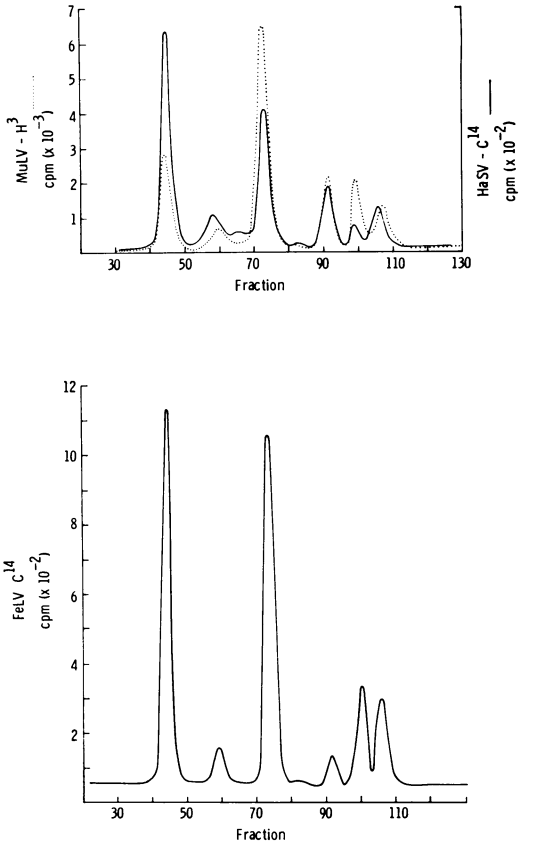


FIG. 2. *Comparative chromatographic separation of radiolabeled proteins from murine leukemia virus (MuLV), hamster sarcoma virus (HaSV), and feline leukemia virus (FeLV). The top panel shows a co-chromatographic separation of proteins from HaSV ($^{14}$C-amino acids) and MuLV ($^{3}$H-amino acids). The lower panel shows a chromatographic separation of proteins from FeLV. The pattern of proteins for these viruses is markedly similar.*

experiment with AvLV-(MC29) ($^{3}$H-amino acid label) and MuLV ($^{14}$C-amino acid label). The protein patterns obtained for the avian and murine viruses resembled each other, but with two exceptions: (i) AvLV had one protein more than MuLV, and (ii) the p1 (=gs1) of AvLV had a smaller molecular weight than the p1 of MuLV. The extra protein observed in the pattern for AvLV is a gs protein and is also found in other viruses of this group (5). The difference in molecular weights of the gs1 proteins from avian and mammalian viruses could also be detected in PAGE (R. C. Nowinski, Ph.D. Thesis, Cornell Univ., Ithaca, N.Y., 1971).

Thus, the leukemia-sarcoma viruses (C-type particles) show essentially the same profile by gel

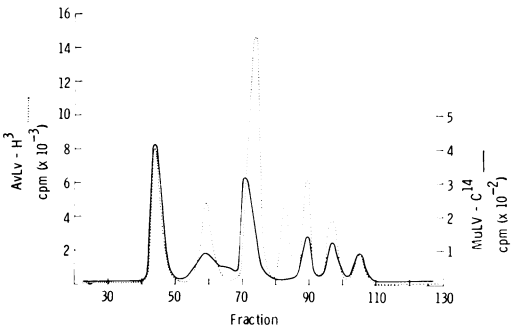


FIG. 3. *Comparative chromatographic separation of radiolabeled proteins from MuLV ($^{14}$C-amino acids) and AvLV ($^{3}$H-amino acids). The pattern of proteins for these viruses is similar, although AvLV contains one protein more than MuLV. Protein p1 (= gs1) of AvLV is smaller than the homolog in MuLV.*

filtration in GuHCl, but the avian viruses differ from the mammalian viruses by having an additional protein. In Table 1 are listed the molecular weights found for the viral proteins, with AvLV as a standard (5). The glycoprotein m1 from AvLV, isolated by gel filtration in GuHCl, disaggregated to a molecular weight of 32,000 in PAGE (5). Since the glycoprotein profiles of MuLV and AvLV in PAGE are similar (4), m1 from mammalian viruses is considered (as in AvLV) to be an aggregate in GuHCl, although it dissociates only partially upon subsequent analysis by PAGE. Glycoprotein m2 of the mammalian viruses (like its avian counterpart) does not dissociate further in PAGE (M. Bernhard and E. Fleissner, *unpublished data*). Column fractions containing protein m1 did not contain protein m2. Furthermore, although the m1 fractions are near the void volume of the column, examination of this region by electron microscopy failed to show either intact virions or intact viral membranes. Thus, viral solubilization appeared to be complete.

**Serological identification of MuLV proteins.** For immunodiffusion, isolated viral proteins were dialyzed free from GuCHl and reducing agent and were tested in immunodiffusion with MuLV antisera. Proteins p1 and p2 reacted with reference typing antiserum for MuLV, whereas proteins m1, m2, p3, and p4 did not. Protein p1 was identified as the MuLV-gs1 antigen (8) (Fig. 4). Thus, as in the case of the avian oncornaviruses, removal of GuHCl permitted recovery of the native antigenic specificities of the viral proteins. Rat antisera prepared against chromatographically isolated proteins formed lines of identity between the ether-treated virus and the respective proteins used for immunization. These antisera identified

TABLE 1. *Molecular weights of viral proteins as determined by chromatography in GuHCl*[a]

| Protein[b] | AvLV | MuLV | HaSV |
|---|---|---|---|
| 1 (m1) | 100,000[c] | 100,000 | 100,000 |
| 2 (m2) | 70,000 | 70,000 | 70,000 |
| 3 (gs1) | 27,000 | 31,000 | 31,000 |
| 4 | 19,000 | | |
| 5 | 15,000 | 15,000 | 15,000 |
| 6 | 12,000 | 12,000 | 12,000 |
| 7 | 10,000 | 10,000 | 10,000 |

[a] Abbreviations: GuHCl, guanidine hydrochloride; AvLV, avian leukosis virus; MuLV, murine leukemia virus; HaSV, hamster sarcoma virus.

[b] Numbered in order of evolution.

[c] For molecular weight in PAGE, see text.

the MuLV-gs1 antigen in the renatured protein p1 and also detected antigenic specificities in p3 and p4 and ether-treated MuLV which were not seen with the reference typing MuLV antiserum. The nature of these new antigens is presently being determined. Rabbit anti-FeLV serum did not react with any of the six viral proteins, and antisera prepared against isolated MuLV proteins did not react with ether-treated FeLV; therefore, it was not possible to determine which of the viral proteins was the MuLV-gs3 antigen (6).

**Cytotoxic tests.** Antisera prepared in (W/Fu × BN)$F_1$ rats against MuLV proteins isolated by column chromatography were first heat-inactivated (56 C for 30 min) to remove the natural anti-GCSA (Gross cell surface antigen) 19*S* antibody that is occasionally found in rat serum and then were tested in the cytotoxic test with the GCSA + typing cell C57BL/6 E♂G2, the GCSA − typing cell C57BL EL4, normal C57BL/6 thymocytes, and normal 129 thymocytes. The cytotoxic activity of these sera was directed against only cell surface antigens of the MuLV + leukemia E♂G2. Thus, cytotoxicity by virtue of natural rat anti-mouse heteroantibody was ruled out. Furthermore, these antisera did not detect the MuLV $G_{IX}$ cell surface antigen (20), since 129 thymocytes were negative. Table 2 shows that antisera prepared against m1 and m2 showed similar levels of cytotoxicity for E♂G2, whereas anti-p1 and anti-p2 sera showed minimal levels, and anti-p3 and p4 were noncytotoxic. These results suggest that m1 and m2 proteins are associated with viral components located on the cell surface (i.e., the viral membrane).

**Immune electron microscopy.** Proteins m1 and m2 were conclusively identified as constituents of the viral membrane by immune electron microscopy; antisera prepared against these two proteins reacted with the membrane of virus budding

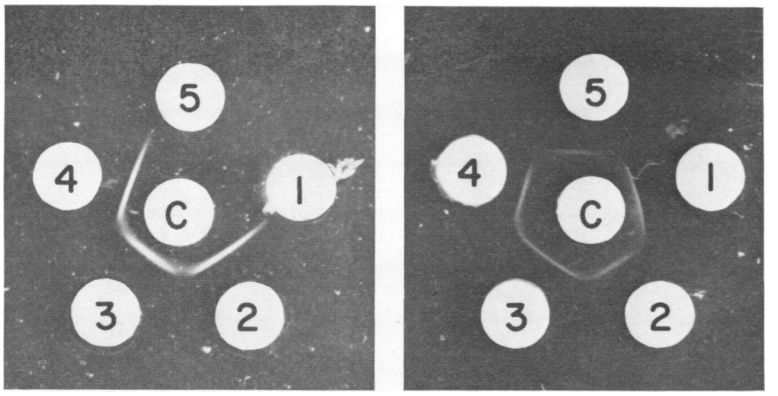


FIG. 4. *Serological identification of p1 as the murine leukemia virus (MuLV)-gs1 antigen. Left: center well contains chromatographically isolated p1; well 2, rat reference typing serum [W/Fu × (BN)F1 anti W/Fu C58(NT)D] for MuLV-gs1; well 3, rabbit antiserum prepared against isolated MuLV-gs1 antigen (13); well 4, rat antiserum prepared against p1 isolated from guanidine-treated virus; wells 1 and 5 are empty. Right: center well contains rat anti-p1 serum; well 1, isolated protein p1; well 2, MuLV-gs1 antigen isolated by Sephadex chromatography from Tween 80-ether-treated virus (13); well 3, ether-treated MuLV; well 4, ether-treated milk of mice from the high leukemia strain C58; and well 5, tissue extract of a leukemia from mice of the high leukemia strain AKR.*

from the MuLV-infected C57BL/6 leukemia E♂G2. Figure 5 shows the typical result of using antiserum directed against m1. Antisera prepared against proteins p1 to 4 did not react with the virion surface. All antisera (although preliminarily heat-inactivated and absorbed with MuLV-negative C57BL/6 EL4 cells) also reacted with the cell surface of E♂G2; as to whether this represents residual natural anti-GCSA antibody or the presence of viral gs antigens on the cell surface remains an open question until the proper absorption studies are available.

**Immunofluorescence.** Rat antisera prepared against the isolated MuLV-proteins contained low titers of nonviral antibodies. This nonviral specificity was eliminated by diluting the antisera past the end point of the undesired reaction; thus, at a dilution of 1:32, these antisera reacted in immunofluorescence with MuLV-infected rat cells (RRTC; Fig. 6) but not with uninfected rat embryo cells.

Antisera detecting antigens of m1, m2, and p1 to 4 gave cytoplasmic fluorescence; no nuclear fluorescence was observed. Anti-m1 serum also reacted strongly with the cell membrane, confirming the observation of the antigen on the cell surface by immune electron microscopy.

**Enzyme inhibition.** Gamma globulins isolated by Sephadex chromatography from reference typing rat antiserum against MuLV, a serum with potent anti-gs activity, were tested by J. Schlom (Columbia University) for inhibitory activity against the RNA-directed DNA polymerase of MuLV-SV, and no inhibition was observed. Similar tests were performed for an avian virus, AMV. Rat antisera prepared against isolated AMV gs proteins (5) were tested by K. Watson (Columbia University) for inhibitory activity against the RNA-directed DNA polymerase of AMV and were found to be uniformly inhibitory for polymerase activity. It was considered likely that this effect was due to inhibitors or the nuclease in whole serum. Therefore, the antisera were fractionated either by chromatography on Sephadex G-200 or by ammonium sulfate (33% saturation) precipitation to separate the gamma globulins from inhibitory substances. Isolated gamma globulins still precipitated the four viral gs antigens but were no longer

TABLE 2. *Cytotoxic activity of rat antisera prepared against isolated MuLV proteins on the MuLV+ leukemia C57B6/6 E♂G2*

| Antiserum[a] | Percentage of cells stained at various dilutions of antiserum | | | | |
|---|---|---|---|---|---|
| | 1:2 | 1:4 | 1:8 | 1:16 | 1:32 |
| Rat anti-m1 | 95 | 95 | 95 | 80 | 46 |
| Rat anti-m2 | 95 | 95 | 95 | 53 | 10 |
| Rat anti-p1 | 40 | 35 | 28 | 20 | 10 |
| Rat anti-p2 | 70 | 60 | 30 | 12 | 10 |
| Rat anti-p3 and p4 | 10 | 10 | | | |

[a] These same antisera were negative in cytotoxic tests with murine leukemia virus-(MuLV)-negative cells from the same mouse strain (16). The two largest proteins from the fractionated virus were designated m1 and m2, and the remaining proteins were designated p1 to p4 in order of decreasing molecular weight.

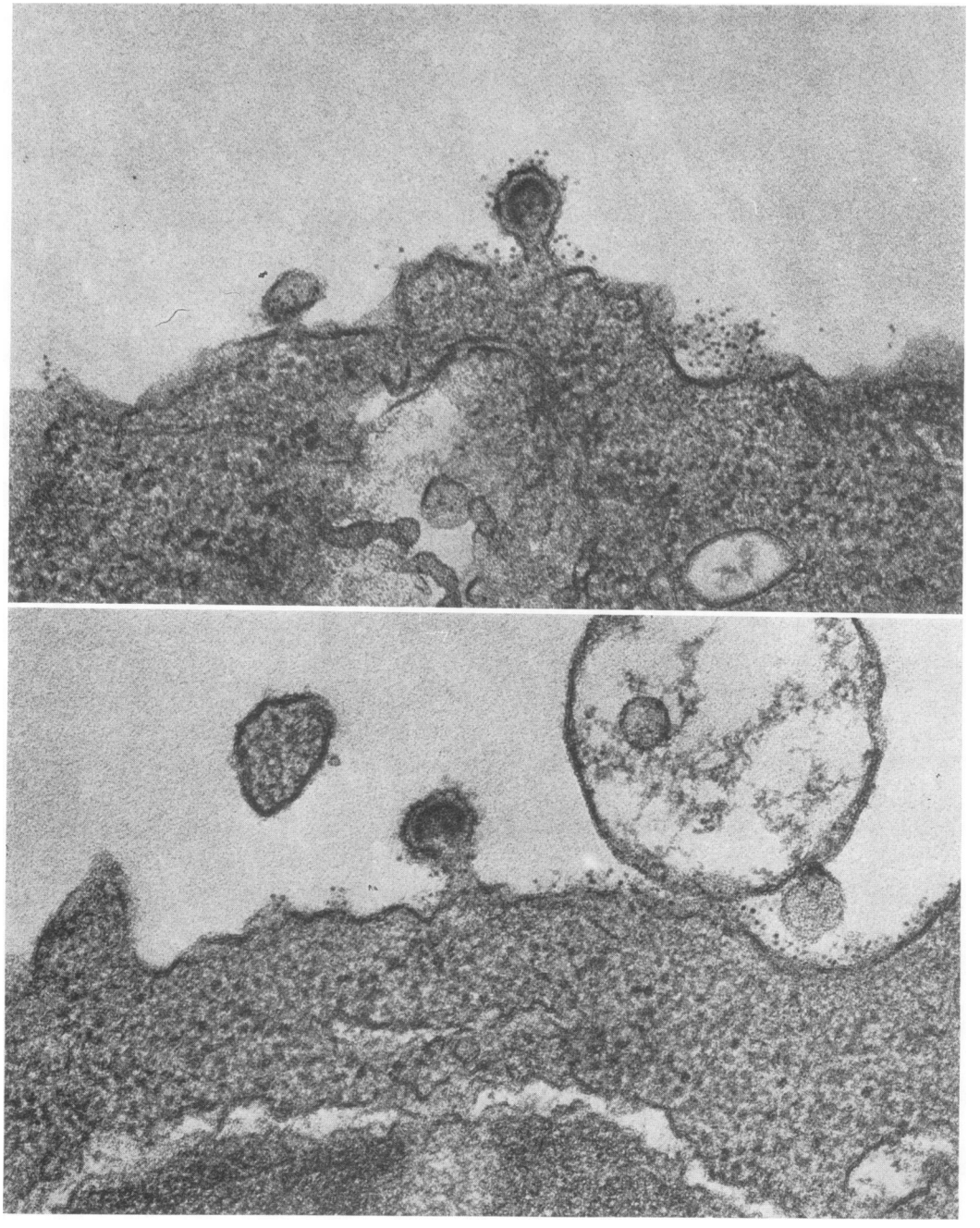

FIG. 5. *Ferritin labeling of budding murine leukemia virus (MuLV) by antiserum prepared against chromatographic fraction m1 from MuLV. MuLV-infected C57BL/6 E♂G2 cells were sequentially treated with (i) rat antiserum prepared against either fraction m1 (top panel) or fraction p1 (bottom panel), (ii) hybrid antibody with specificity against rat gamma G and ferritin, and (iii) ferritin. In the top panel, with anti-m1 serum, the ferritin label is observed on the viral membrane and on the cell surface as well; in the bottom panel, with anti-p1 serum, the labeling is only on the cell surface. Antiserum prepared against m2 also reacted with the viral membrane but not antisera prepared against p2 to p4. For details on method, see reference 1.*

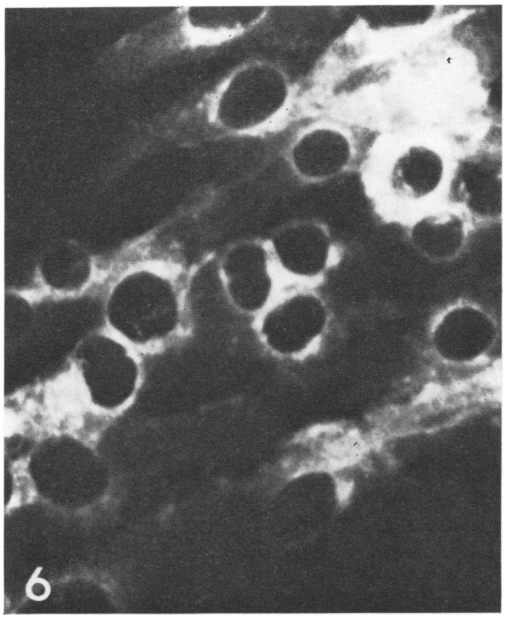


FIG. 6. *Immunofluorescence tests with anti-MuLV-gs1 antiserum on rat cells infected with murine leukemia virus (MuLV). The viral antigen is only found in the cytoplasm.*

inhibitory for the polymerase. Furthermore, potent gs antisera did not react in immunodiffusion tests with highly concentrated preparations of isolated AMV viral polymerase (kindly provided by K. Watson).

Thus, the RNA-directed DNA polymerase does not appear to be antigenically related to the internal gs antigens of the virion.

## DISCUSSION

The studies described here show that C-type viruses isolated from diverse species have striking similarities in their protein compositions. Chromatography (gel filtration) of leukemia-sarcoma viruses in 6 M GuHCl reveals that viruses from three mammalian species each contain six major proteins, whereas the avian viruses contain seven. The molecular weights of six of these proteins coincide closely for the four species tested. Since there is an extra gs protein (gs2; see reference 5) in the avian oncornaviruses, it will be of interest to determine whether an analogous gs protein exists in the reptilian C-type viruses.

In general, estimates of molecular weights determined by chromatography in GuHCl agree with those of authors who have used PAGE (3, 4, 10, 16, 19), with the qualification that protein m1 behaves as an aggregate in GuHCl (5). However, analysis of mammalian oncornaviruses in PAGE reveals three major proteins not containing carbohydrate, whereas analysis in GuHCl detects four such proteins. Studies by E. Fleissner (5) indicate that the smallest protein observed in PAGE with AvLV is actually two proteins (gs3 and p5) which have a similar mobility in polyacrylamide gels but which are resolved by chromatography in 6 M GuHCl. A similar shift in the apparent molecular weight of protein p2 of the mammalian leukemia-sarcoma viruses also occurs in PAGE, superimposing this protein on protein in p4 (M. Bernhard and E. Fleissner, *unpublished data*). Thus, this alteration in the expected mobility of p2 in PAGE appears to be characteristic for the equivalent protein in oncornaviruses of diverse origin. The resultant superposition of these two proteins accounts for the finding of one less protein by PAGE than is found by gel filtration.

Native antigens of the mammalian oncornaviruses can be recovered by dialysis after exposure to 6 M GuHCl. This result parallels the findings with the avian viruses (5). Immunological analysis of the isolated, renatured viral proteins from mammalian sources indicates that, as found by other workers (8, 16), the major polypeptide of the virion (molecular weight 31,000 to 33,000) coincides with the gs1 antigen. This analysis also shows that the two largest viral proteins (which are in fact glycoproteins) are constituents of the viral membrane and are accessible to specific antibody. These findings coincide with other reports that glycoproteins are constituents of viral membranes in AvLV (3, 4, 5, 10), MuLV (16), HaSV (16), and FeLV (19). The remaining lower-molecular-weight proteins are in the interior of the virus, since they are not accessible to specific antibody. In the case of AvLV, these have been identified as serologically distinct group-specific antigens (5); a detailed study of this sort is now being performed with the murine leukemia-sarcoma viruses.

Although definitive similarities are observed between the leukemia-sarcoma viruses of diverse origins, these agents clearly differ from another representative of the oncornavirus group, the mouse mammary tumor virus (14). Thus, the virus particles associated with leukemias and sarcomas in the mouse (C-type viruses) are morphologically and antigenically distinguishable from virus associated with mouse mammary tumors (B-type virus). The murine B-type viruses are characterized by having prominant spikes on the viral membrane and by having a condensed eccentric nucleoid; C-type viruses of mice, on the other hand, have a smooth viral membrane and a condensed central nucleoid. It appears that the

morphological differences between these viruses are also reflected in the structural proteins of these agents. Perhaps the most significant difference in the proteins of these viruses is that the C-type viruses contain a number of low-molecular-weight (11,000 to 30,000) internal proteins, whereas the B-type virus contains a major internal protein of molecular-weight 52,000 (14). Since viruses of both types have a 60 to 70S viral RNA (17) and have similar nucleocapsid structures (13, 18), it is possible that the major protein component of the nucleocapsid of C-type virus is formed by a combination of gs protein molecules (5) and that the major internal protein of mouse mammary tumor virus forms a morphologically analogous structure from fewer polypeptide chains.

## ACKNOWLEDGMENTS

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