Proteins of Kirsten Murine Leukaemia-sarcoma Virus: localization within the Virus Particle by Iodination and Fractionation Techniques

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SUMMARY
The protein and glycoprotein composition of Kirsten murine leukaemia-sarcoma virus [KiMSV(KiMuLV)] was studied using SDS-polyacrylamide gel electrophoresis. Twenty-three polypeptides and three glycoproteins were detected following electrophoresis by staining with Coomassie blue and PAS or by autoradiography of isotopically labelled virus. Protein components were assigned positions in the virus particle, envelope, nucleoid or intermediate area based on iodination with lactoperoxidase and sedimentation in potassium citrate equilibrium gradients. The KiMSV(KiMuLV) envelope contained 11 polypeptides and three glycoproteins. The virus nucleoid and intermediate area were each composed of six proteins. The protein composition of KiMSV(KiMuLV) was highly reproducible when virus was harvested from cells of the same subculture generation. However, the protein profiles were altered with repeated in vitro passages of the virus-producing cell line.

INTRODUCTION
The protein composition of avian and murine oncornaviruses has been extensively studied (Duesberg, Martin & Vogt, 1970; McDugald, Panem & Kirsten, 1970; Fleissner, 1971; Nowinski et al. 1971, 1972; Strand & August, 1971; Bolognesi et al. 1972; Moroni, 1972; Robinson & Robinson, 1972, Schaefer et al. 1972; Bolognesi, Luftig & Sharper, 1973; Lange et al. 1973; Panem, Schwartz & Kirsten, 1973; Green & Bolognesi, 1974). Most reports deal with the functions of structural proteins from this virus group whereas few reports are available on the location of these proteins in the virus particle. We have attempted to correlate the polypeptide and glycoprotein components of Kirsten murine leukaemia-sarcoma virus [KiMSV(KiMuLV)] with the morphologic compartments of the virus particles. The proteins and glycoproteins were analysed by an improved technique of SDS-polyacrylamide gel electrophoresis following iodination and fractionation of purified virus particles. The results differ from earlier reports in the number of proteins solubilized and resolved by this method.

During the course of this work, we noted that the protein composition of KiMSV-
(KiMuLV) changed considerably with serial virus passages in a chronically infected cell line. This communication deals with a topographic assignment of the structural proteins as well as their modification and alterations with *in vitro* passages.

**METHODS**

**Cells and virus.** Normal rat kidney (NRK) cells were obtained from Dr K. D. Somers, Houston, Texas (Duc-Nguyen, Rosenblum & Zeigel, 1966; Somers & Kit, 1971). NRK cells were infected with KiMSV(KiMuLV) to produce a virus-releasing cell line referred to as NRK-K cells (Somers & Kirsten, 1969). Cells were grown in glass roller bottles (Belco, Vineland, N.J.) at 37 °C in an atmosphere of 5% CO₂. Minimal essential medium (MEM) was used, containing 10% foetal calf serum, 100 μg/ml penicillin and 100 units/ml streptomycin (GIBCO, Grand Island, N.Y.). Cells were subcultured weekly and tested weekly for mycoplasma contamination as described (McClain & Kirsten, 1974). Only mycoplasma-free cultures were used.

Virus was collected from culture fluids 6 to 21 subcultures after the initial infection of NRK cells with KiMSV(KiMuLV). Virus was harvested from approx. 300 ml spent culture fluids at 6 to 12 h after the last medium change. The fluids were clarified by sedimentation at 10000 g for 20 min in an IEC B-20 refrigerated centrifuge (International Equipment Co., Needham, Mass.). The clarified material was centrifuged through 5% sucrose in STE buffer (0.1 M-NaCl, 0.01 M-tris, pH 7.2 and 0.001 M-EDTA, pH 7.3) at 100000 g for 90 min in a Spinco model L-50 ultracentrifuge at 4 °C. The pellet was resuspended in 0.2 to 0.4 ml STE and centrifuged for 3 h in a SW 50.1 rotor at 165000 g at 4 °C through 5 ml of 30 to 60% (w/v) sucrose gradients prepared in STE (Panem & Kirsten, 1973 b). The visible virus band at the buoyant density of 1.155 to 1.17 g/ml was removed and used immediately or after disruption with SDS at a final concentration of 1%. Disrupted virus was dialysed overnight against STE at room temperature.

Iodination was performed by a modification of the technique described by Stanley & Haslam (1971). Galactose and galactose oxidase were used to continually generate hydrogen peroxide during the reaction. The reaction was performed by adding to 60 μl of virus sample in STE, 5 μl each of 0.32 mg/ml lactoperoxidase (Worthington Biochemical, Freehold, N.J.), 9 mg/ml galactose; 10 international units (iu)/ml galactose oxidase (Worthington Biochemicals, Freehold, N.J.) and 0.5 mCi/ml [125I]-labelled NaI (Amersham Searle, Arlington Heights, Ill.). The reaction mixture was incubated for 30 min on ice and stopped by the addition of 5 μl of 0.75 mg/ml cysteine hydrochloride (Sigma Chemical Co., St Louis, Mo.).

**Fractionation of virus.** KiMSV(KiMuLV) was disrupted with the non-ionic detergent Nonidet P40 (NP-40, Shell Oil Co., New York, N.Y.) (Panem & Kirsten, 1973 b). The detergent was added to the virus sample to a final concentration of 0.5%, and the mixture incubated for 10 min on ice. 0.5 ml of disrupted virus was layered on to 5 ml of a 10 to 50% (w/w) potassium citrate gradient prepared in STE, and the gradients were spun for 110 min at 165000 g in the SW 50.1 head of a Spinco model L-2 ultracentrifuge (McDugald et al. 1970; Panem & Kirsten, 1973 b). Fractions were collected and dialysed overnight in the cold against STE. A sample of each fraction was removed for protein determination by the method of Lowry *et al.* (1951). The remaining samples were solubilized for electrophoresis as described below.

**Polyacrylamide gel electrophoresis.** Samples were solubilized by bringing them to final concentration of 1% SDS, 5 to 10% sucrose, 0.001 M-EDTA, 0.01 M-tris-HCl, pH 8, 0.04 M-dithiothreitol (DTT) and 10 μg/ml pyronin Y. Samples were incubated for 15 min at
Proteins of KiMSV(KiMuLV)  

Proteins of KiMSV(KiMuLV) were solubilized and analyzed by electrophoresis. Solubilization was achieved by incubation with 0.4 M DTT for 15 min at 37 °C. Electrophoresis was performed using 5% acrylamide-bisacrylamide gels containing 0.2% SDS. Glycoproteins were detected by staining with PAS after fixation in isopropanol-acetic acid and recorded by densitometry. The gels were stained with Coomassie blue to determine total protein.

RESULTS

Protein and glycoprotein composition of KiMSV(KiMuLV)

Virus was purified from NRK-K culture fluids by equilibrium sedimentation in sucrose gradients. The proteins were solubilized by incubation with 1% SDS and 0.4 M DTT for 15 min at 37 °C and analysed by electrophoresis on 5% acrylamide-bisacrylamide gels containing 0.2% SDS. Glycoproteins were detected by staining with PAS after fixation in isopropanol-acetic acid and recorded by densitometry. The gels were stained with Coomassie blue to determine total protein. The protein profiles were highly reproducible between virus preparations collected from different sources.
Fig. 1. SDS-polyacrylamide gel electrophoretic analysis of iodinated KiMSV(KiMuLV). KiMSV-
(KiMuLV) was purified by sedimentation in sucrose equilibrium gradients, iodinated with lacto-
peroxidase and [¹²⁵I] and a sample was solubilized for electrophoretic analysis. Following electrophoresis on a 5% acrylamide-bisacrylamide gel containing 0.2% SDS at 100 V for 90 min when the
tracking dye (TD) had migrated 7.5 cm, the gel was stained with periodic acid–Schiff’s and then
with Coomassie blue. Stain profiles were recorded densitometrically and the gel was then sectioned
into 1 mm slices for determination of [¹²⁵I] radioactivity. (a) Photograph of KiMSV(KiMuLV) proteins stained with Coomassie blue; (b) densitometric scans of periodic acid–Schiff’s and Coom-
assie blue staining of KiMSV(KiMuLV); (c) distribution of [¹²⁵I] in KiMSV(KiMuLV) proteins.
—,—, E₅₅₀ Coomassie blue; ———, E₅₅₀ periodic acid–Schiff’s; O——O, [¹²⁵I] distribution. a, b and c
are the identical gel at different stages of analysis.
Table 1. Proteins of KiMSV(KiMuLV)*

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<th>Position‡</th>
<th>Mol. wt.§</th>
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<th>PAS‡</th>
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* KiMSV(KiMuLV) was purified on sucrose gradients and iodinated with lactoperoxidase. The intact iodinated virus was disrupted with NP-40 or Triton X-100 and fractionated on potassium citrate gradients. After sedimentation three fractions at densities ≤ 1.08 g/ml, 1.108 g/ml and 1.23 g/ml were collected, dialysed to remove salt and solubilized for SDS-polyacrylamide gel electrophoresis. Following electrophoresis, the gels were analysed for staining with PAS and Coomassie blue, and [125I] distribution.

† The relative mobility of each protein band was defined as the ratio of distance of band migration to the distance of migration by the tracking dye.

‡ Periodic acid–Schiff’s base staining for glycoproteins.

§ Mol. wt. were estimated relative to the migration of standard proteins (Methods).

¶ Iodine-125 labelling of proteins.

Proteins were assigned positions in the virus envelope (E), nucleoid (N) or region between nucleoid and envelope (I) on the basis of iodination and position in potassium citrate gradients. Proteins were designated E if they were iodinated and banded at ρ ≤ 1.08, and 1.108 g/ml or both. Unlabelled proteins banding at ρ ≤ 1.08 and 1.108 g/ml; ρ ≤ 1.08 and 1.23 g/ml or exclusively at ρ ≤ 1.08 g/ml were designated I. Proteins were designated N if they were unlabelled and located exclusively at ρ = 1.23 g/ml or predominantly at ρ = 1.23 g/ml.

NRK-K cells of the same subculture generation. The profiles were also identical when virus was prepared from the same NRK-K subculture generation after the cultures had been stored for 7 months at -70 °C. Solubilization of virus proteins with 1% SDS and 0.4 M DTT at 37 °C for 15, 30 or 60 min or at 60 °C for 30 min gave scans identical to those shown in Fig. 1 (b). Virus purification by sedimentation through equilibrium gradients of 10 to 50% (w/w) potassium citrate did not alter the protein profiles (data not shown). However, variations were detected with repeated in vitro passages of NRK-K cells (Fig. 2, Table 2). The proportion of total virus protein detected by staining with Coomassie blue in the mol. wt. range of 110 to 50 × 10^3 increased from 28% to 49% after 15 serial passages, whereas proteins in the mol. wt. range of 50 to 23 × 10^3 decreased from 41% to 22%. This pattern was
Fig. 2. Alterations of KiMSV(KiMuLV) proteins with in vitro serial passage. KiMSV(KiMuLV) was purified by sucrose gradient sedimentation from culture fluids of serially passaged NRK-K cells. The virus was analysed by SDS-polyacrylamide gel electrophoresis as described in the legend to Fig. 1. Protein was detected by Coomassie blue staining and profiles were recorded by densitometry at 550 nm. Protein migration was determined relative to the pyronin Y tracking dye (TD). Virus was analysed from six different cell generations: (a) 6 generations after virus infection; (b) 7 generations after virus infection; (c) 13 generations; (d) 15 generations; (e) 17 generations; (f) 21 generations.

Fig. 3. Comparison of autoradiographic and stain profiles of KiMSV(KiMuLV) proteins. KiMSV(KiMuLV) was labelled with [14C]-amino acids and purified by sucrose density-gradient sedimentation. Following electrophoresis on a 5% acrylamide-bisacrylamide gel containing 0.2% SDS as described in the legend to Fig. 1, virus proteins were detected by staining with Coomassie blue and recorded densitometrically. The gel was then analysed for [14C]-amino acid distribution by autoradiography and the autoradiogram was scanned densitometrically. ---, $E_{550}$ scan of Coomassie blue stained proteins; ---, $E_{550}$ scan of autoradiogram.

confirmed when proteins from early virus passages (six subculture generations after virus infection) were compared with those from a late virus passage (22 subculture generations later). Further experiments are in progress to relate these virus protein alterations with bioactivity.

Contamination

It is well recognized that proteins from the culture medium or cell debris may be included in the final virus band or pellet of gradient-purified oncornaviruses (Moroni, 1972). The electrophoretic chromatogram of Fig. 1(b) revealed an intense staining with Coomassie blue in the mol. wt. range 80 to $65 \times 10^3$ which may have represented a protein derived from the culture medium. In order to exclude such non-virus proteins from the present analysis,
Table 2. Alterations of virus proteins during serial in vitro passages

<table>
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<th>Subpassages after virus infection*</th>
<th>Coomassie blue staining† (%)</th>
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<tr>
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<tr>
<td>21</td>
<td>17</td>
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</table>

* NRK cells were infected with KiMSV(KiMuLV) as described, and the infected cells were subcultured at weekly intervals.
† KiMSV(KiMuLV) was purified from culture fluids of serially passaged NRK-K cells and analysed by SDS-polyacrylamide gel electrophoresis. Proteins were detected by staining with Coomassie blue and gel profiles were recorded by densitometry. The percentage of protein detected by Coomassie blue staining in the regions 0.00 to 0.25, 0.25 to 0.50, 0.50 to 0.75 and 0.75 to 1.00 relative to the tracking dye was determined piconometrically from the profiles presented in Fig. 3.

NRK-K cells were labelled with [14C]-amino acids (see Methods). The radiogram of the [14C]-labelled virus was compared with the protein profiles resolved by staining with Coomassie blue on the same gel. Fig. 3 demonstrates that all proteins detected by staining were also present when examined by autoradiography. It should be noted, however, that proteins of lower mol. wt. (< 50 × 10^3) appeared to stain less strongly than did the high mol. wt. proteins. The reasons for this disparity are, at present, unclear.

Iodination of KiMSV(KiMuLV)

Lactoperoxidase-mediated protein iodination has been used to distinguish surface proteins from internal virus proteins (Stanley & Haslam, 1971; Robinson & Robinson, 1972; Katz & Margalith, 1973; Witte, Weissman & Kaplan 1973; Fritz, 1974). The iodine labelling technique can be used as a criterion for the surface location of virus proteins if the conditions of iodination are selective so that internal protein components are not iodinated. The selectivity of the labelling procedure was tested as follows. KiMSV(KiMuLV) was purified from NRK-K culture fluids on a sucrose gradient, and the virus band was collected. One half was immediately iodinated, solubilized for electrophoresis with SDS and DTT as described and stored at −20 °C. This fraction will be referred to as 'iodinated intact virus'. The other half was disrupted by incubation with 1% SDS, dialysed overnight, iodinated and then solubilized for electrophoresis. This virus sample will be called 'iodinated disrupted virus'. The iodinated intact and disrupted virus samples were again equally divided: one sample of each was analysed by electrophoresis under standard conditions for 90 min, while the other sample was electrophoresed for 135 min. Iodination of whole virus resulted in a variable ratio of [125I] labelling to Coomassie blue staining (Fig. 4a, 5a). Some proteins were heavily labelled whereas others were not. In contrast, the distribution of the [125I]-label closely followed the Coomassie blue profile after disrupted virus was iodinated (Fig. 4b, 5b). We conclude that iodination of intact virus labelled a selective group of proteins, whereas iodination of disrupted virus labelled all proteins which were resolved by staining. These findings also indicated that the standard electrophoretic conditions used were sufficiently sensitive to distinguish iodinated from unlabelled proteins since gels run for standard or prolonged times gave identical results. Moreover, the enzymes used to catalyse iodination (lactoperoxidase...
Fig. 4. Selective labelling of virus proteins by iodination: standard electrophoretic conditions. Sucrose-gradient-purified KiMSV(KiMuLV) was divided in half. One portion was iodinated with lactoperoxidase and $^{[125]I}$, and solubilized for electrophoresis (iodinated intact virus). The other portion was disrupted with 1% SDS, dialysed overnight, then iodinated and solubilized for electrophoresis (iodinated disrupted virus). The two portions were divided in half following solubilization and a sample of each was analysed on 5% acrylamide-bisacrylamide gels containing 0.2% SDS for 90 min at 100 V when the tracking dye (TD) had migrated 7.5 cm. Virus proteins were detected by Coomassie blue staining and recorded densitometrically. Gels were then sectioned into 1 mm slices for determination of $^{[125]I}$. (a) Iodinated intact virus; (b) iodinated disrupted virus. $--$, $E_{550}$ Coomassie blue; $O--O$, $^{[125]I}$ distribution.
Fig. 5. Selective labelling of virus proteins by iodination: expanded electrophoretic conditions. A sample of solubilized iodinated intact and iodinated disrupted virus described in the legend to Fig. 4 was electrophoresed on 5% acrylamide-bisacrylamide gels containing 0.2% SDS for 135 min at 100 V. Virus proteins were detected by Coomassie blue staining and the gels were then sectioned for $[^{125}]I$ determination. (a) Iodinated intact virus; (b) iodinated disrupted virus. — , $E_{600}$ Coomassie blue; ○—○, $[^{125}]I$ distribution.
Fig. 6. SDS-polyacrylamide gel electrophoretic analysis of iodinated and fractionated KiMSV-
(KiMuLV). A sample of the gradient purified, iodinated KiMSV(KiMuLV) presented in Fig. 1 was
disrupted with 0.5% NP-40 and centrifuged for 1.8 h at 165,000 g on a 10 to 50% (w/w) potassium
citrate gradient. Fractions with buoyant densities = 1.08 g/ml, 1.108 g/ml and 1.23 g/ml were
collected, dialysed overnight against STE and solubilized for electrophoresis as described. Follow-
ing electrophoresis and analysis as described in the legend to Fig. 1, the [125I] and Coomassie blue
distribution was determined. (a) Virus proteins in density fraction 1.08 g/ml; (b) virus proteins in
density fraction 1.108 g/ml; (c) virus proteins in density fraction 1.23 g/ml —, $E_{250}$ Coomassie
blue; ◦ — ◦, [125I] distribution.
Proteins of KiMSV(KiMuLV)

and galactose oxidase) were not detected as stained or labelled bands in the gels (data not presented).

**Fractionation of iodinated KiMSV(KiMuLV)**

The location of virus proteins was examined by using iodinated intact virus which was subsequently disrupted with the non-ionic detergent NP-40. The virus split products were separated by sedimentation in 10 to 50% (w/w) potassium citrate density gradients (McDugald et al. 1970; Panem & Kirsten, 1973b). Three protein fractions could be resolved by this procedure: (i) proteins floating at the gradient-sample interface with the buoyant density of 1.08 g/ml; (ii) proteins with the buoyant density of the virus envelope (1.108 g/ml) and (iii) proteins with the buoyant density of virus nucleoid (1.23 g/ml) (McDugald et al. 1970; Bolognesi et al. 1973). The fractions were dialysed overnight against STE, solubilized and examined by electrophoresis in polyacrylamide gels. A sample of the iodinated intact virus which had been stored frozen was simultaneously electrophoresed as shown in Fig. 1 c. Gels were stained for protein with Coomassie blue, recorded by densitometry, and sliced for the determination of [125I]-radioactivity. Fig. 6(c) reveals that [125I]-radioactivity was not associated with proteins from the density region of virus nucleoids (1.23 g/ml). This finding confirmed that the iodination procedure selectively labelled the surface proteins of KiMSV(KiMuLV). Iodinated proteins were only associated with the density fractions of the gradient-sample interface (1.08 g/ml) and the virus envelope (1.108 g/ml) (Fig. 6a, b). Identical results were obtained when virus was disrupted with Triton X-100 rather than NP-40.

Composite data from ten experiments with iodinated, fractionated KiMSV(KiMuLV) are summarized in Table I. The virus used was harvested from NRK-K cell culture fluids 9 and 11 subculture generations following infection. The proteins are listed according to their migration in gels relative to standard proteins, staining with PAS, estimated mol. wt., iodination patterns and location in equilibrium density gradients of potassium citrate. On the basis of these data each protein was assigned a position in the virus envelope, nucleoid or the intermediate area between nucleoid and envelope.

The following artifacts must be considered in such topographic assignments. Structural surface components may become dislodged during virus purification and may become associated with the fraction found at the gradient-sample interface (Nermut, Frank & Schaefer, 1972). Internal virus proteins may adsorb to the nucleoid, envelope or both during the preparatory steps. In order to minimize these artifacts we designated proteins as envelope proteins if they were iodinated and sedimented to densities of \( \leq 1.08 \) g/ml, 1.108 g/ml or both. Proteins were considered as belonging to the intermediate area if they were unlabelled by iodine and found at densities \( \leq 1.08 \) g/ml and 1.108 g/ml. Nucleoid-associated proteins were unlabelled and banded at the density of 1.25 g/ml.

**DISCUSSION**

The proteins of KiMSV(KiMuLV) can be assigned positions in the envelope, nucleoid or intermediate area on the basis of the present iodination and fractionation experiments (Table I). Fourteen envelope proteins with mol. wt. ranging from 25 to \( 112 \times 10^3 \) were iodinated in intact, gradient-purified virus. Any contaminating proteins from the culture medium or cell debris would be expected to be iodinated and would therefore appear among these proteins which we classified as envelope constituents. The following findings argue that a significant proportion of the designated envelope proteins are virus particle components or very tightly adsorbed cell contaminants: (i) all proteins detected by staining with Coomassie blue were also detected by autoradiography of labelled virus; (ii) changes in the protein
profiles were recorded with continuous passages of the NRK-K cells although the growth medium was unaltered in composition; (iii) the protein profiles of KiMSV(KiMuLV) did not change when the virus was purified through sucrose equilibrium gradients and then re-centrifuged on potassium citrate equilibrium gradients. We also have evidence to indicate that the protein profiles of KiMSV(KiMuLV) were not altered when virus was grown in serum-free culture medium (unpublished data).

The 14 iodinated protein components of the virus envelope can be further divided into two groups according to their sedimentation behaviour in equilibrium gradients of potassium citrate. A group of loosely attached proteins sedimented at a buoyant density of 1.08 g/ml and included four polypeptides (mol. wt. 105, 37, 25 and 22 x 10^3) as well as two glycoproteins (mol. wt. 80 and 65 x 10^3). The glycoproteins are probably representative of the attached surface knobs of murine oncornaviruses (Nermut et al. 1972), while the four polypeptides are considered components of the exterior envelope layer. Previous work has shown that the surface projections of avian and murine oncornaviruses contain at least two glycoproteins (Bolognesi et al. 1972; Witter et al. 1973a, b). The second group of KiMSV-(KiMuLV) envelope proteins sedimented predominantly at a buoyant density of 1.108 g/ml. These proteins are tightly bound and may be considered components of the deeper envelope layers. The functions of these groups are unknown; several type-specific antigens have been shown to reside in the envelope of oncornaviruses (Eckner & Steeves, 1972).

The area between envelope and nucleoid of oncornaviruses is poorly understood at present. Some group-specific antigens of MuLV probably occupy this intermediate zone because they are released after virus disruption with Tween-ether or non-ionic detergents (Gregoriades & Old, 1969). Furthermore, a protein (30 x 10^3 mol. wt.) containing both interspecies-specific and species-specific antigenicity has recently been identified in the intermediate area of Friend MuLV (Bolognesi et al. 1973). We have recovered proteins with mol. wt. of 44, 23, 21 and 17 x 10^3 from the intermediate zone. Bolognesi et al. (1973) concluded from studies with avian myeloblastosis virus and Friend MuLV that a protein of a given mol. wt. might be localized in more than one virus particle component. This conclusion is relevant to our data as proteins with mol. wt. of 68, 56, 44 and 37 x 10^3 were recovered from both the envelope and intermediate zone. While this finding may represent four proteins found at two locations each, an equally valid interpretation is that each of these bands may be composed of two distinct proteins of equal mol. wt. Alternatively, these may be internal proteins partially adsorbed to the core side of the virus envelope, or loosely bound envelope components not exposed at the virus particle surface.

The nucleoid fraction of KiMSV(KiMuLV) contains six proteins (mol. wt. 85, 75, 56, 37 and 20 x 10^3). Proteins of 37 and 20 x 10^3 probably correspond to the low mol. wt proteins recently isolated from the nucleoid fraction of Friend MuLV (Lange et al. 1973).

The electrophoretic profiles of KiMSV(KiMuLV) proteins changed with serial subcultures of the virus-producing NRK-K cells. The proportion of total virus proteins with a mol. wt. range of 24 to 55 x 10^3 gradually decreased with a concomittant increase of proteins with mol. wt. of 55 to 110 x 10^3 daltons. This pattern was reproduced when proteins were solubilized from frozen virus of early in vitro passages of NRK-K cells and compared with proteins of virus progeny sampled fifteen subcultures later. To the best of our knowledge, this is the first report of an altered protein composition with in vitro growth of an oncornavirus. It should be recalled that KiMSV(KiMuLV) is a pseudotype sarcomagenic virus and that the culture fluids of NRK-K cells also contain KiMuLV (Huebner et al. 1966; Panem & Kirsten, 1973a). In addition, NRK cells harbour an endogenous rat C-type virus (Klement et al. 1972). We suggest the following explanations to account for the altered protein pro-
Proteins of KiMSV(KiMuLV) files. (i) Each virus may have a distinct protein profile and the alterations with serial subcultures reflect changes in the proportion of the three viruses. (ii) The virus population remains constant throughout serial subculture but late-passage virus may be more difficult to dissociate than early-passage virus, giving rise to larger protein subunits on electrophoretic analysis. (iii) Late passage virus may differ from early-passage virus in the loss of the loosely associated envelope protein with mol. wt. 37 and 25 \times 10^5. Further work is necessary to distinguish between these alternatives.

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