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Tryptic Peptide Analysis of *gag* Gene Proteins of Endogenous Mouse Type C Viruses†

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Tryptic digests of the internal proteins p30, p15, p12, and p10 of mouse xenotropic, ecotropic, and amphotropic type C viruses were subjected to cation-exchange chromatography. Analysis of these maps revealed that the p30 proteins from representative isolates of all three viral subgroups were distinguishable. The p15 proteins were all unique. The p12 proteins of NZB xenotropic and wild-mouse amphotropic viruses were not identical and yielded peptide maps remarkably different from that of the ecotropic virus. The p10 proteins of xenotropic and ecotropic viruses were identical and were dissimilar to that of the wild-mouse amphotropic virus.

The multiplicity of viruses in mouse strains, coupled with the ability of such closely related viruses to undergo efficient phenotypic mixing and genetic recombination, can give rise to unique virus types with novel biochemical or biological properties (12, 21, 22, 40). Consequently, the definitive analysis of virus functions requires stringent identification of virus species. Type C viruses can be differentiated on the basis of various parameters, including biological properties (17), immunochemical analysis of viral proteins (14), nucleic acid hybridization (7), and peptide mapping of viral proteins (5, 6). All members of the murine type C virus group have substantial interrelatedness as evidenced by the presence of conserved group-specific antigenic determinants on internal structural proteins (1, 27). These proteins are derived from a common precursor polyprotein that is a translational product of the *gag* gene, one of three recognized genes of naturally occurring murine type C viruses (2, 3). The purpose of the present study was to analyze and compare the internal viral proteins of known endogenous mouse type C viruses, including xenotropic viruses of the embryo cells of NZB mice and (NZW × NZB)F_1 hybrids and the ecotropic virus of the nominally healthy NZW strain. The main strategy of this study was the use of tryptic peptide mapping as developed by Buchhagen et al., who first showed that this technique can distinguish between peptides of closely related mouse type C viral proteins (6).

We prepared tryptic digests of the internal proteins p30, p15, p12, and p10 of xenotropic, ecotropic, and wild-mouse amphotropic viruses and subjected those digests to cation-exchange chromatography. Our findings showed that the p30 proteins from virus isolates of each of these subgroups are strongly conserved but are finely different. The p10 proteins of NZB xenotropic and two N-tropic isolates were identical but differed from the amphotropic p10. The p12 proteins of both NZB xenotropic and amphotropic viruses were remarkably similar and clearly distinguishable from the ecotropic p12. The p15 proteins were all unique. These results permit an initial comparison of a number of endogenous mouse viruses comprising the three main interference and host range groups and contribute information bearing on the evolution of these viruses.

MATERIALS AND METHODS

Viruses and cells. Initial stocks of the following cloned virus-infected cells were obtained from P.V. O'Donnell of the Sloan-Kettering Institute (cell line given first, followed by virus type): SC-1/amphotropic-1504A, a virus originally isolated from an outbred mouse by Hartley and Rowe (16), exhibiting dual tropism; SC-1/MCF-247, a dual-tropic genetic recombinant isolated from AKR thymocytes (18); SC-1/WN1802N, an N-tropic virus isolated from normal BALB/c mouse spleen (17); mink lung (CCL-64)/AT124, a xenotropic virus isolated from human rhabdomyosarcoma cells transplanted into immunosuppressed NIH/Swiss mice (39). The following viruses were isolated in this laboratory (uninfected cells used for propagation were obtained from P. V. O'Donnell): mink lung/NZB-X, a xenotropic virus spontaneously produced by NZB embryo cells; mink lung/(NZW × NZB)F_1-X, a xenotropic virus spontaneously produced by (NZW × NZB)F_1 embryo cells;

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SC-1/NZW-N, an N-tropic virus induced from NZW embryo cells by 5-bromodeoxyuridine (25); SC-1/(NZW × NZB)F_1-N, an N-tropic virus spontaneously produced by (NZW × NZB)F_1 embryo cells. NZB, NZW, and (NZW × NZB)F_1 mice were from specific-pathogen-free colonies maintained at this institute. All cells were maintained with Dulbecco modified Eagle medium (Grand Island Biological Co.) supplemented with 10% heat-inactivated fetal calf serum (Microbiological Associates) and grown in a 5% CO_2 atmosphere.

Characterization of viruses. The viruses used in this study had been biologically characterized as to host range, interference groups, and neutralization patterns. Virus growth was assayed by monitoring infectious viral titers and/or production of particle-associated RNA-dependent DNA polymerase (29, 37). All xenotropic virus grew on mink lung cells (CCL-64; reference 19), mink lung cells transformed by Kirsten sarcoma virus (64J1; reference 19), and SIRC cells and did not grow on either NIH/Swiss or BALB/c secondary mouse embryo cells or SC-1 cells, an established mouse cell line lacking Fv-1 restriction mechanisms (15).

Interference experiments performed as described by Hartley and Rowe (16) showed that all xenotropic viruses were of the same interference group and were distinct from ecotropic and amphotropic mouse groups and from the endogenous feline RD-114 group. Furthermore, all xenotropic viruses were neutralized by group-specific anti-Rauscher murine leukemia virus gp70 serum and by pooled sera from 5-month-old NZB mice, which contain a non-immunoglobulin factor capable of selectively neutralizing xenotropic viruses (23).

The ecotropic viruses were all N-tropic, capable of inducing XC plaques on NIH/Swiss and SC-1 cells (34), unable to infect CCL-64, SIRC, or 64J1 cells, neutralized by anti-Rauscher gp70 serum but not by NZB sera, and showed interference only with other ecotropic viruses. Finally, wild-mouse amphotropic virus (1504A) could infect NIH/Swiss mouse cells as well as CCL-64 and 64J1 cells, did not induce XC plaques, and showed no interference with either ecotropic or xenotropic viruses. The MCF-247 virus was not biologically typed in this laboratory. Viruses so defined were subsequently labeled with radioactive amino acids, purified by sucrose density gradient centrifugation, and chromatographed by agarose gel filtration in the presence of 6.0 M guanidine hydrochloride.

Radiolabeling of virus. Cells were grown until approximately 80% confluent in plastic dishes (100 by 15 mm). Fresh medium containing one-fifth of the normal amino acid concentration plus 10 to 20 μCi of ^{3}H-amino acids per ml or 1 to 2 μCi of ^{14}C-amino acids per ml (Amersham Corp.) was added to the cultures for 24 h at 37°C.

Isolation of virus. Culture fluids containing radiolabeled virus were centrifuged at 5,000 × *g* for 15 min at 4°C in a GSA rotor and then recentrifuged at 100,000 × *g* for 65 min at 4°C in an SW27 rotor in a Beckman L5-65 ultracentrifuge. The pellets were suspended in a total of 2 to 4 ml of NTE buffer (0.1 M NaCl, 0.01 M Tris, 0.001 M EDTA; pH 7.4), overlaid on a 10 to 50% (wt/wt) linear sucrose gradient, and centrifuged for 3 to 4 h at 100,000 × *g* in an SW27 rotor. The gradient was fractionated into 1.5-ml samples and diluted 1:1 with NTE buffer. Portions of 50 to 100 μl each were taken to determine the position of the viral band. Peak tubes were pooled, diluted with NTE buffer, and centrifuged at 100,000 × *g* for 120 min in an SW27 rotor. The final pellet was either used directly or stored at −70°C.

Gel filtration in 6 M guanidine hydrochloride. Gel filtration in 6 M guanidine hydrochloride (12) was performed as described by Fleissner (13). Purified ^{3}H- or ^{14}C-amino acid-labeled virus was dissolved in 1.0 ml of 8 M guanidine hydrochloride–0.05 M Tris-hydrochloride–0.01 M EDTA–2% 2-mercaptoethanol containing 100 mg of sucrose. The viral proteins were denatured by heating for 4 min at 100°C.

The sample was chromatographed through a Pharmacia column (1.5 by 100 cm) of Bio-Gel A-5M (200–400 mesh; Bio-Rad Laboratories), utilizing an elution buffer of 6 M guanidine hydrochloride–0.01 M dithiothreitol–0.02 M sodium phosphate (pH 6.5). A constant flow rate of 0.7 ml/h was attained by gravity flow under a constant pressure head of 15 cm of elution buffer. Fractions (1.0 ml) were collected, and 25- to 50-μl samples were assayed from each tube by dissolving in 10 ml of ACS scintillation fluid (Amersham) plus 0.4 ml of water. Samples were counted in glass vials in a Packard 3255 scintillation counter. The elution profiles contained six major polypeptide species of molecular weights 100,000 (void volume), 70,000 (gp70), 30,000 (p30), 15,000 (p15), 12,000 (p12), and 10,000 (p10), in addition to a minor 45,000-molecular weight component. Fractions representing a radioactive viral polypeptide peak were pooled, mixed with 0.5 to 1.0 mg of bovine serum albumin, and dialyzed against 0.01 M ammonium bicarbonate (pH 8.5) for 5 days with two changes per day. The dialyzed samples were then lyophilized, dissolved in 2.0 ml of distilled water, and either stored at −70°C or mixed with appropriate viral proteins of a different label, relyophilized, and used as sample preparation for either gel electrophoresis or tryptic peptide mapping.

Tryptic peptide mapping. Samples of polypeptides to be compared by tryptic mapping were mixed, relyophilized, and trypsinized by the method of Buchhagen et al. (6), using TPCK trypsin (Worthington Biochemicals Corp.) at a final enzyme concentration of 0.00105% (wt/vol). Digestion was for 2 h at 23°C. Digests were lyophilized and chromatographed.

Chromatographic separation of tryptic peptides. Tryptic digests were dissolved in 270 μl of 0.1 M pyridine–acetate buffer (pH 2.7), and 250 μl was applied by a manual sample injector (Beckman Instruments, Inc.) to a jacketed chromatography column (0.9 by 15.0 cm) containing PA35 cation-exchange resin (Beckman Instruments, Inc.) maintained at 56°C. A concave gradient was formed by a three-chambered Autograd (Technicon) mixing apparatus in the ratios on 200 ml of 0.1 M pyridine–acetate (pH 2.7) in each of two chambers and 200 ml of 2.0 M pyridine–acetate (pH 5.0) in a third, and pumped through a column by a Beckman Accu-Flo pump at a flow rate of 25 ml/h under 75 to 100 lb/in^2. All buffers contained 0.1% thiodiglycol (Eastman Kodak) as an

antioxidant. Fractions (5.0 ml) were collected and dried in scintillation vials in an electric oven at 80°C. The residues were dissolved in 10 ml of ACS scintillation fluid and counted for 20 min each in a Packard 3255 scintillation counter under a $^{3}H/^{14}C$ window whose spillover values, counting efficiencies, and quench characteristics were determined beforehand.

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of viral proteins purified by guanidine hydrochloride gel filtration was performed in 7.5 or 10% gels as described by Dunker and Rueckert (9). Viral polypeptides were prepared by heating at 100°C for 5 min in an equal volume of 2% (wt/vol) sodium dodecyl sulfate–2 mM dithiothreitol–8 M urea, and the mixture was subjected to electrophoresis in the presence of 0.1% sodium dodecyl sulfate–0.1 M sodium phosphate buffer (pH 7.1) circulating between the reservoirs at 4 ml/min at a constant temperature of 19.5°C. Electrophoresis was performed at 5 to 7 mA/tube. The position of the proteins in the gels was revealed by fractionating the gels into 1- to 2-mm slices with a Gilson gel fractionater (Gilson Medical Electronics) and determining the radioactivity in each fraction. Each polypeptide used for tryptic peptide analysis gave a single, sharp symmetrical peak, indicating homogeneity in terms of molecular weight and corresponding charge density.

RESULTS

Peptide mapping of p30. NZB cells spontaneously released infectious xenotropic virus (24; unpublished data). NZW cells did not spontaneously release either xenotropic or ecotropic virus, but these viruses were readily induced by halogenated pyrimidines (25). Stephenson et al. reported that both classes of viruses were spontaneously produced by NZW cells from mice of their colony (38), a possible reflection of strain differences between the two mouse colonies. (NZW × NZB)F_1 cells spontaneously released both xenotropic and ecotropic viruses, but it is not known whether the same cell type produced both viruses. Virus proteins were individually purified by guanidine hydrochloride chromatography, trypsinized, and chromatographed on PA35 cation-exchange column. It was first determined that the same viral protein labeled separately with two different isotopes gave identical peptide maps.

Figure 1 compares the peptide maps of the p30 proteins of NZW ecotropic and NZB xenotropic viruses. The patterns are very similar but

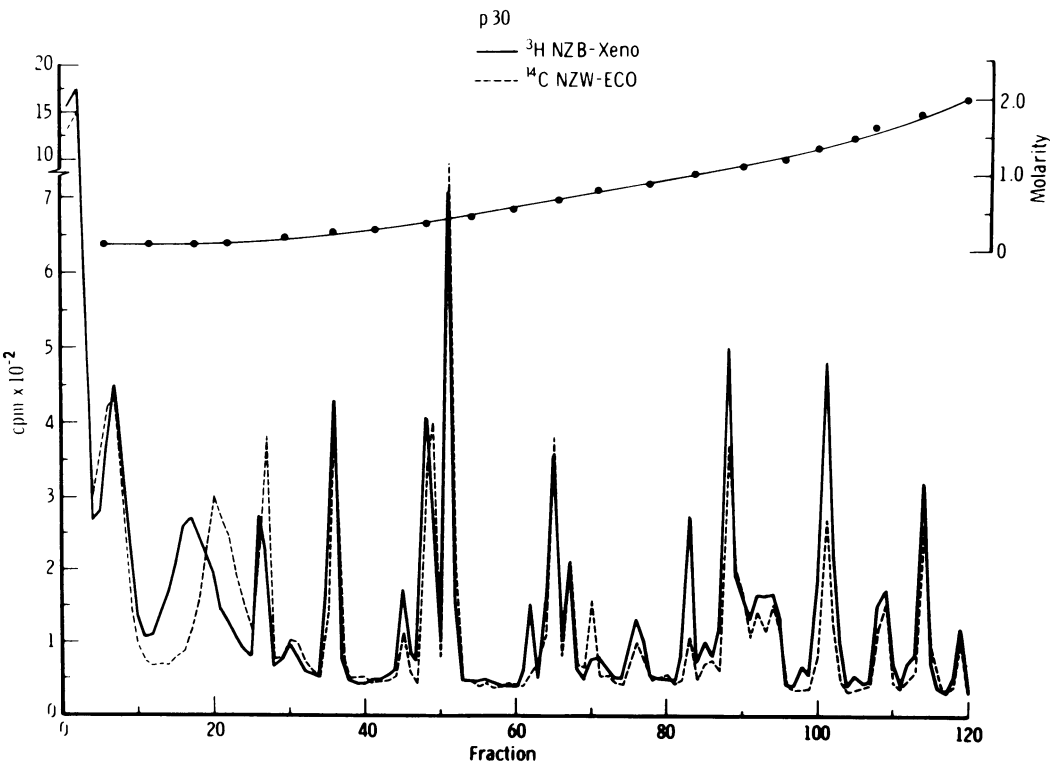

FIG. 1. *Peptide maps obtained by trypsinization of purified murine leukemia virus p30 proteins chromatographed on guanidine hydrochloride-equilibrated agarose columns. ^{3}H-labeled NZB xenotropic (NZB-Xeno) viral p30* (——); *^{14}C-labeled NZW ecotropic (NZW-ECO) viral p30* (– – –).

show: (i) an apparent shift in a large peptide eluting as a peak at fractions 17 (NZB) and 20 (NZW); (ii) the presence of a peptide at position 70 of the NZW map with a less apparent, broader peak in the NZB map; and (iii), the presence of a peptide at position 62 of the NZB map, without a visible corresponding peptide in the NZW map (but see Fig. 2 below). There are also minor shifts in elution positions of peaks at fractions 26–27 and 48–49. Ecotropic and xenotropic viruses isolated from (NZW × NZB)F_1 mice yielded p30 proteins which mapped identically to those of NZW and NZB.

Figure 2 shows maps of NZW ecotropic and wild-mouse amphotropic (1504A) p30 proteins. The patterns differ only in: (i) the presence of a peptide at position 67 of the NZW map and absence of a corresponding peptide in the 1504A map (as was the case for the NZB xenotropic map in Fig. 1); and (ii) the resolution of a peptide at position 59 of the 1504A map from a peptide eluting one position later in the NZW map. The latter peptide may correspond to the missing peak in the NZW map of Fig. 1.

Figure 3 compares the p30 proteins of NZB and AT124 xenotropic viruses. The map shows only one major region in which peptides do not coelute, at fractions 15 (AT124) and 18 (NZB). The variations in this region of the map here and in Fig. 1 indicate what appears to be a particularly type-specific region of the protein structure of the p30 molecules analyzed. Direct analysis of AT124 and 1504A p30 proteins (not shown) reveals a lack of coelution of the large peptide at positions 15 (AT124) and 20 (1504A). In all cases this peptide elutes as a single, unusually broad peak.

MCF-247 virus appears to be fundamentally different from other endogenous viruses in that it is apparently generated de novo during the life of the animal by recombinational events involving at least the envelope genes of endogenous ecotropic and xenotropic genomes (10, 33). Figure 4 shows the comparison of WN1802N eco-

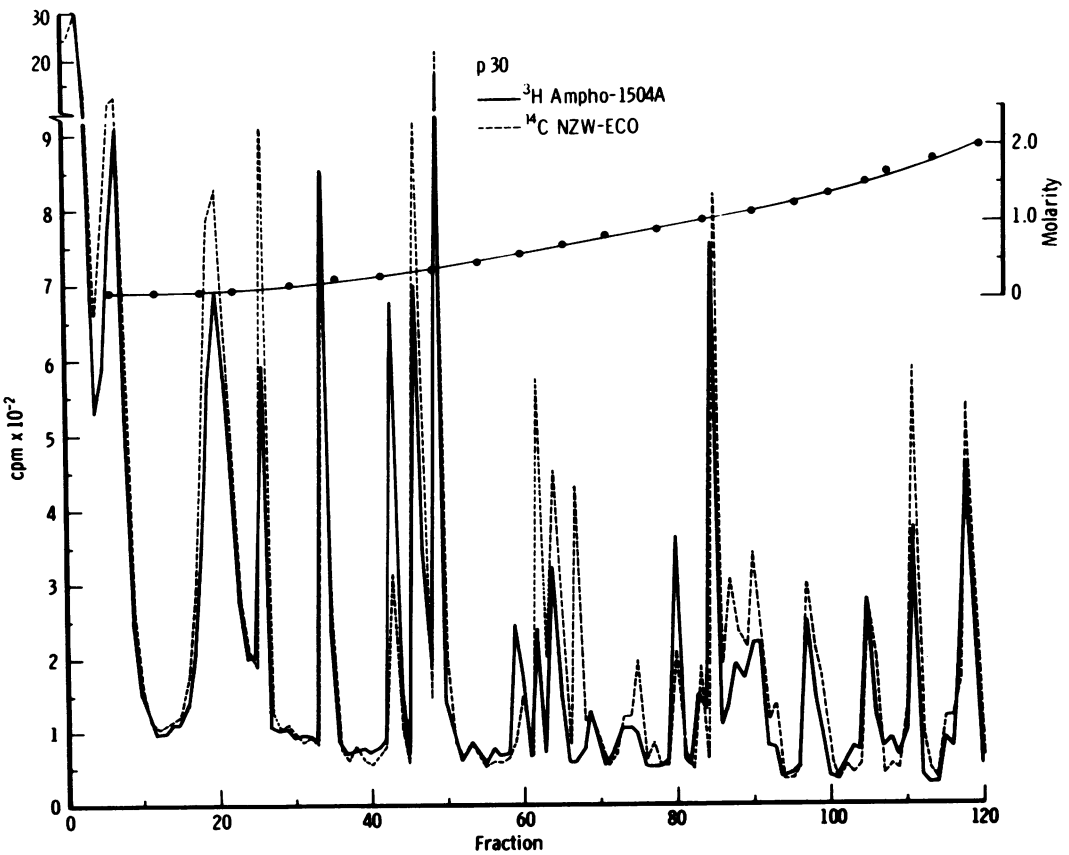

FIG. 2. *Peptide maps of purified p30's. This chromatograph represents a mixture of ^{3}H-labeled wild-mouse amphotropic-1504A (Ampho-1504A) viral p30* (——) *and ^{14}C-labeled NZW ecotropic (NZW-ECO) viral p30* (- - -).

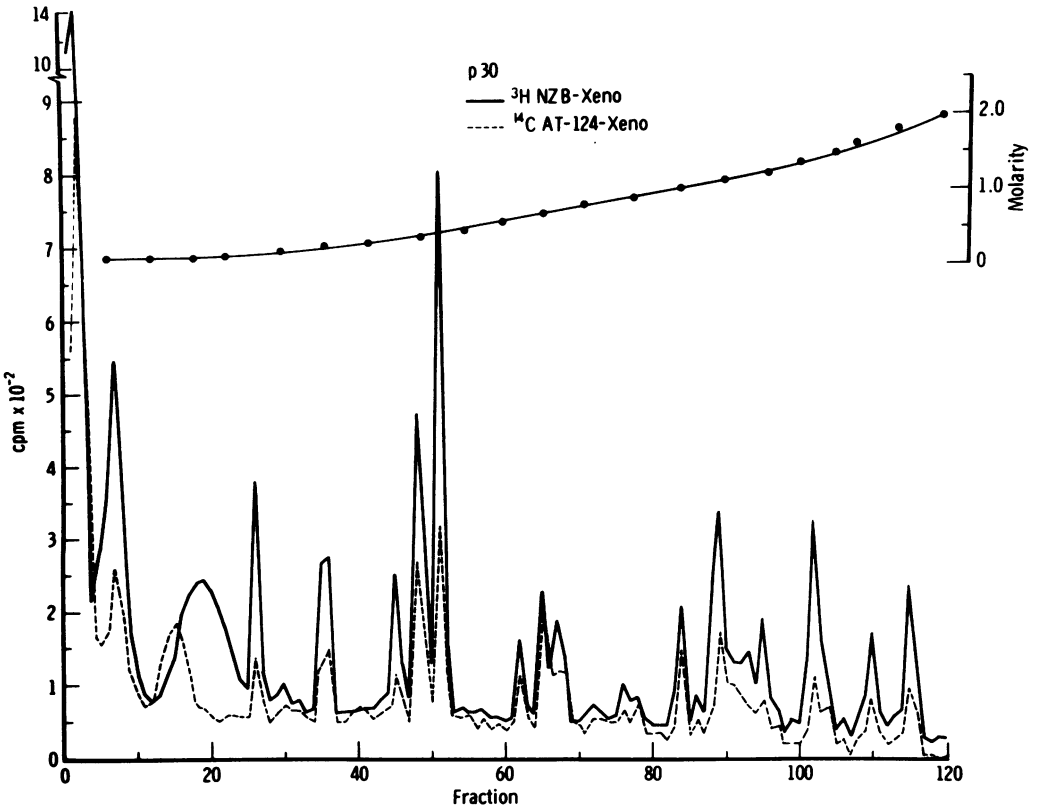

FIG. 3. *Peptide maps of purified p30's. This chromatograph represents a mixture of ^{3}H-labeled NZB xenotropic (NZB-Xeno) viral p30* (——) *and ^{14}C-labeled AT124 xenotropic (AT-124-Xeno) viral p30* (- - -).

tropic p30 from BALB/c cells and MCF-247 dual-tropic p30 from AKR thymocytes. Only one major peptide difference is evident, at fractions 63 (WN1802N) and 65 (MCF-247); minor changes in the patterns are seen at positions 45–46 and 76–77. When MCF-247 p30 was chromatographed with NZB xenotropic p30, the resulting profiles (not shown) were essentially identical to those shown in Fig. 1; i.e., MCF-247 was indistinguishable from NZW ecotropic p30, indicating that it is WN1802N p30, which displays a unique peptide at position 63.

p15 mapping. The very similar chromatographs of NZW ecotropic and NZB xenotropic p15 proteins shown in Fig. 5 differ in the following respects: (i) the presence of a unique peptide in the NZW map at position 30 not found in either NZB or amphotropic (see Fig. 6) p15 proteins; and (ii) a small peptide eluting at position 34 of the NZB and amphotropic (see Fig. 6) maps but not in the NZW profile. The NZB and NZW profiles show a major peptide eluting at position 23, while all three viral p15 maps have coeluting peptides at positions 38 and 58. Figure 6 compares the p15 proteins from NZW ecotropic and wild-mouse amphotropic viruses. The profiles are clearly dissimilar, with only one major peptide in common at position 38. A direct comparison of the p15 protein from NZW and WN1802N ecotropic viruses shows them to be identical (not shown). In general, the p15 proteins of mouse type C viruses are difficult to analyze by this technique due to the fact that trypsinization yields very few peptides that bind to a cation-exchange column.

p12 mapping. Figure 7 shows that the p12 proteins of NZW ecotropic and NZB xenotropic viruses are very dissimilar and have only two major peptides in common at positions 20 and 107. A comparison of NZW ecotropic and amphotropic 1504A p12 proteins given in Fig. 8 reveals distinct differences, with major peptides in common only at positions 7, 93, and 104. NZB p12 is similar in its mapping characteristics to the amphotropic 1504A p12. Figure 9 indicates that the p12 proteins of NZW and WN1802N

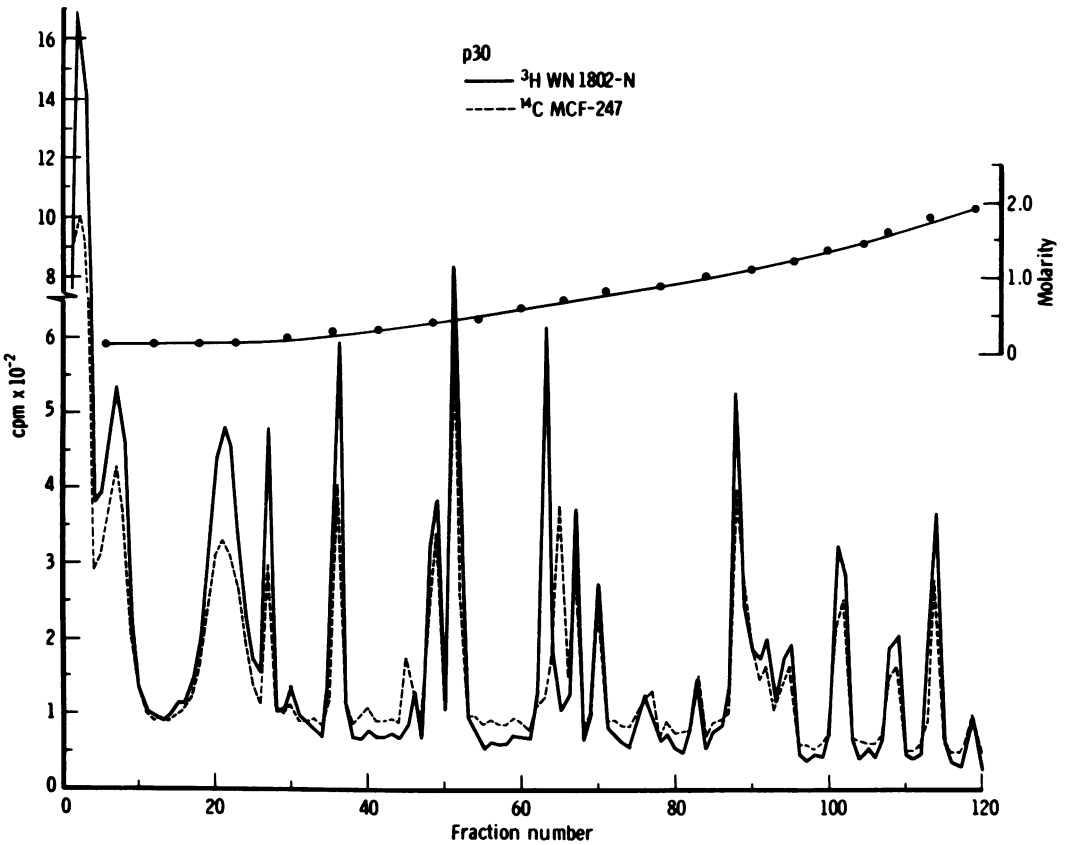

FIG. 4. *Peptide maps of purified p30's. This chromatograph represents a mixture of ^{3}H-labeled WN1802N ecotropic viral p30 (——) and ^{14}C-labeled MCF-247 dual-tropic viral p30 (- - -).*

ecotropic viruses are identical. Likewise, the p12 proteins of NZW ecotropic and MCF-247 viruses are identical (not shown).

p10 mapping. Figure 10 shows that the p10 proteins of NZW ecotropic and wild-mouse amphotropic 1504A viruses are not identical: each contains several unique peptides, with the rest being held in common. The p10 map of NZB xenotropic virus (not shown) is identical to the NZW p10 map (as is WN1802N p10), indicating the strong conservation of the p10 sequences in other murine leukemia virus isolates.

DISCUSSION

In this study we prepared peptide maps of tryptic digests of the *gag* gene products (p30, p15, p12, and p10) of the three principal classes of endogenous murine leukemia viruses: ecotropic, xenotropic, and amphotropic. Peptide maps of the envelope proteins (gp70) of these three classes have recently been compared in other studies (5, 11). Our results, summarized in Table 1, further delineate both the interrelatedness and polymorphism of these *gag* gene proteins. The p30 proteins of representative viruses in these three main classes are strongly conserved and show structural heterogeneity in only two regions, the first one eluting at low normality (fractions 15–20) and the second one eluting at an intermediate normality (fractions 58–72).

The major peptide difference among p30 proteins, at fractions 15–20, reflects a highly type-specific portion of these molecules. Although a more complete interpretation of this structural difference will be possible after analysis of alpha xenotropic viruses, N- and B-tropic viruses, and other amphotropic isolates, it is reasonable to consider whether this peptide is related to Fv-1-mediated tropism of the virus (30). The ecotropic, amphotropic, and dual-tropic viruses studied are all N-tropic and possess identical peptides in this region, whereas the corresponding peptide of the xenotropic viruses eluted ear-

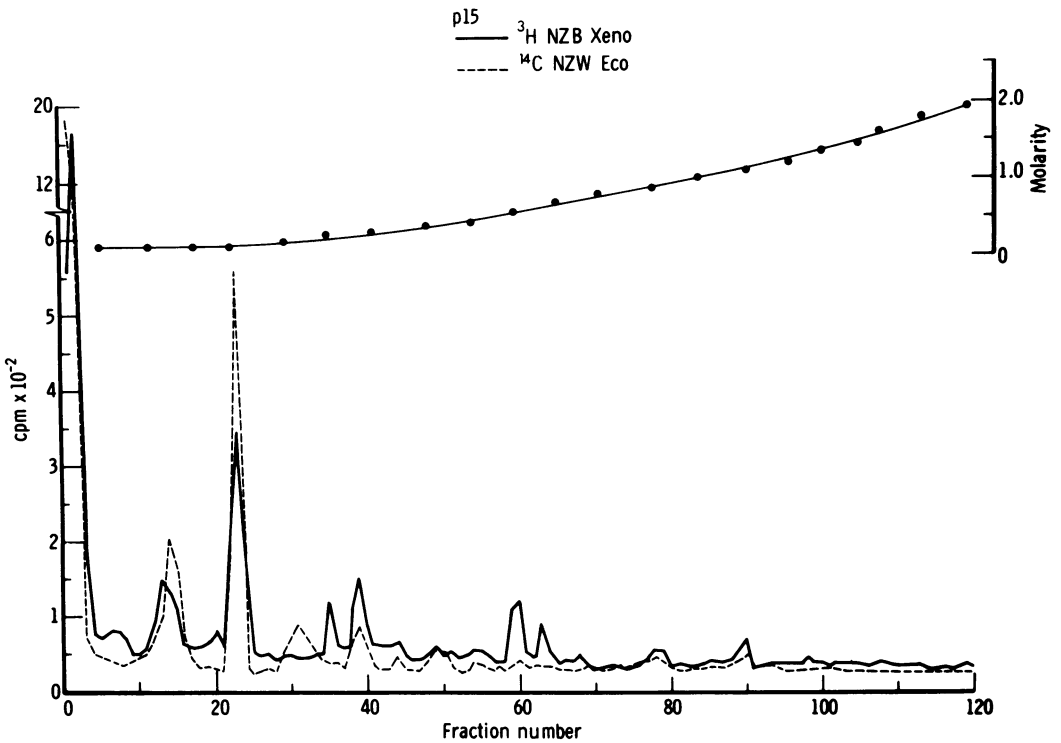

FIG. 5. *Peptide maps of purified p15's. This chromatograph represents a mixture of ^{3}H-labeled NZB xenotropic (Xeno) viral p15* (——) *and ^{14}C-labeled NZW ecotropic (Eco) viral p15* (- - -).

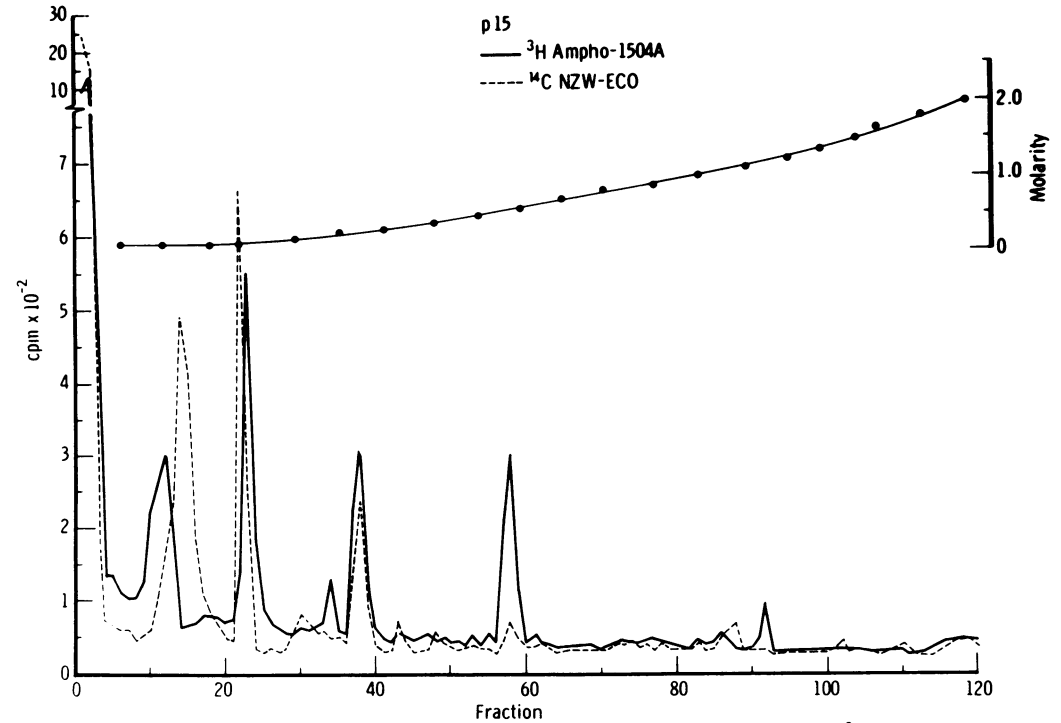

FIG. 6. *Peptide maps of purified p15's. This chromatograph represents a mixture of ^{3}H-labeled wild-mouse amphotropic-1504A (Ampho-1504A) viral p15* (——) *and ^{14}C-labeled NZW ecotropic (NZW-ECO) viral p15* (- - -).

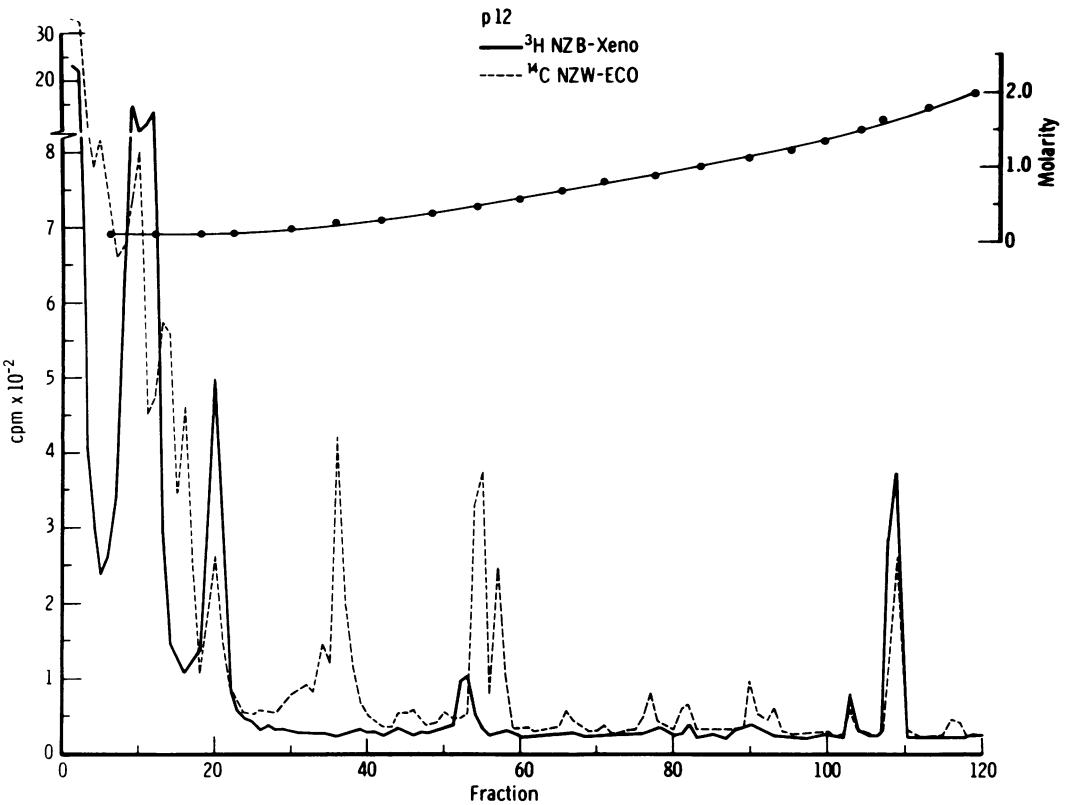

FIG. 7. *Peptide maps of purified p12's. This chromatograph represents a mixture of ^{3}H-labeled NZB xenotropic (NZB-Xeno) viral p12* (——) *and ^{14}C-labeled NZW ecotropic (NZW-ECO) viral p12* (- - -).

lier, although the latter viruses appear more polymorphic in this region. These data further indicate that the NZB and AT124 beta viruses, although clearly one class based on nucleic acid hybridization (7) and p12 immunoassays (36), can be differentiated into two subclasses as has been recently suggested by O'Donnell and Stockert (26). Buchhagen et al. found a similar lack of comigration of this early eluting marker peptide (i.e., fractions 15–20) obtained from the p30 proteins of N- and B-tropic viruses from BALB/c cells (6). Further data of ours (manuscript in preparation) indicate that a cloned B-tropic isolate from C57BL spleen (26) showed the same lack of comigration of this peptide (fractions 15–20) when compared to the N-tropic isolate from NZW cells. Rein et al. implicate a virus protein as a determinant of tropism (32). Hopkins et al. (20) and Schindler et al. (35), have evidence suggesting that this determinant is the p30 molecule or another protein specified by a viral gene closely linked to the gene for p30.

The second elution region (fractions 58–72) of the p30 maps displaying peptide heterogeneity indicates that the xenotropic and amphotropic p30 proteins are identical in this region and distinguishable from the ecotropic p30 protein. Moreover, since amphotropic, dual-tropic MCF, and phenotypically mixed ecotropic viruses grow equally well in mouse and non-mouse cells (16, 18, 21, 31), it appears that the polymorphism seen in this region of the p30 molecule does not affect viral multiplication. The p30 proteins of WN1802N compared with those of MCF-247 contained a unique peptide in this second elution region, possibly indicating that, during its long passage history, this virus has undergone genetic recombination or mutation in the sequences coding for p30. The p15, p12, and p10 proteins of WN1802N had peptide maps identical to those of corresponding proteins of NZW ecotropic virus. The p30 proteins of MCF-247, NZW, and (NZW × NZB)F_1 ecotropic viruses were also shown to be identical, as were the p12 proteins from these viruses. These results further indicate that MCF-247 virus has not undergone detectable genetic recombination in the *gag* gene regions coding for these peptides. Based on analysis of the tyrosine-containing peptides, Elder et al. have determined that NZB p30 is distinguishable from AKV-1 and AKV-2 ecotropic viruses and MCF-247 dual-tropic virus which

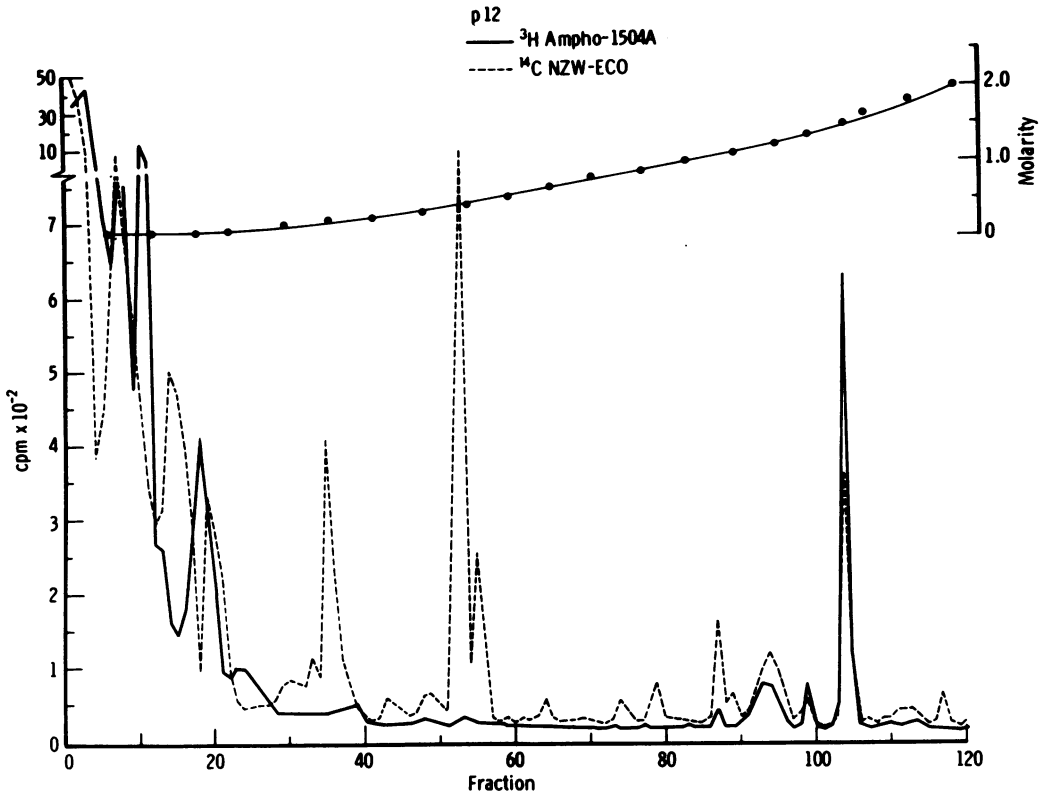

FIG. 8. *Peptide maps of purified p12's. This chromatograph represents a mixture of ^{3}H-labeled wild-mouse amphotropic-1504A (Ampho-1504A) p12* (——) *and ^{14}C-labeled NZW ecotropic (NZW-ECO) viral p12* (- - -).

were themselves indistinguishable (10).

The p15 protein of NZW and BALB/c ecotropic viruses studied were identical and clearly dissimilar to amphotropic p15. While the p15 proteins from ecotropic and xenotropic viruses studied showed considerable homology, they were distinguishable. Elder et al. have found similar conservation among NZB, AKV-1, and MCF-247 p15 proteins (10). The NZW ecotropic and NZB xenotropic p10 proteins were identical and dissimilar to amphotropic p10, indicating the possible subgroup-specific nature of this protein.

Biochemical and immunological data have indicated the close relatedness among p12 proteins of individual viral subgroups as well as the strong type specificity of this molecule (6, 28, 38). The data presented here confirm these reports by showing extensive homology among the p12 proteins of the ecotropic viruses studied. The p12 proteins from both the NZB xenotropic and wild-mouse amphotropic viruses were clearly very similar in their mapping characteristics and substantially different from the ecotropic p12 proteins. These results could indicate a partial immunological cross-reactivity between xenotropic and amphotropic viruses not shared by ecotropic viruses. Finally, our data offer strong evidence of amino acid sequence differences between the p12 proteins of xenotropic, amphotropic, and ecotropic viral subgroups.

Based on the ubiquity of xenotropic and amphotropic genome sequences in the cellular DNA of all mouse strains studied, it appears that they antedate the ecotropic viruses evolutionarily (7, 8). Our results indicate that virtually all proteins encoded by the amphotropic 1504A *gag* gene contain unique peptides. If parts of the amphotropic *gag* gene were acquired via genetic recombination with existing viral or cellular sequences, it appears not to have been a recent event as there is clear structural divergence of all the *gag* gene products compared with similar products of the endogenous xenotropic and ecotropic viral genomes. This indicates that the amphotropic virus has had an independent evolution for a long time. Chattopadhyay et al. have recently shown by nucleic acid hybridization that the 1504A amphotropic virus is in fact a unique isolate having at least 20% of its genomic sequences which are not shared by AKR-type ecotropic or three types of xenotropic type C viruses (8).

It has recently been reported that mink lung

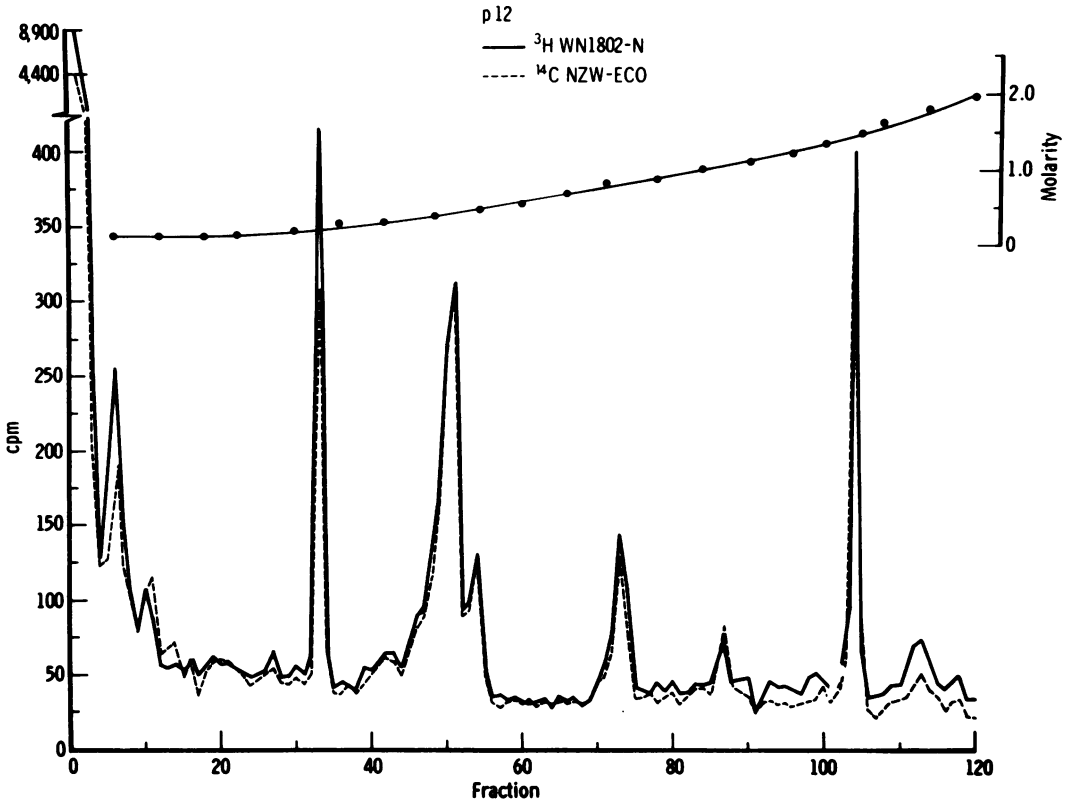

FIG. 9. *Peptide maps of purified p12's. This chromatograph represents a mixture of* ^{3}*H-labeled WN1802N ecotropic viral p12* (——) *and* ^{14}*C-labeled NZW ecotropic (NZW-ECO) viral p12* (- - -).

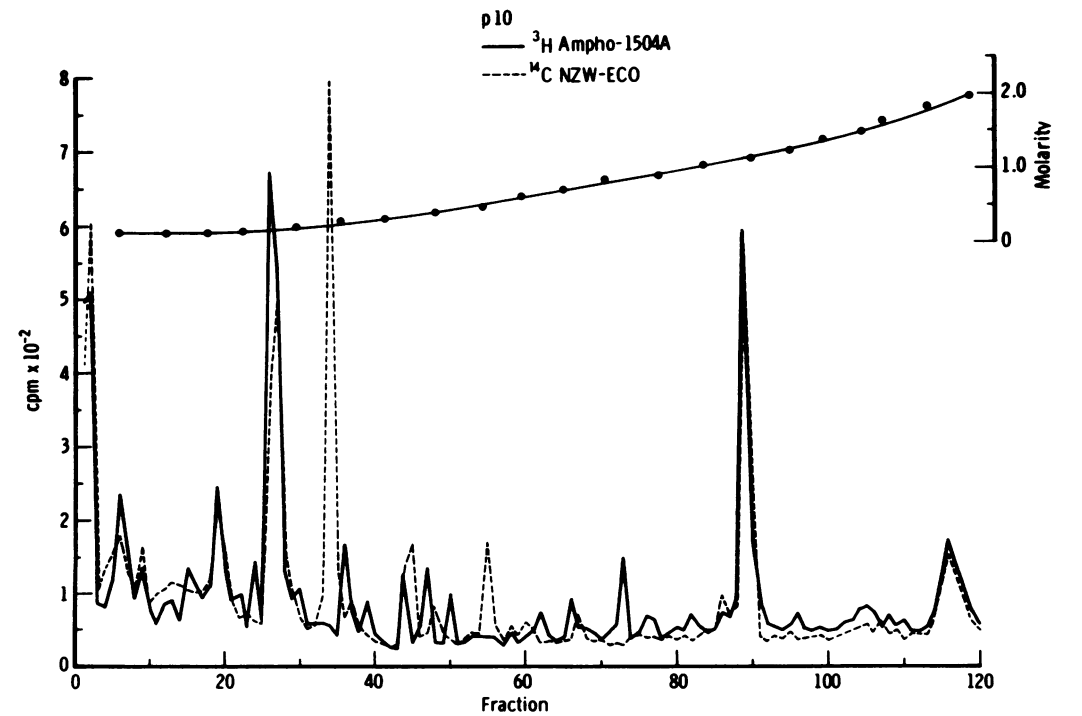

FIG. 10. *Peptide maps of purified p10's. This chromatograph represents a mixture of* ^{3}*H-labeled wild-mouse amphotropic-1504A (Ampho-1504A) viral p10* (——) *and* ^{14}*C-labeled NZW ecotropic (NZW-ECO) viral p10* (- - -).

TABLE 1. *Comparison of the gag proteins of type C viruses*

Maps showing:	p30	p15	p12	p10
Complete homology	NZW N-tropic MCF-247 dual-tropic (W/B)F_1 N-tropic[a, b]	NZW N-tropic WN1802N	NZW N-tropic WN1802N MCF-247[b] (W/B)F_1 N-tropic[a, b]	NZW N-tropic WN1802N[b] NZB xenotropic[b]
Unique peptides	Amphotropic 1504A NZB xenotropic[c] AT124 WN1802N	Amphotropic 1504A NZB xenotropic	Amphotropic 1504A NZB xenotropic	Amphotropic 1504A

[a] Virus from (NZW × NZB)F_1 cells.
[b] Maps not shown in this paper.
[c] (NZW × NZB)F_1 xenotropic p30 is identical to NZB xenotropic p30.

(CCL-64) cells contain an endogenous virus of mink origin (4), which is expressed after long-term tissue culturing. We feel that the data presented here are unaffected by the mink virus for the following reasons: (i) uninfected mink cell cultures were consistently negative for production of type C viruses; (ii) cultures of mink cells were utilized at relatively early passage levels and only for a limited number of passages before being discarded; (iii) NZW ecotropic virus produced in mouse SC-1 cells and NZB xenotropic virus produced in mink lung cells had identical p10 protein peptide maps; and (iv) NZB xenotropic p30 grown in mink lung cells and amphotropic p30 grown in SC-1 cells had identical peptide maps with the exception of one major peptide which appeared to correlate with viral tropism.

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