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Membrane Glycopeptides from Virus-Transformed Hamster Fibroblasts and the Normal Counterpart†

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ABSTRACT: Comparisons of membrane glycopeptides from baby hamster kidney fibroblasts (BHK_{21}/C_{13}) and a clone transformed by Rous sarcoma virus (C_{13}/B_4) were made by using cells metabolically labeled with radioactive D-glucose and L-fucose. Most of the glycopeptides were metabolically labeled with both the general and the specific glycoprotein precursors. The glycopeptides obtained from the cell surface by controlled trypsinization were representative of the surface membrane as shown by comparing them with those of purified membrane preparations. The trypsin-removable glycopeptides from both cell types were further processed and examined by successive chromatography on Sephadex G-50 and DEAE-cellulose. The chromatographic distribution patterns showed that each cell type had glycopeptides of similar characteristics, although the proportions of the glycopeptides differed dramatically between the two cell types. After transformation there was an increase in the larger, more highly charged glycopeptides. This was verified by the increased sialic acid content in these glycopeptides. Some of the glycopeptides were homogeneous after the size and charge separations, since a variety of procedures did not separate them further. The apparent homogeneity and reasonably few species obtained may be due to the methods of isolation, with the procedures selecting particular glycopeptides from the external portion of the membrane. These results corroborate the concept and show for the first time that virus transformation is accompanied by an increase in certain species of glycopeptides rather than de novo synthesis.

Membrane glycoproteins from virus-transformed and tumor cells differ from those of the normal counterparts. This has been shown in many systems by using a variety of methods. Among the direct methods used have been polyacrylamide gel electrophoresis of purified surface membranes, using metabolic labeling or Coomassie blue staining for the detection of the proteins and glycoproteins, or whole-cell preparations after surface labeling by different techniques. Others have examined the glycopeptides obtained after protease digestions of a variety of cell types (Fishman & Brady, 1976; Glick, 1976a; Emmelot et al., 1977).

The glycopeptides which were removed from the surface of virus-transformed cells with controlled trypsinization were shown, after subsequent Pronase digestion, to be different from those of the norman counterparts by the criterion of the elution patterns from Sephadex G-50 columns (Glick, 1974a; Warren et al., 1978). These observations were extended to show that the appearance of particular glycopeptides was directly correlated with tumorigenesis (Glick et al., 1974) and that human tumors (Glick, 1976b), including leukemias (Van Beek et al., 1975), showed similar alterations. In fact, the altered surface glycopeptides have been reported to be the most consistent change accompanying malignancy (Van Beek et al., 1977) and have been reviewed (Warren et al., 1978). It must be remembered that these glycopeptides were derived from glycoproteins on the external side of the membrane and indeed, for this very reason, are extremely important in defining a tumor cell.

In spite of the many cell types which have been reported to show these altered glycopeptides [see Warren et al. (1978) for a review], no data have been reported to determine the unique or ubiquitous nature of the glycopeptides. Are the glycopeptide alterations quantitative or qualitative? In order to answer this question, one must examine the heterogeneity of the glycopeptides. Only a brief report (Glick, 1974a) showed that the glycopeptides can be further separated on DEAE-cellulose and another showed their behavior on Con A–Sepharose (Ogata et al., 1976). The studies reported here suggest that the latter separation on Con A–Sepharose was not sufficient and must yield a mixture of glycopeptides. Moreover, the separation on Sephadex G-50, followed by DEAE-cellulose chromatography, revealed a spectrum of glycopeptides.

The glycopeptides were removed by trypsin from the surface of transformed hamster cells (C_{13}/B_4) and the normal counterpart (BHK_{21}/C_{13}). Subsequent digestion of the surface glycopeptides with Pronase as well as further processing left species of glycopeptides representative of the surface of both cell types and showing clearly the alterations observed originally after virus transformation (Buck et al., 1970). As a result of further purification of these glycopeptides, evidence is presented here that all species appeared in both cell types, although the percentages were vastly different. In addition, although there was some heterogeneity of size and charge, the species of glycopeptides were amazingly few. It has thus been possible to isolate some of these glycopeptides in homogeneous

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Table I: L-[^{3}H]Fucose Incorporation into BHK_{21}/C_{13} and C_{13}/B_4 Cell Fractions[a]

cell type	cpm/10^4 cells: trypsinate,[b] $n = 8$	cpm/10^4 cells: cell, $n = 8$	cpm/10^4 organelles[c]: membrane, $n = 3$	cpm/10^4 organelles[c]: nucleus, $n = 2$	cpm/10^4 organelles[c]: remaining[d]
BHK_{21}/C_{13}	3.9 ± 1.1 (37%)	6.7 ± 2.1 (63%)	4.0 (38%)	0.5 (5%)	(20%)
C_{13}/B_4	6.9 ± 2.2 (21%)	25.3 ± 7.1 (79%)	3.4 (11%)	0.9 (3%)	(65%)

[a] The numbers in parentheses represent the percentage of the total radioactivity which was the cell and trypsinate. [b] Calculated from the total cell content. [c] Surface membranes and nuclei were counted in the hemocytometer. [d] Minus the trypsinate which was removed before the membranes were prepared.

form and begin to derive the oligosaccharide structure (Santer & Glick, 1979).

Materials and Methods

Cell Growth and Harvest. A clone of baby hamster kidney fibroblasts (BHK_{21}/C_{13}) and the same clone transformed by the Bryan strain of Rous sarcoma virus (C_{13}/B_4) were grown to upper log phase on a Bellco roller apparatus (654.51-cm^2 growth area) as described previously (Buck et al., 1970). Both cell types showed similar growth properties and growth related changes (Buck et al., 1971; Glick & Buck, 1973); therefore, the conditions of culture were controlled rigidly. All cells used for these experiments were below passage 9 and free of mycoplasma. The cells were made radioactive by growth for 72 h with the appropriate isotope, washed 4 times with TBS,[1] and harvested from the monolayers with trypsin (10 mg in TBS per roller), followed by a neutralizing amount of soy bean inhibitor. The cells were centrifuged at 800*g* for 5 min, and the supernatant material, referred to as trypsinate, was further purified and the cells were used to prepare surface membranes.

Processing of Trypsinate. The trypsinate containing the membrane glycopeptides was further centrifuged at 49000*g* for 20 min and lyophilized. Lyophilized trypsinates obtained from (2–3) × 10^8 cells were suspended in 1.5 mL of water and digested with Pronase (preincubated at 37 °C for 30 min) for 5 days with daily additions of Pronase (Buck et al., 1970). The glycopeptide-containing incubation mixture was precipitated with 5% trichloroacetic acid, and the supernatant material was dialyzed against water for 18 h and lyophilized.

Preparation of Surface Membranes and Nuclei. The cell pellets were washed 3 times in 20 mL of 0.16 M NaCl and suspended finally at 5 × 10^7 cells/mL of 0.16 M NaCl. Aliquots were removed for proteins and radioactivity. Surface membranes were prepared by the Zn ion technique (Warren & Glick, 1969), and after purification, aliquots were removed for counting in the hemocytometer, proteins, and radioactivity. Pronase digestion of the surface membranes to obtain the glycopeptides was as described for the trypsinates, with the exception that the membranes were dissolved in 0.1% $NaDodSO_4$ prior to Pronase treatment. Nuclei were prepared from the cells after removal of the surface membrane, and the final preparation was counted in the hemocytometer (Keshgegian & Glick, 1973).

Purification of Glycopeptides. To screen the glycopeptides, chromatography was on Sephadex G-50 (Pharmacia) as described (Buck et al., 1970). For large-scale experiments, to isolate radioactive Pronase-digested glycopeptides, a column (2.5 × 120 cm) of Sephadex G-50 was eluted with 0.1 M Tris–acetate buffer, pH 9.0, containing 0.1% $NaDodSO_4$, 0.01% EDTA, and 0.1% mercaptoethanol, and fractions of 2.0 mL were collected between Blue Dextran 2000 and phenol red which served as markers. Fractions were combined according to the radioactivity, dialyzed for 18 h against water at 5 °C, lyophilized, suspended in 0.5 mM sodium phosphate buffer, pH 6.8, and further separated on a column (1.3 × 22.5 cm) of DEAE-cellulose with 40 mL of 0.5 mM sodium phosphate buffer, pH 6.8, followed by a gradient containing 100 mL each of 0.5 and 30 mM sodium phosphate buffer, pH 6.8. Final elution was with 60 mL of 100 mM sodium phosphate buffer, pH 6.8. The fractions of 2.0 mL which were collected were combined according to the radioactivity, dialyzed, lyophilized, and frozen at −80 °C until further use. Where specified, these fractions were rechromatographed on a column (0.8 × 20 cm) of DEAE-cellulose with a gradient of 50 mL of each buffer under conditions similar to those used for the larger column or with gradients of 50 mL each of 50–120 or 20–200 mM sodium phosphate buffer, pH 6.8, as specified, with a final elution with 200 mM sodium phosphate buffer.

Chemical Analysis. Sialic acid was determined by a microadaptation of the thiobarbituric acid assay (Glick, 1974b). Crystalline *N*-acetylneuraminic acid (Calbiochem) served as the standard. Proteins were determined by the method of Lowry et al. (1951). All radioactive counting was performed after suspending the aqueous solutions in Aquasol (New England Nuclear).

Materials. New England Nuclear Corp. was the source of the following compounds: L-[1-^{14}C]fucose (50.8 mCi/mmol); L-[^{3}H]fucose (Gl, 4.3 Ci/mmol); D-[^{14}C]glucose (U, 200 mCi/mmol); D-[1-^{3}H]- and D-[6-^{3}H]glucose (7 Ci/mmol), which were added to the cell growth media at 25 and 50 μCi/roller for ^{14}C and ^{3}H compounds, respectively. Trypsin (3× crystallized) and purified soy bean inhibitor were obtained from Worthington Biochemicals, Pronase was from Calbiochem, and Con A–Sepharose was from Pharmacia.

Results

Incorporation of Radioactive Fucose. Table I gives the percentage of radioactive fucose incorporated into the BHK_{21}/C_{13} and C_{13}/B_4 cells and cell fractions. Approximately fourfold more radioactivity was incorporated within 72 h into the C_{13}/B_4 fibroblasts when calculated per cell. However, because of the cell size, when the incorporation was calculated per milligram of protein, only a twofold increase was apparent. Again, although the amounts differed per cell, similar amounts were found in trypsinates from both cell types when expressed per milligram of cell protein. However, the amount of radioactive fucose in the trypsinate from BHK_{21}/C_{13} cells represented a higher percentage of the total fucose incorporated, that is, 37% as compared to 21% (Table I).

The surface membranes and nuclei isolated from both cell types were whole and were counted in a hemocytometer so the amount of radioactivity incorporated was expressed as a percentage of the whole cell. The surface membranes contained approximately 10% of the total cell protein, 2.5 × 10^{-8} and 4.5 × 10^{-8} mg for BHK_{21}/C_{13} and C_{13}/B_4 membranes, respectively. As with the trypsinate, a higher percentage of the total radioactivity was incorporated into the surface

[1] Abbreviations used: TBS, 0.15 M NaCl and 0.02 M Tris-HCl (pH 7.5); $NaDodSO_4$, sodium dodecyl sulfate.

Table II: Separation of Membrane Glycopeptides on Sephadex G-50 and DEAE-Cellulose

Sephadex G-50 fraction[a]		% of total radioactivity	subsequent recovery on DEAE-cellulose,[b] % of total radioactivity							
			100[e]	27-30[e]	23-25[e]	18-22[e]	16-17[e]	10-15[e]	8-9[e]	0.5[e]
1	NT[c]	3	17	26	31		0	10	0	9
	T[d]	8	15	27		42		10		4
2	NT	18	4	19	36	41	0	0	0	0
	T	33	6	16	39	38				
3	NT	12	0	9	0	18	13	38	10	0
	T	12		1		26	14	37	10	
4	NT	32	0	0	0	0	10	44	34	7
	T	22					10	48	32	5
5	NT	23	0	0	0	0	0	13	27	39
	T	11						14	21	52
6	NT	11								
	T	13								

[a] See Figure 3 for examples of a similar experiment. [b] See Figures 4 and 5 for examples of similar experiments. [c] NT = nontransformed BHK_{21}/C_{13} cells metabolically labeled with L-[^{3}H]fucose. [d] T = transformed C_{13}/B_4 cells metabolically labeled with L-[^{14}C]fucose. [e] mM sodium phosphate buffer, pH 6.8.

membranes of the BHK_{21}/C_{13} cells than into those of the transformed cells. Only 20% of the radioactivity was found in the remaining cell fractions in contrast to C_{13}/B_4 cells which had 65% of the radioactivity incorporated into the remaining fractions. Thus, the trypsinates and surface membranes of BHK_{21}/C_{13} cells were more enriched in fucose-containing components than those of the C_{13}/B_4 cells, although the absolute amount was more in the transformed cells.

Differences between the Membrane Glycopeptides of BHK_{21}/C_{13} and C_{13}/B_4 Cells. Trypsinates from the transformed cells (C_{13}/B_4) and the normal counterpart (BHK_{21}/C_{13}), metabolically labeled with L-[^{14}C]- or L-[^{3}H]fucose, respectively, were combined on the basis of radioactivity, digested with Pronase, precipitated with trichloroacetic acid, dialyzed, and chromatographed on DEAE-cellulose. The fucose-containing glycopeptides from the transformed fibroblasts separated into seven or more groups while those from the nontransformed cells separated into only three major groups (Figure 1). The glycopeptides which eluted from DEAE-cellulose with approximately 18–30 mM sodium phosphate buffer, pH 6.8, were more predominant in the transformed cells than in the normal counterpart. These are represented by three radioactive areas: fractions 80–95, 100–120, and 125–134, respectively, in Figure 1. The fucose-containing material which was eluted in less than 17 mM phosphate buffer was common to both cell types. The material which was eluted with 100 mM phosphate buffer varied in relative amount in different experiments but was always present in both cell types.

The glycopeptides which were removed from the cell surface with trypsin were representative of those which remained in the surface membranes. The patterns obtained by chromatography on DEAE-cellulose of Pronase-digested trypsinate and surface membranes from C_{13}/B_4 cells were similar (Figure 2). The trypsinate was from cells metabolically labeled with L-[^{14}C]fucose, and the surface membranes were from cells labeled with L-[^{3}H]fucose. The fractions were combined prior to Pronase digestion. The surface membranes and trypsinates from BHK_{21}/C_{13} were also similar to each other. Interchange of the radioactivity or use of radioactive glucose gave the same results.

Detailed Differences in the Trypsinates from Virus-Transformed Cells and Their Normal Counterpart. Trypsinates were prepared from BHK_{21}/C_{13} and transformed C_{13}/B_4 cells made radioactive by growth for 72 h in the presence of L-[^{3}H]- or L-[^{14}C]fucose, respectively. Chromatography of the Pronase-digested trypsinates on Sephadex

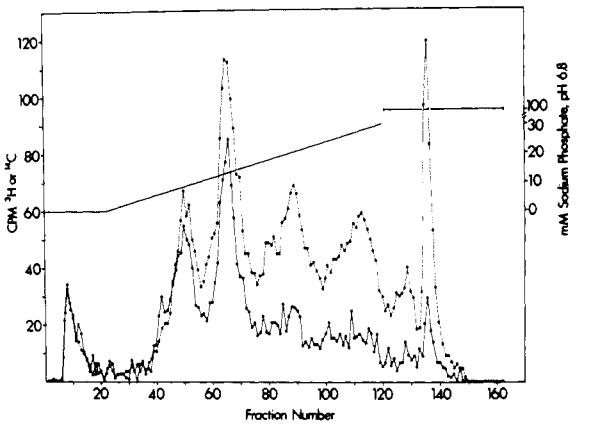

FIGURE 1: Chromatography on DEAE-cellulose of membrane glycopeptides. Trypsinates from BHK_{21}/C_{13} (●) and C_{13}/B_4 (○) cells, metabolically labeled with L-[^{3}H]- or L-[^{14}C]fucose, respectively, were combined, digested with Pronase, processed, and chromatographed on DEAE-cellulose. The glycopeptides were eluted with increasing molarity of sodium phosphate buffer, pH 6.8 (see Materials and Methods for details).

G-50 showed that the fractions which were eluted more rapidly from the column were more prominent in the virus-transformed cells (C_{13}/B_4) than in the normal counterpart. This result was similar to previous reports (Glick, 1974a; Buck et al., 1970). Only the fractions included within the Sephadex G-50 column were separated and used for further purification. Material which was eluted in the void volume, such as shown in Figure 3b, was not labeled with fucose and included hyaluronidase-digestible material as described previously (Buck et al., 1970). It was not considered further.

The first two columns of Table II give the fractions which were included within the Sephadex G-50 column and were separated on the basis of radioactivity. The fractions obtained from this column of Sephadex G-50 were similar to the fucose-labeled material shown in Figure 3a for C_{13}/B_4 trypsinate and Figure 3b for BHK_{21}/C_{13} trypsinate. More than 50% of the radioactivity in the C_{13}/B_4 trypsinate was found in Sephadex fractions 1–3, and the predominant fraction, fraction 2, contained 33% of the total radioactivity. In contrast, fraction 2 from the nontransformed cells contained only 18% of the total radioactivity. Moreover, the reverse proportions were found in fraction 4 which contained 32% of the radioactivity of BHK_{21}/C_{13} trypsinate, in contrast to only 22% found in fraction 4 of C_{13}/B_4 trypsinate (Table II).

Fractions 1–5 from Sephadex G-50 which separated as shown in Table II were isolated, and the corresponding

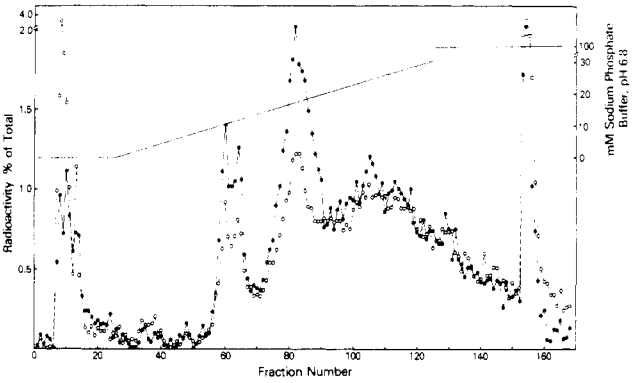

FIGURE 2: Comparison of glycopeptides from trypsinates and surface membranes. Surface membranes prepared from C_{13}/B_4 cells metabolically labeled with L-[^{3}H]fucose (●) were combined with a trypsinate from C_{13}/B_4 cells, metabolically labeled with L-[^{14}C]fucose (○), Pronase digested, and chromatographed on DEAE-cellulose. Elution was with increasing molarity of sodium phosphate buffer, pH 6.8 (see Materials and Methods).

fractions derived from the transformed or nontransformed cells were combined on the basis of radioactivity and chromatographed on DEAE-cellulose. Fraction 6 was dialyzable and was not examined further. Table II shows the percentage of each fraction which was recovered after these combined fractions were eluted from DEAE-cellulose with sodium phosphate buffer, pH 6.8. Similar fractions but in different proportions were obtained from the glycopeptides of both cell types. The glycopeptides which were eluted with 18–30 mM phosphate buffer from DEAE-cellulose represented the bulk of the radioactivity in the Sephadex fractions 1 and 2. Those glycopeptides which were eluted with 0.5–18 mM buffer were the bulk of the radioactivity in Sephadex fractions 3, 4, and 5.

The differences in the glycopeptides between the two cell types such as shown after filtration on Sephadex G-50 (Figure 3) were further clarified by chromatography on DEAE-cellulose (Table II). The transformed trypsinate contained significantly more of the total radioactive material which eluted with 18–30 mM phosphate buffer. For example, the bulk of the radioactive material in Sephadex fraction 2 of both trypsinates was eluted with 18–25 mM phosphate buffer; however, this material represented 25% of the trypsinate from the transformed cells and only 12% of that from the nontransformed cells (Table II). On the other hand, in Sephadex fraction 4 where the bulk of the radioactivity was eluted with 8–15 mM phosphate buffer, the radioactivity represented similar percentages (20–25%) of both trypsinates. Even more striking were the actual amounts: the radioactive material from fraction 2 of the transformed-cell trypsinate which was eluted with 23–25 mM phosphate buffer was 3.5 times more than that from the normal counterpart, while the amount of radioactivity from fraction 4 which eluted with 10–15 mM phosphate buffer was only 1.5 times more than that from the normal counterpart.

Heterogeneity of the Glycopeptides. The first separation on Sephadex G-50 was made presumably on the basis of size, while that on DEAE-cellulose reflected the charge of the glycopeptides. In general, glycopeptides which were eluted from Sephadex G-50 as larger in size (Sephadex fractions 1–3) were also eluted from DEAE-cellulose as more highly charged (Table II). However, a few glycopeptides with apparently large size differences, i.e., Sephadex fractions 1 and 5, had a similar charge, in that some of the radioactivity from both fractions was eluted from DEAE-cellulose with 10–15 or 0.5 mM phosphate buffer. An example of size similarity but

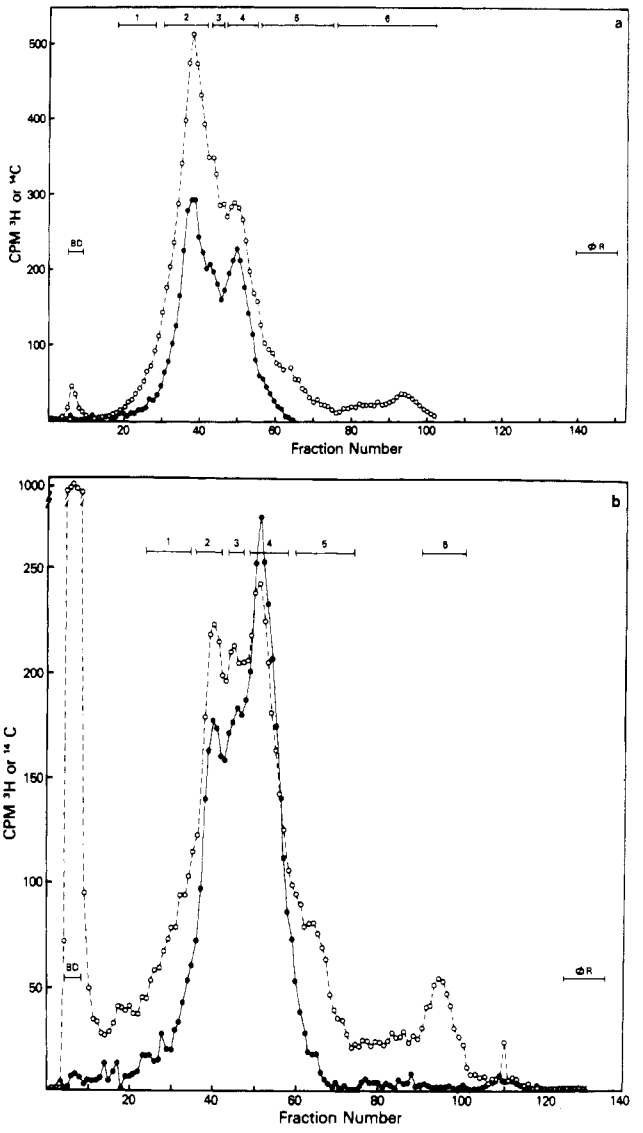

FIGURE 3: Separation of membrane glycopeptides by filtration on Sephadex G-50. (a) C_{13}/B_4 and (b) BHK_{21}/C_{13} cells were each metabolically labeled with both D-[^{14}C]glucose (○) and L-[^{3}H]fucose (●). The Pronase-digested trypsinates from both cell types were eluted from the column of Sephadex G-50, an aliquot was removed for radioactive counting, and fractions were subsequently combined according to areas designated 1–6. BD, Blue Dextran 2000, and ϕR, phenol red, served as markers. All details are described under Materials and Methods.

charge difference was found in Sephadex fractions 4 and 5. Fraction 5 represented the trailing edge of fraction 4 (Figure 3); however, the bulk of the radioactivity was eluted from fraction 5 as uncharged, while that from fraction 4 was eluted with 10–15 mM phosphate buffer.

The most heterogeneous glycopeptides were found in Sephadex fraction 1 (Table II and Figure 4a). Although the bulk of this fraction was eluted as highly charged (Figure 4a and 5a), occasionally a smaller amount of heterogeneously charged material was eluted (Figure 4a). Since this whole fraction represented less than 8% of the total radioactivity in the trypsinates, the reasons for the discrepancy have not been pursued.

Additional Characterization of the Glycopeptides. Trypsinates were obtained from BHK_{21}/C_{13} and C_{13}/B_4 cells which were each metabolically labeled simultaneously with two isotopes, D-[^{14}C]glucose and L-[^{3}H]fucose. The glycopeptides were digested with Pronase and after precipitation

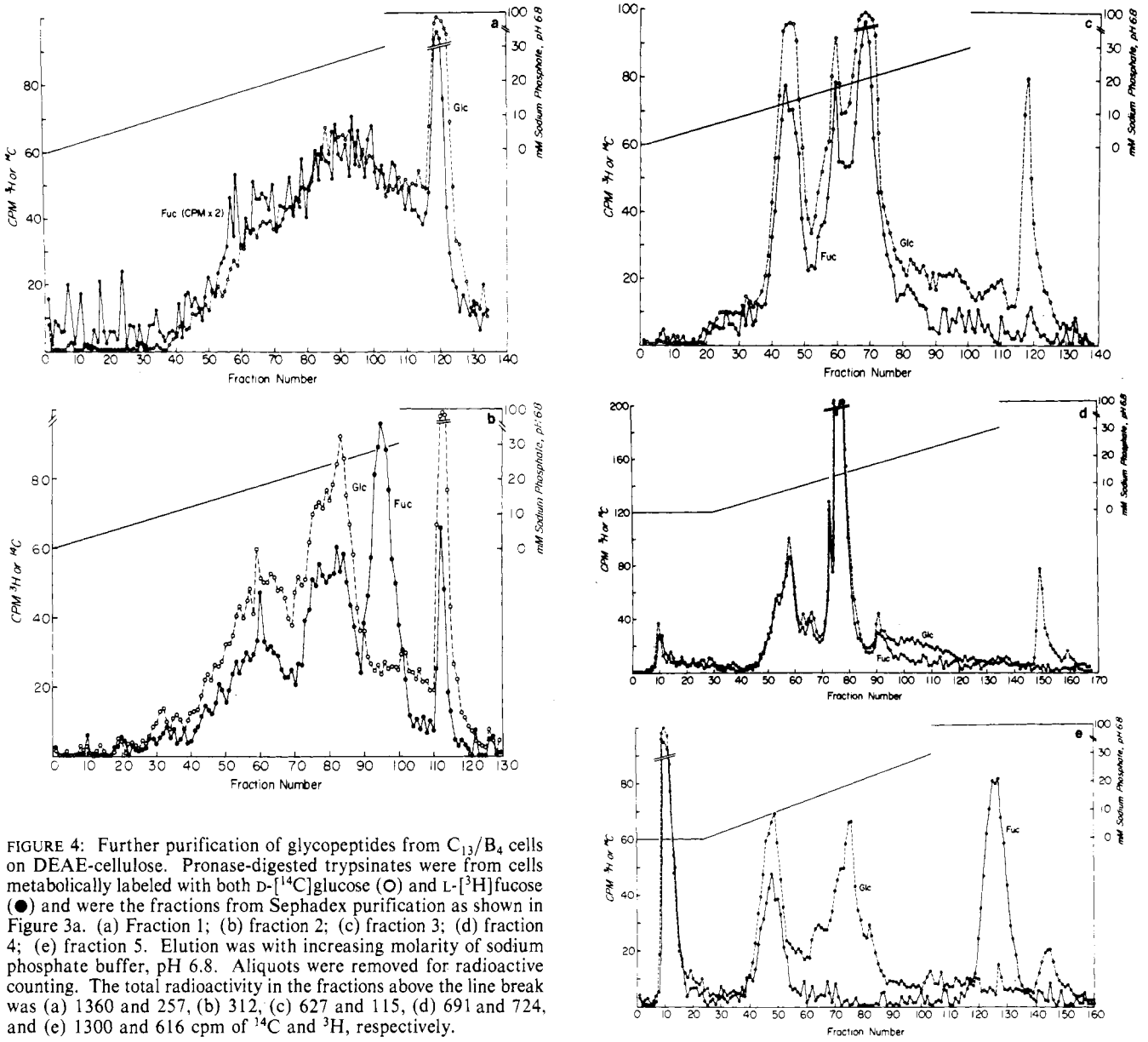

FIGURE 4: Further purification of glycopeptides from C_{13}/B_4 cells on DEAE-cellulose. Pronase-digested trypsinates were from cells metabolically labeled with both D-[^{14}C]glucose (○) and L-[^{3}H]fucose (●) and were the fractions from Sephadex purification as shown in Figure 3a. (a) Fraction 1; (b) fraction 2; (c) fraction 3; (d) fraction 4; (e) fraction 5. Elution was with increasing molarity of sodium phosphate buffer, pH 6.8. Aliquots were removed for radioactive counting. The total radioactivity in the fractions above the line break was (a) 1360 and 257, (b) 312, (c) 627 and 115, (d) 691 and 724, and (e) 1300 and 616 cpm of ^{14}C and ^{3}H, respectively.

with trichloroacetic acid were separated on Sephadex G-50, followed by further separation on DEAE-cellulose. The results point out that L-fucose as a metabolic label marked an equal number of glycopeptides in approximately the same proportions as the more general label, glucose (Figures 3, 4, and 5).

Figure 3a shows the pattern of the doubly labeled glycopeptides obtained from the Pronase-digested trypsinates of C_{13}/B_4 cells and the separation made on Sephadex G-50. Similar to the results in Table II, fraction 2 contained the largest fraction of glycopeptides. In contrast, fraction 4 from the BHK_{21}/C_{13} trypsinates (Figure 3b) contained the bulk of the glycopeptides from this latter cell type.

When each of the fractions from Sephadex G-50 (Figure 3a,b) was further separated on DEAE-cellulose (Figures 4a–e and 5a–e), the percentage of radioactivity was similar in most cases whether radioactive glucose or fucose served as the glycoprotein precursor. The most notable exceptions were with the material which was eluted with 100 mM phosphate buffer, where an increased percentage of glucose-derived radioactivity was found (Figures 4 and 5). Other exceptions were in Sephadex fraction 5, when glucose-derived material only was eluted with 13–16 mM phosphate buffer from both cell types (Figures 4e and 5e). Prominent fucose-labeled material was eluted as highly charged (Figure 4e) but was not always seen (Table II) and represented a minor amount of radioactivity in Sephadex fraction 5. A significant amount of similar material was found in Sephadex fraction 2 from the transformed cells (Figure 4b) and is under further study.

The radioactive material which was eluted with 100 mM phosphate buffer (Figures 4a and 5a) was rechromatographed on DEAE-cellulose and eluted with higher salt concentrations. Seventy percent of the glucose-derived material of BHK_{21}/C_{13} origin eluted with 65 mM phosphate buffer, and 25% was eluted with 78 mM phosphate buffer, while all of the fucose-labeled material was eluted with 65 mM phosphate buffer. Similar material from C_{13}/B_4 cells was eluted as one peak with 68 mM phosphate buffer whether the label was derived from glucose or fucose.

Thus, use of the more general label, glucose, again supported the data obtained with fucose that similar glycopeptides were present on both transformed and nontransformed cell surfaces but that the major difference was in the proportions of these glycopeptides.

Further Purification of the Glycopeptides. Rechromatography of any of the separated glycopeptides showed that they maintained their original characteristics on either

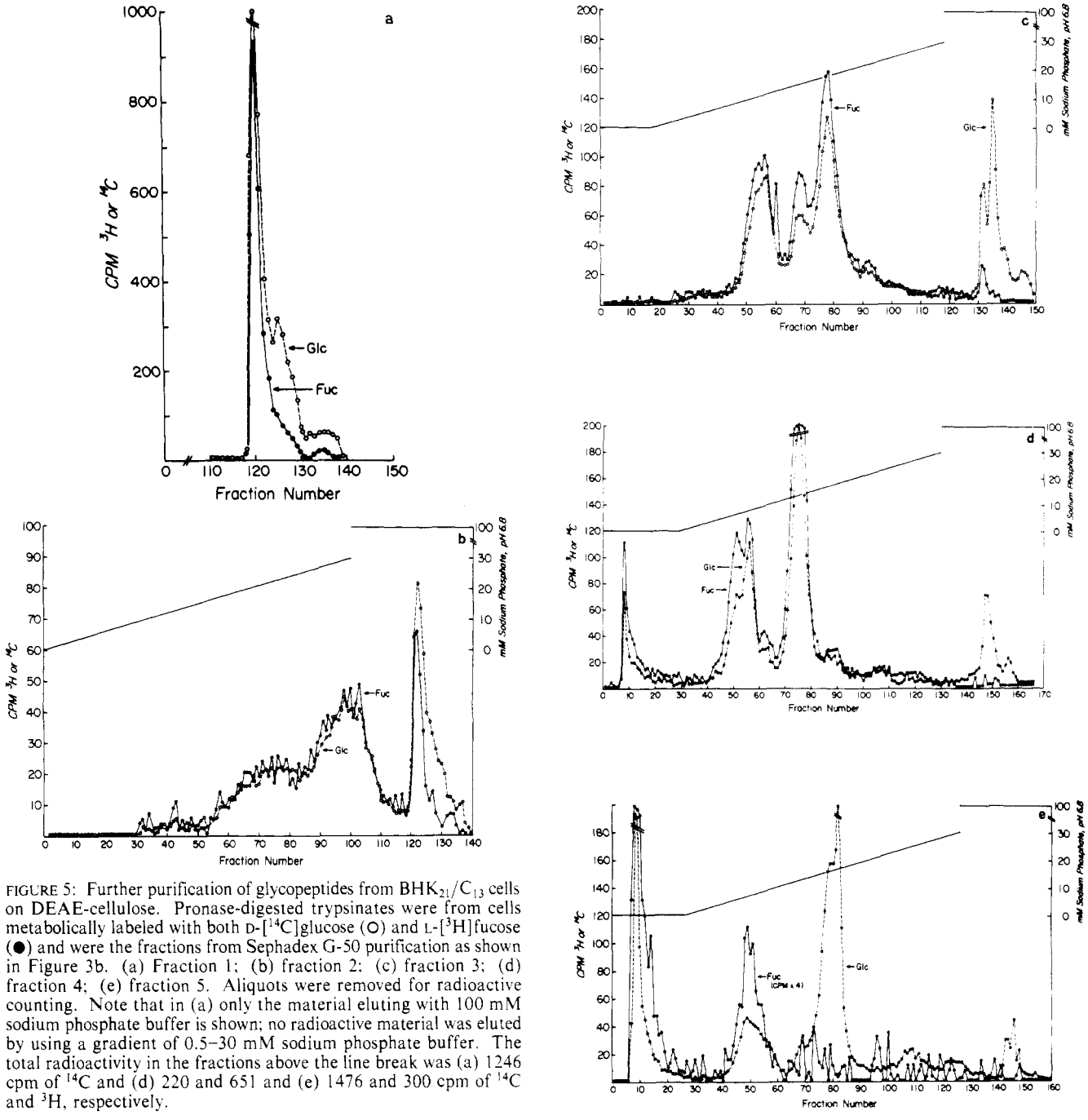

FIGURE 5: Further purification of glycopeptides from BHK_{21}/C_{13} cells on DEAE-cellulose. Pronase-digested trypsinates were from cells metabolically labeled with both D-[^{14}C]glucose (○) and L-[^{3}H]fucose (●) and were the fractions from Sephadex G-50 purification as shown in Figure 3b. (a) Fraction 1; (b) fraction 2; (c) fraction 3; (d) fraction 4; (e) fraction 5. Aliquots were removed for radioactive counting. Note that in (a) only the material eluting with 100 mM sodium phosphate buffer is shown; no radioactive material was eluted by using a gradient of 0.5–30 mM sodium phosphate buffer. The total radioactivity in the fractions above the line break was (a) 1246 cpm of ^{14}C and (d) 220 and 651 and (e) 1476 and 300 cpm of ^{14}C and ^{3}H, respectively.

DEAE-cellulose or Sephadex G-50. When the four fucose-containing glycopeptides which separated on DEAE-cellulose from Sephadex fraction 2 (Table II) of BHK_{21}/C_{13} and C_{13}/B_4 cells were recombined and rechromatographed on Sephadex G-50, one peak was eluted which corresponded to the original fractions. When the fractions which were eluted from DEAE-cellulose with 100, 23–25, and 17–20 mM phosphate buffer were chromatographed separately on Sephadex G-50, they were eluted as single peaks from the column with decreasing rapidity, in fraction numbers 29, 31, and 33 as would be expected according to Figure 3a,b. Thus, the separation of each fraction, obtained after gel filtration, into three or more glycopeptides by chromatography on DEAE-cellulose was not a procedural artifact.

Further separation on DEAE-cellulose of the fraction of Figure 4b which was eluted with 23–28 mM phosphate buffer is given in Figure 6. Fucose-containing glycopeptides similar to those which were eluted between 23 and 25 mM phosphate buffer (Figure 6) were used for enzymatic sequencing, to be described in the following paper (Santer & Glick, 1979).

Additional procedures failed to give further separation of these glycopeptides (Figure 6). Less than 10% of the radioactivity was retained on affinity columns of concanavalin A and soybean agglutinin. Conversely, greater than 90% of the radioactivity was retained on Fucosylex while approximately 85% was recovered after eluting with 0.1 M L-fucose. Columns of Sephadex G-25 or DEAE-cellulose using other molarities of elution buffer yielded no further separations.

Sialic Acid Containing Glycopeptides. The glycopeptides containing sialic acid should be labeled with radioactive glucose. However, in order to determine if differences existed which were not represented by the radioactivity, we determined the sialic acid content of each of the glycopeptides from the Pronase-digested trypsinates which were separated on DEAE-cellulose (see Figure 1). Table III expresses this data as nanomoles of sialic acid per 10^9 cells. The data can be

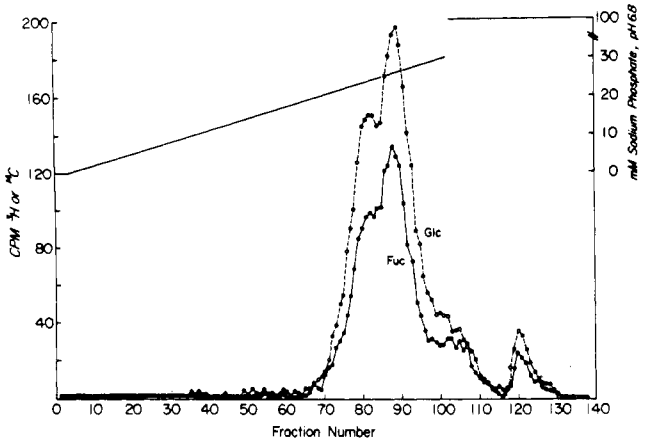

FIGURE 6: Rechromatography of glycopeptides from trypsinates of C_{13}/B_4 cells on DEAE-cellulose. Fractions which were eluted with 23–28 mM sodium phosphate buffer from the column of DEAE-cellulose shown on Figure 4b were combined, dialyzed, and rechromatographed on a column of DEAE-cellulose. The radioactive material was eluted with increasing molarity of sodium phosphate buffer, pH 6.8, as determined by the counting of aliquots.

summarized as follows. (1) The total surface material from BHK_{21}/C_{13} cells contained less than half the sialic acid of that from the transformed cells. (2) Glycopeptides which were eluted with less than 15 mM phosphate buffer each contained similar amounts of sialic acid in both the transformed and nontransformed cells. (3) The glycopeptides which were eluted with greater than 15 mM phosphate buffer showed increased amounts of sialic acid after transformation. (4) The C_{13}/B_4 derived surface material had 250% more sialic acid in the glycopeptides which were eluted with 15–30 mM phosphate buffer than that found in those which were eluted with less than 15 mM buffer, while BHK_{21}/C_{13} surface material had only 50% more sialic acid. (5) The increased negative charge of the glycopeptides requiring higher molarity of buffer to be eluted from the column of DEAE-cellulose (Figure 1) was verified by the increased sialic acid in the C_{13}/B_4 derived material and reflected a lesser amount of glycopeptide in these fractions from BHK_{21}/C_{13} surface material.

Discussion

The hypothesis that virus transformation is accompanied by an increase in certain types of existing glycopeptides on the cell surface and the internal membranes (Keshgegian & Glick, 1973; Glick, 1974a) was corroborated by the data reported here. That is, the alteration which has been observed appeared to be one of amount rather than the de novo synthesis of unique types of glycopeptides. Although many cell systems have been shown to have altered membrane glycopeptides accompanying virus transformation and tumorigenesis [see Warren et al. (1978) for a review], until this report it was not known if the change was qualitative or quantitative. In the studies reported here, glycopeptides of similar size and charge distribution were isolated from a clone of transformed hamster cells (C_{13}/B_4) and the normal counterpart (BHK_{21}/C_{13}). Clearly, however, some of these glycopeptides were more prominent on the cell surface after virus transformation. The three glycopeptides which comprised the main difference in the transformed cell surface were 50–60% of the total glycopeptides removed by trypsin. Three glycopeptides with size and charge distribution similar to those of the transformed cells were isolated from the surface of BHK_{21}/C_{13} fibroblasts but represented only 25–30% of the surface glycopeptides. On the basis of the radioactivity incorporated and the content of sialic acid per cell, these glycopeptides from the transformed cells were 3 times more prevalent than those from the normal counterpart (Tables II and III).

Table III: Sialic Acid Composition of Glycopeptides Isolated from the Surface of BHK_{21}/C_{13} and C_{13}/B_4 Cells

DEAE-cellulose fractions (mM phosphate buffer)	sialic acid[a] (nmol/10^9 cells)		$(C_{13}/B_4)/(BHK_{21}/C_{13})$ molar ratio
	BHK_{21}/C_{13}	C_{13}/B_4	
0.5	19	18	1.0
8–10	35	37	1.0
13–15	107	140	1.3
18–22	105	219	2.1
23–25	77	214	2.7
26–30	39	92	2.3
100	60	158	2.6

[a] Average of two determinations.

Others have suggested that the glycopeptides from the transformed cells were more highly sialylated than those of the normal counterpart (Van Beek et al., 1973; Warren et al., 1974). Table III appears to verify these latter results; however, compositional analyses showed that the other monosaccharides also differed in amounts (Glick, 1974a; Santer & Glick, 1979). Therefore, the alteration seen or transformation is more likely in the branching of the oligosaccharide units, with at least some of the more highly sialylated glycopeptides representing also more highly branched species (Glick & Santer, 1978).

The glycopeptides, isolated with the use of trypsin, were from the external surface of the cells and were representative of the surface membrane (Figure 2). The similarity to the membranes is interpreted to show that the differences observed were not totally due to the absence of Fibronectin (Hynes, 1976) on the transformed cell surface, therefore exposing different glycoproteins. Trypsinization affords higher yields of glycopeptides than can be obtained by membrane isolation and makes it possible to characterize the oligosaccharide units. Moreover, at least some of the individual glycoproteins have been reported to contain the appropriate species of glycopeptides which characterized them as transformed or nontransformed (Van Nest & Grimes, 1977; Tuszynski et al., 1978).

Most of the glycopeptides which were labeled metabolically with glucose were also labeled with fucose. Fucose is not degraded in animals (Bocci & Winzler, 1969) and cell-culture systems (Kaufman & Ginsburg, 1968; Lazo et al., 1977), and indeed all of the radioactive fucose incorporated into the trypsinates of the cells reported here was recovered as fucose.[2] Many glycoproteins have been reported which contain no fucose (Kornfeld & Kornfeld, 1976); however, with few exceptions, the simultaneous use of both radioactive glucose and fucose marked similar glycopeptides (Figures 4 and 5). Thus, it is possible that most of the externally oriented glycopeptides which are removed from the cell surface under our controlled conditions contained fucose. In fact, it has been proposed that fucose may be on the externally oriented glycoproteins for a specific function (Glick, 1978). A large sialic acid containing glycopeptide which apparently contained little or no fucose was among the glycopeptides isolated from rat hepatoma cells (Walborg et al., 1976). Similar material may have been eluted in the void volume of Sephadex G-50 but was not detected when radioactive fucose was used as a glycoprotein precursor (Figure 3b). This current study made use of only the radioactive material which was retained on Sephadex G-50 or which was not precipitated with trichloroacetic acid. A

[2] A. Fischer and M. C. Glick, unpublished experiments.

difference noted in a few of the glycopeptides from cells metabolically labeled with both radioactive L-fucose and radioactive D-glucose was that several of the glycopeptides contained predominantly only one isotope. Figures 4 and 5 show examples; however, these components represented less than 5% of the total radioactivity so were not examined further.

The glycopeptide units from both cell types were surprisingly homogeneous after these separations, and attempts at further separation have not succeeded. This was further verified by the detailed analyses of one of the glycopeptides (Santer & Glick, 1979). Similarly, Kornfeld (1978) reported that only three glycopeptides constituted 50% of the membrane glycopeptides from thymocytes, using similar isolation procedures.

Some heterogeneity of the glycopeptides existed on the basis of size and charge by the chromatographic separations which were used. The most heterogeneous fraction was that which was eluted most rapidly from Sephadex G-50 and comprised only 8% or less of the glycopeptides (see Figure 4a and Table II for two different experiments). It is possible that some of the heterogeneity resulted from different amounts of amino acids due to incomplete digestion of the polypeptides with Pronase or, alternately, that a spectrum of oligosaccharide units was present. Another exception to the size–charge separation was noted in Sephadex fractions 2 and 5 where material with an apparent different size distribution showed similar charge characteristics (Table II). It has become popular to use sizing of glycopeptides to corroborate structure (Pesonen & Renkonen, 1976; Ogata et al., 1976). Caution must be used in assigning molecular weights to molecules of different charges, since anomalous molecular weights may result, depending on the substituents on the oligosaccharide units[3] (Etchison et al., 1977; Ceccarini & Atkinson, 1977).

The relatively few glycopeptides described here may be due in large part to the methods of isolation, with the procedures selecting particular populations from the external portion of the surface membrane. If this is so it has worked to our advantage because our ultimate goal is to show the exact nature of the difference observed when the glycopeptides from transformed cells were compared with those of the normal counterpart. It has been reported for the first time that all glycopeptides are present in both cell types and that a quantitative difference is the alteration in the membrane glycopeptides of the virus-transformed cells. However, it remains to be shown if the glycopeptides quantitated here on the basis of size and charge truly reflect these characteristics. Sequential enzyme degradation of one of these glycopeptides thus far confirms these results and supports the concept that the predominant glycopeptide in the transformed cell membrane is highly branched (Santer & Glick, 1979).

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[3] U. V. Santer and M. C. Glick, unpublished experiments.