Glycosaminoglycans and Other Carbohydrate Groups Bound to Proteins of Control and Transformed Cells*

(Received for publication, January 18, 1980)

Susan R. Baker,† Diana L. Blithe, Clayton A. Buck, and Leonard Warren
From the Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

The membrane glycoproteins from control (BHK21/C13) and Rous sarcoma virus-transformed (C13/B4) baby hamster kidney cells labeled with D-[14C]- or D-[3H]glucosamine, respectively, were purified by means of polyacrylamide electrophoresis and gel electrofocusing. The homogeneity of the isolated glycoproteins was demonstrated by analysis of the N-terminal peptides. Some purified glycoproteins were found to be hybrid molecules in terms of the type of oligosaccharides they bear. The majority of the oligosaccharides (approximately 90%) on these glycoproteins are N-glycosidically linked (M, ~ 3000 to 5000). Another 5% appears to be small groups linked O-glycosidically to several adjacent or closely spaced amino acid residues. The remainder (5%) of the carbohydrate groups appears to be small, covalently bound glycosaminoglycans. This is the first report of hybrid molecules bearing glycosaminoglycans in the cell surface. The ratio of the types of oligosaccharides varies among different glycoproteins. There is slightly more glycosaminoglycan present on glycoproteins from malignant cells.

A remarkably complex but similar array of N-glycosidically linked oligosaccharides is bound to different individual membrane glycoproteins. Each individual polypeptide must contain only a small number of the total observed carbohydrate groups, i.e. the carbohydrate groups on individual polypeptides are grossly heterogeneous. This implies that purification is based largely on the characteristics of the polypeptide, and that overall charge and size of the carbohydrate groups are relatively constant in a single population of glycoproteins.

Our results suggest that the differences between the carbohydrate groups derived from glycoproteins from control and transformed cells are mainly quantitative.

The composition and structure of membrane glycoproteins from normal and malignant cells have been of considerable interest because of their presumed role in growth control (1, 2), intercellular adhesiveness (3, 4), and cell recognition (3-6). Malignant cells bear relatively more bound carbohydrate groups of a larger molecular weight than those of corresponding control cells. This phenomenon has been observed in a number of tissue cultures and tumor systems (7, 8). The glycoproteins of subcellular organelles from virally trans-
Rockville, Md.; phenol was from Mallinckrodt, St. Louis, Mo.; and LiSal, acrylamide, \(N,N'\)-methylenebisacrylamide, and 1-hexadecylpyridinium chloride were from Eastman, Rochester, N. Y. All isotopes and precipitation fluid were purchased from New England Nuclear Corp., Boston, Mass., or Bio-Rad, Richmond, Calif.; disaccharide standards and \textit{Staphylococcus aureus} V8 protease were from Miles Laboratories, Elkhart, Ind.; and leech hyaluronidase was from Biotics Inc., Arlington, Mass. The glycosidase mixture from \textit{Streptococcus pneumoniae} was the gift of Dr. M. Lowe, National Institutes of Health. A mycoplasma standard was the gift of Dr. D. Safer, Wistar Institute, and purified hyaluronic acid came from Dr. E. A. Balazs, Columbia University.

**Cell Culture and Harvesting**—Baby hamster kidney fibroblasts, BHK21/C13, and a cell line derived from the same clone transformed by the Bryan high titer strain of Rous sarcoma virus, C13/B4, were cultured and harvested as described previously (16). Routine tests for the presence of mycoplasma in the cell cultures were performed at the Institute for Medical Research, Camden, N. J., by Dr. Elliot Levine of the Wistar Institute and by Flow Laboratories, Rockville, Md. (17). Cells were cultured to a density at which both cell types were in the process of growth and cell density was 80–90% of cells/roller bottle. Phase-contrast patterns of two cell types were used as a guide to cut out the desired bands from which the glycoproteins could be dissociated under conditions which minimize noncovalent interactions, a dissociative cesium chloride density gradient from 1.3 to 1.1 was prepared (27). Equal volumes of a "heavy" solution (4
\text{M} \text{guanidine hydrochloride in 0.05} \text{M sodium acetate, pH 5.3}) was retained on the ion exchange columns after elution with the 0 to 0.1 M sodium borate gradient. To remove these glycopeptides from the column, a second gradient from 0 to 1.0 M sodium acetate in 0.1 M sodium borate and 0.01 M pyridine acetate, pH 4.5, was used. Tonic strength was measured by refractive index on a Bausch and Lomb refractometer and radioactivity was assayed in a scintillation counter (Intertechnique model SL300).

**Pronase glycopeptides** that were excluded from the Sephadex G-50 column were analyzed to determine the glycosaminoglycan content and the type of oligosaccharide linkage to the protein. Material from the void volume was pooled, lyophilized, and subjected to the treatments described below which result in conversion of susceptible carbohydrate from a large form that is excluded by Sephadex G-50 to a smaller form that is included. After each treatment, the reaction mixture was assayed by Sephadex G-50 chromatography.

Alkaline sulfite treatment was carried out in order to determine whether O-glycosidically-linked material was present. The glycoproteins were incubated for 48 h in 0.1 \text{NaOH} and 0.5 \text{Na}_2\text{SO}_4 at 37°C.

Glycopeptides were digested with 100 \text{µg} of testicular hyaluronidase in a solution of \text{NaH}_2\text{PO}_4 (0.1 \text{M}) and \text{NaCl} (0.15 \text{M}), \text{pH} 5.3, at 37°C for 18 h. For leech hyaluronidase digestion, an aliquot containing 20 \text{µg} of enzyme in 100 \text{µl} of 0.1 \text{M phosphate/citrate buffer, pH 5.6}, was added to the glycopeptides four times at zero time and at 1-h intervals thereafter. The enzyme preparation did not contain activity against chondroitin 4 and 6-sulfate (J. W. Lasb, University of Pennsylvania) nor against heparan sulfate. Chondroitinase ABC (0.125 unit) in 10 \text{µl} of Tris buffer (3 g of Tris, 2.4 g of sodium acetate, 1.46 \text{g} of sodium chloride, and 90 \text{µg} of crystalline bovine serum in 100 \text{ml} of \text{H}_2\text{O}) was incubated for 3 h at 37°C with a glycopeptide mixture in 20 \text{µl} of distilled water (25).

Samples were treated with nitrous acid as described by Hart (29) to determine whether N-sulfated glycosaminoglycans were present. A 0.2-ml sample was mixed with 0.1 \text{ml} of \text{HCl} (1 \text{N}) and 0.1 \text{ml} of freshly prepared \text{n-butyl nitrite (20\% v/v, in absolute ethanol)} and incubated for 2 h at room temperature with occasional shaking. Glycosidase treatment of the glycopeptides was carried out with 100 \text{µl} of a glycosidase mixture in 0.1 M citrate phosphate buffer for 48 h at 37°C. The glycosidase preparation is from the culture filtrate of \textit{S. pneumoniae}. It contains no protease activity and at least seven known \text{N-glycosidase} activities. Three fractions gave rise to a smaller form that was included. After each treatment, the glycopeptides were excluded from the Sephadex G-50 column were analyzed to determine the glycosaminoglycan content and the type of oligosaccharide linkage to the protein. Material from the same conditions as the experimental samples and were analyzed by Sephadex G-50 chromatography at the same time as the experimental samples. As positive controls, the enzymes and chemical reactions were tested for activity by incubation under the prescribed conditions with authentic polysaccharides for which each fraction is specific.

To determine whether the glycosaminoglycan material bound to the glycopeptides could be dissociated under conditions which minimize noncovalent interactions, a dissociative cesium chloride density gradient from 1.3 to 1.1 was prepared (27). Equal volumes of a "heavy" solution (4 M guanidine hydrochloride in 0.05 M sodium acetate, pH 5.8, with density adjusted to 1.5 \text{g/cm}^3 with cesium chloride) and a "light" solution (density adjusted to 1.3 \text{g/cm}^3 with \text{H}_2\text{O}) with the samples were centrifuged for 18 h at 350,000 \text{g} \text{x} g in a Sorvall TI-75 centrifuge at 10°C. The sample containing isolated glycoprotein and purified, authentic \text{N-acetylglucosaminidase (2.8 units/ml), \text{β-galactosidase (5.66 units/ml), and \text{β-N-acetylglucosaminidase (0.1 units/ml)}} also contain \text{α-n-fucosidase, \text{β-N-galactosidase, \text{β-β-acetylgalactosaminidase D, and \text{endo-α-N-acetylgalactosaminidase D}}. Control vesicles of glycopeptide samples containing no enzymes could easily be distinguished under the same conditions as the experimental samples and were analyzed by Sephadex G-50 chromatography at the same time as the experimental samples. As positive controls, the enzymes and chemical reactions were tested for activity by incubation under the prescribed conditions with authentic polysaccharides for which each fraction is specific.

**Comparison of the Oligosaccharides Linked to the Isolated Glycopeptides**—For comparison of the oligosaccharides linked to the glycopeptides, the co-isolated glycopeptides were subjected to extensive pronase digestion and chromatography on columns of Sephadex G-50 (85 × 1 cm) eluted with 0.01 \text{M ammonium acetate in 20\% ethanol, pH 6.5, or 0.1 \text{M pyridine acetate, pH 4.0}}. Glycopeptide groups that were included in the Sephadex G-50 column were pooled, dialyzed, and lyophilized, and then analyzed for affinity to Con A as described by Blithe et al. Con A-Sepharose in a column (10 × 1 cm) was preconditioned by adsorbing the nonspecific binding sites with bovine serum albumin (5 \text{mg/ml} in 0.001 \text{M MnCl}_2 and 10 \text{ml of buffer (0.05 \text{M Tris/acetate, 0.001 \text{M CaCl}_2, 0.001 \text{M NaCl, 0.003 \text{M NaF, and 0.075 \text{M NaCl}}}) were added to the sample, and the affinity was then measured by the procedure as described previously (14). Glycopeptide bands that was bound by Con A (Con A(+)) was eluted with the same buffer containing a-methyl-n-mannoside (40 \text{mg/ml}). Fractions of bound and unbound material were each pooled, dialyzed extensively against water, and lyophilized. The glycoproteins fracitoned by size and affinity for Con A were further analyzed by DEAE-Sephadex A-50 chromatography employing a linear gradient from 0 to 0.1 M sodium borate in 0.01 M pyridine acetate, pH 4.5. A portion of the glycoside mixture that did not bind to Con A (Con A(-)) was retained on the ion exchange columns after elution with the 0 to 0.1 M sodium borate gradient. To remove these glycopeptides from the column, a second gradient from 0 to 1.0 M sodium acetate in 0.1 M sodium borate and 0.01 M pyridine acetate, pH 4.5, was used. Tonic strength was measured by refractive index on a Bausch and Lomb refractometer and radioactivity was assayed in a scintillation counter (Intertechnique model SL300).

For a total carbohydrate analysis, glycoproteins from gels were precipitated with trichloroacetic acid and hydrolyzed. The released
sugars were converted to alditol acetate derivatives for gas-liquid chromatography by the procedure of Leinhardt and Windler (29). The samples were analyzed on a Hewlett-Packard 5890A gas chromatograph. Sialic acid was determined by the thiobarbituric acid assay (30).

Glycopeptides that were excluded from Sephadex G-50 were analyzed using the methods described by Saio et al. (26) to determine the amount of carbohydrate that was present in the form of hyaluronic acid and chondroitin. After chondroitinase digestion of the glycopeptides, 25 μg of each of four known digestion productions, ΔDi-6S, ΔDi-4S, ΔDi-6S, and ΔDi-0HA, were added to the digest. ΔDi-0HA was prepared by chondroitinase ABC digestion of authentic purified hyaluronic acid. The mixtures were spotted on Whatman No. 1 paper and were desalted by descending paper chromatography for 48 h in a solvent consisting of 1-butanol/ethanol/water (52:32:16). Separations were carried out by descending chromatography overnight in 1-butanol/acetic acid/1 N NH₄OH (2:3:1), at room temperature. The disaccharide standards were visualized under short wavelength ultraviolet with a Mineralight. Each path was cut into 0.5-cm segments. Radioactive material was eluted from the strips in 0.5 ml of 0.1 N HCl on a rotary shaker at 37°C for 3 h. Radioactivity was determined by scintillation counting.

NH₂-terminal Peptide Analysis—NH₂-terminal peptide analysis was performed to determine the degree of homogeneity of the isolated glycopeptides. Glycopeptides were dialyzed extensively and detergent was removed by incubation for 15 min with Bio-Rad SM-2 Biobeads (31). Dansylation of the salt- and detergent-free glycopeptides was carried out in a solution of 20 μl of 0.2 M sodium carbonate buffer, pH 10, and 20 μl of a 0.1% solution of dansyl chloride in acetone at 37°C in the dark for 30 min (32). The mixture was then dried by lyophilization and treated enzymatically with either 1 μg of trypsin plus tosylphenylalanil chloromethyl ketone in 50 μl of 0.1 M ammonium carbonate buffer, pH 8.0, at 37°C for 1 h (32) or with 1 μg of B. aureus V8 protease in 50 μl of 0.1 M ammonium acetate buffer, pH 7.9, at 37°C for 18 h (33). To the dried, dansylated peptides was added 20 μl of absolute ethanol. The mixture was centrifuged for 5 min at 150 x g to pellet the insoluble salts. The ethanol supernatant which contained dansyl peptides was investigated. Background fluorescent material arising from the glycopeptide purification procedure was visualized in the absence of protein-derived material by dansylating samples of isolated glycopeptides and then chromatographing the material without treating with protease. The glycopeptides were not enzyme treated and, therefore, not ethanol-soluble. Only in this way could a valid test for background fluorescent material be made since the concentration of various impurities varied among samples. The dansylated, enzyme-treated material was subjected to two-dimensional chromatography on polyamide layer sheets (5 x 5 cm) (Accurate Chemical and Scientific Corp., Hicksville, N. Y.). Routinely, 1 to 3 μl of the material was applied to give a spot with a diameter of 1 mm. The polyamide plates were coated on both sides, so the peptides from homologous glycoprotein pairs were co-chromatographed on opposite sides of the plate. Histological staining cuvettes containing 10 to 15 μl of solvent were used to develop the chromatograms. The plates were developed for 5 min in the first dimension with a 10% solution of formic acid in water and for 12 min in the second dimension with a benzene/acetic acid mixture (9:1 or 3:1, v/v) (32). The chromatograms were dried and fluorescent spots were visualized under an ultraviolet lamp.

RESULTS

Isolation of Membrane Glycoproteins from Control and Transformed Cells—Total cell membrane preparations obtained from [14C]glucosamine-labeled BHK21/C13 and [3H]glucosamine-labeled C13/B4 cells were extracted separately with solutions of LiSal as previously reported (14). Glycopeptides from the LiSal aqueous phase of BHK21/C13 and C13/B4 cells were pooled and subjected to preparative SDS-PAGE in slab form (14). The two high molecular weight regions, designated A1 (160,000) and A2 (110,000) in Fig. 1, were treated from the preparative gels and were reanalyzed by SDS-PAGE to determine apparent molecular weight and the extent of homogeneity based on molecular weight. Glycoprotein bands from BHK21/C13 and C13/B4 cells that migrate as single peaks (see Fig. 4 in Ref. 18 for example) were subjected to a second preparative SDS-PAGE. The eluted glycoprotein bands were then subjected to isoelectric focusing (Fig. 2). The peaks indicated by numbered arrows were cut out and eluted for further analysis. A portion of several of these bands was reanalyzed by SDS-PAGE to determine

![Fig. 1. SDS-PAGE profiles of aqueous phase glycoproteins labeled with radioactive glucosamine. Glycopeptides were extracted from crude membrane pellets of BHK21/C13 and C13/B4 cells by the LiSal-phenol procedure (14). Aqueous phase glycopeptides from BHK21/C13 cells labeled with 3H]glucosamine (---) and from C13/B4 cells labeled with 14C]glucosamine (---) were mixed and analyzed on an 8.7% acrylamide-SDS slab gel. The hatched areas represent the bands that were cut out and further purified by electrofocusing. In this experiment, two bands, A1 and A2, were further purified.](image1)

![Fig. 2. Isoelectric focusing gel profiles of aqueous phase glycopeptides, the SDS gel profiles of which are shown in Fig. 1. Samples were originally fractionated by preparative SDS-PAGE to minimize any overlap between adjacent peaks. A1 and A2, corresponding aqueous phase glycopeptides from BHK21/C13 cells labeled with 14C]glucosamine (---) and C13/B4 cells labeled with 3H]glucosamine (---) were mixed and analyzed on an 8.7% acrylamide-SDS slab gel. The hatched areas represent the bands that were cut out and further purified by electrofocusing. In this experiment, two bands, A1 and A2, were further purified.](image2)
whether any degradation had occurred and to assess the degree of homogeneity based on molecular weight following isoelectric focusing. Since the profiles were similar to those obtained before isoelectric focusing, we concluded that no appreciable degradation had taken place.

**Purification of Surface-labeled Glycoproteins**—To assure that the A1 and A2 glycoproteins were from the cell surface, intact C13/B4 and BHK21/C13 cells were labeled by the galactose oxidase NaB[3H]4 (19) or by the 125I-lactoperoxidase (18) methods. Cells labeled by the galactose oxidase NaB[3H]4 method were mixed with LiSal aqueous phase material from D-[14C]glucosamine-labeled cells and were then subjected to SDS-PAGE (Fig. 3, a and b). With both cell lines, the major LiSal aqueous peaks co-migrated with the major peaks of surface-labeled material. This same result was obtained when cells were labeled by the 125I-lactoperoxidase method (Fig. 3c). In order to ascertain whether these co-migrating, surface-labeled peaks would fractionate into the LiSal aqueous phase, 125I-labeled C13/B4 and BHK21/C13 cells were extracted in the normal way and subjected to SDS-PAGE (Fig. 3d). The two major aqueous phase peaks, A1 and A2, were observed. These 125I-labeled bands were eluted and subjected to isoelectric focusing analysis. The profiles obtained were qualitatively similar to those obtained from glucosamine-labeled LiSal aqueous bands (Fig. 2). Thus, we found that the major glycoproteins of the LiSal aqueous phase which we are examining are accessible to labeling by 125I in the intact cell and are presumably located on the cell surface.

**Peptide Analysis of Isolated Glycoproteins**—The degree of homogeneity of glycoproteins was determined by the fingerprinting method of Tichy (32). Glycoproteins of identical molecular weight and pI were dansylated and subjected to digestion with either trypsin or S. aureus V8 protease. Peptides derived from glycoproteins A1-2, A1-3, and A2-2 (see Fig. 2) from control and transformed cells were chromatographed on opposite sides of polyamide plates and, thus, directly compared. When standard proteins, egg white lysozyme and papain, were labeled and processed in this manner, only one dansylated peptide was observed. Tests for background fluorescent material arising from protease revealed no fluorescent spot when a ratio of enzyme to glycoprotein of 1:50 was used. Ampholites from the isoelectric focusing procedure which co-purify with the glycoproteins were labeled with dansyl chloride and caused some interference (Fig. 4g). However, fluorescent NH2-terminal peptides could easily be distinguished from fluorescent nonprotein substances. These were found in the central region of the chromatogram, while most of the peptides migrated to other regions.

Using two different proteolytic enzymes, two independent NH2-terminal peptide determinations were made on three glycoproteins (Fig. 4, a to f). The homogeneity of these glycoproteins was demonstrated by the appearance of a single Dns-peptide from each glycoprotein after digestion with either

---

**Fig. 3.** SDS-polyacrylamide profiles of aqueous phase glycoproteins electrophoresed on the same gel with surface-labeled glycoproteins. a, aqueous phase glycoproteins from BHK21/C13 cells labeled with D-[^14C]glucosamine (---) and BHK21/C13 cells surface labeled by the NaB[3H]4/galactose oxidase method (----) were mixed and analyzed on an 8.7% acrylamide-SDS gel. b, aqueous phase glycoproteins from BHK21/C13 cells labeled with D-[^14C]glucosamine (---) and C13/B4 cells surface labeled by the NaB[3H]4/galactose oxidase method (----) were mixed and analyzed on an 8.7% acrylamide-SDS slab gel. c, aqueous phase glycoproteins from BHK21/C13 cells labeled with D-[^14C]glucosamine (---) and C13/B4 cells surface labeled by the NaB[3H]4/galactose oxidase method (----) were analyzed side by side on an 8.7% acrylamide-SDS slab gel. d, BHK21/C13 (----) and C13/B4 (-----) cells surface labeled by the 125I-lactoperoxidase method were extracted with LiSal-phenol and the aqueous phase was subjected to SDS-PAGE. The glycoproteins of interest are labeled A1 (160,000) and A2 (110,000).
Fig. 4. Peptide maps of purified glycoproteins. Glycoproteins were purified by SDS-PAGE and isoelectric focusing gels. Dansylation followed by treatment with trypsin or with S. aureus V8 protease was carried out as described under "Experimental Procedures." The polyamide plates were coated on both sides so that the peptides from homologous glycoprotein pairs could be co-chromatographed. The fluorescent peptides on both sides of the plates could be seen at the same time by fixing the eye and ultraviolet light on the edge of the plate and flipping the plate back and forth a few degrees. Thus, any differences could be easily visualized. a, Dns-NH₂-terminal peptides from trypsin or b, S. aureus V8 protease treatment, of a purified glycoprotein, molecular weight 160,000, pI = 4.3, designated A1-2. c, Dns-NH₂-terminal peptides from trypsin or d, S. aureus V8 protease treatment, of a purified glycoprotein, molecular weight 160,000, pI = 4.0, designated A1-3. e, Dns-NH₂-terminal peptides from trypsin or f, S. aureus V8 protease treatment, of A2-2, molecular weight 110,000, pI = 4.0. g, impurities and side products blank. The glycoprotein was dansylated and chromatographed. No peptides from the glycoprotein were seen because it was not enzyme-treated. The background fluorescent spots were subtracted from all glycoprotein peptide maps. +, point of application of ethanol solution containing DNA derivatives. Dotted circles, peptides from C13/B4 cells; solid circles, peptides from BHK21/C13 cells.

trypsin or S. aureus V8 protease. Upon trypsinization, the Dns-peptide derived from BHK21/C13 co-migrates with the Dns-peptide from C13/B4, indicating that the same trypsin peptides (presumably NH₂-terminal) of the glycoproteins from C13/B4 cells are present in the homologous glycoproteins from control cells. The peptides resulting from S. aureus V8 protease treatment migrate to different locations on the polyamide plate than the trypsic peptides. Thus, the production of single, chromatographically identical dansylated peptides following digestion with two different proteases further demonstrates the similarity of the glycoproteins compared.

Glycopeptides of Individual Glycoproteins—In previous studies, the glycopeptides from crude fractions of control and transformed cells were compared (7, 12, 34–37). We wished to analyze the glycopeptides from individual membrane glycoproteins to see whether the patterns were simpler and whether they varied from one glycoprotein to another.

Bands containing glycopeptides from both BHK21/C13 and C13/B4 cells were eluted from isoelectric focusing gels and subjected to exhaustive proteolysis by pronase. The resulting digests were chromatographed on columns of Sephadex G-50 as described previously (14). The glycopeptides fractionated by Sephadex G-50 were pooled into two groups, Group A (M, 4000 to 5500) and Group B (3000 to 4000), in accordance with earlier studies on the distribution of membrane glycoproteins on Sephadex G-50 columns (16) (see Fig. 6 in Ref. 14). The material eluting, in the void volume of the Sephadex G-50 column designated Group EV (exclusion volume), was also examined further. The Group A and B glycopeptides were further fractionated by affinity for Con A-Sepharose. When Group A glycopeptides were tested for affinity for Con A, virtually no Group A-Con A(+) material was found. The distribution of the total Group A and B material from each of six individual glycoproteins recovered after lectin affinity chromatography is shown in Table I. In every pair of glycoproteins examined, there is a smaller amount of glycopeptide material from the transformed cell compared to the control that can bind to Con A-Sepharose. A relative increase in the amount of the large molecular weight glycopeptide material (Group A) from glycoproteins of transformed cell relative to controls (Figs. 5 and 6 of Ref. 14) is also apparent.

Remarkably complex patterns were obtained (Figs. 5 to 7) when the glycopeptides co-purified by size and lectin affinity were analyzed by ion exchange chromatography. The profiles obtained for all glycoproteins examined were qualitatively similar. There appear to be no distinct qualitative differences between the glycopeptides derived from homologous glycoproteins present in the membranes of control and malignant cells. Virtually identical results have been obtained with three other membrane glycoproteins of BHK21/C13 and C13/B4 cells (data not shown). The quantitative differences observed in the Sephadex G-50 profiles and Con A-Sepharose affinity are also reflected in the ion exchange chromatographic profiles. The patterns obtained with glycopeptides from individual glycoproteins are remarkably similar to those observed with glycopeptides derived by trypsin digestion of the cell surface. There appear to be at least 10 to 15 different glyco-

| Glycoprotein | Cellular sources | Group A(+) | Group B(+) |
|-------------|-----------------|------------|------------|
| A1-1        | BHK21/C13       | 34         | 43         | 23         |
| M₁ = 160,000 | C13/B4         | 43         | 44         | 13         |
| A1-2        | BHK21/C13       | 33         | 47         | 20         |
| M₁ = 160,000 | C13/B4         | 41         | 51         | 8          |
| A1-3        | BHK21/C13       | 41         | 52         | 7          |
| M₁ = 160,000 | C13/B4         | 47         | 51         | 2          |
| A2-1        | BHK21/C13       | 12         | 12         | 76         |
| M₁ = 110,000 | C13/B4         | 26         | 18         | 56         |
| A2-2        | BHK21/C13       | 40         | 38         | 22         |
| M₁ = 110,000 | C13/B4         | 57         | 31         | 12         |
| A2-3        | BHK21/C13       | 36         | 51         | 13         |
| M₁ = 110,000 | C13/B4         | 52         | 40         | 8          |

* Con A(−) refers to material that did not bind to Con A-Sepharose.

* Con A(+) refers to material that did bind to Con A-Sepharose and was eluted with α-methyl mannoside.

The percentages were calculated on the basis of 100% recovery of Group A and Group B glycopeptide material from each purified glycoprotein.
peptides present on each purified glycoprotein. The higher molecular weight glycopeptides, Group A, are less well resolved than the Group B glycopeptides. This may be due to the greater influence of intramolecular forces and shape on the elution from DEAE-Sephadex of the larger molecules, or the groups could be more heterogeneous with increasing molecular size. Our results suggest that there are quantitative shifts in the populations of carbohydrate groups on polypeptide chains resulting in more Peak A glycopeptides on the polypeptides of transformed cells. There are also far more individual carbohydrate groups on these glycoproteins than can be accommodated by their polypeptide chains. These findings will be discussed further.

**Analysis of Glycopeptides Excluded from Sephadex G-50 (Group EV)**—In this and previous studies (14), it has been found that aqueous phase glycoproteins with an isoelectric point of 4.0 or less contain glycopeptide material that is excluded from Sephadex G-50. About 60% of the Band A1 glycoproteins of $M_r = 160,000$ have a pI of 4.0 or less as estimated by the area under each isoelectric focused peak (Fig. 2a). The presence of this large glycopeptide material on some glycoproteins and not on others suggests that its occurrence is specific for some macromolecules. This glycopeptide material does not enter the organic phase upon extraction with chloroform/methanol/water (2:1:1) and accounts for 10 to 20% of the bound carbohydrate of the glycoprotein as determined by the radioactivity of the glucosamine-labeled glycopeptides. Five glycoprotein pairs which bear these large carbohydrate groups were examined further (Table II). The glycoproteins from transformed cells appear to yield 5 to 40% more of these glycopeptides than do glycoproteins from control cells.

The amount of Group EV glycopeptide material bearing carbohydrate that is linked $O$-glycosidically to protein was determined by mild alkaline reduction treatment and subsequent chromatography on columns of Sephadex G-50. Of the Group EV material from the individual glycoproteins examined, between 45% and 88% of the carbohydrate was included in a Sephadex G-50 column after this treatment (Table II). The cleavage of the oligosaccharide from the peptide results in the formation of a significantly smaller molecule that is Sephadex G-50 chromatography appears to have a molecular weight of approximately 800. Possibly these large glycopeptide structures contain several adjacent or closely spaced serine- or threonine-bearing carbohydrate groups that hinder the action of pronase (16, 38, 40). Group EV glycopeptides make up 10 to 20% of the bound carbohydrate of the individual glycoproteins that have an isoelectric point of 4.0 or lower. It appears then that approximately 5 to 17% of the carbohydrate of these glycoproteins that give rise to Group EV material is probably in the form of relatively small molecular weight oligosaccharides linked $O$-glycosidically to many adjacent serine and/or threonine residues.

The Group EV glycopeptide material remaining in the exclusion volume of Sephadex G-50 after mild alkaline reduction was subjected to treatment with enzymes that degrade hyaluronic acid and chondroitin. The percentage of leech hyaluronidase-sensitive material in the Group EV glycopeptides ranges between 9% and 36% in the five glycoproteins examined (Table II). Material that was not small enough to be included by Sephadex G-50 after digestion by leech hyaluronidase was treated exhaustively with chondroitinase ABC.
Sequential degradation of Group EV glycopeptides from purified glycoproteins

| Glycoprotein | Cellular sources | Mild alkaline reduction | Leech hyaluronidase* | Chondroitinase ABC* | Glycosidase from S. pneumoniae* | Nitrous acid* |
|--------------|-----------------|------------------------|---------------------|---------------------|-------------------------------|--------------|
| A1-3 BHK21/C13 | C13/B4          | %                      | %                   | %                   | %                             | %            |
| A1-5 BHK21/C13 | C13/B4          | %                      | %                   | %                   | %                             | %            |
| A2-2 BHK21/C13 | C13/B4          | %                      | %                   | %                   | %                             | %            |
| A2-3 BHK21/C13 | C13/B4          | %                      | %                   | %                   | %                             | %            |
| A3-4 BHK21/C13 | C13/B4          | %                      | %                   | %                   | %                             | %            |

* The co-purified glycoproteins from C13/B4 and BHK21/C13 cells were pronase-digested and subjected to Sephadex G-50 chromatography. Several of the glycoproteins analyzed in this way were observed to bear large glycopeptides eluting in the void volume. Group EV material from five glycoproteins was analyzed further.

1. Group EV glycopeptides were subjected to mild alkaline reduction treatment and subsequent chromatography on columns of Sephadex G-50 to determine the percentage of the material degraded by the treatment and thereby eluting from the column in the included volume.

2. The material excluded from Sephadex G-50 after mild alkaline reduction was pooled and digested with leech hyaluronidase. The percentage of the total original Group EV material that is included by Sephadex G-50 was calculated after Sephadex G-50 chromatography.

3. The material excluded from Sephadex G-50 after mild alkaline reduction and then treated with leech hyaluronidase was pooled, digested with chondroitinase ABC, and followed by Sephadex G-50 chromatography. The percentage of the total Group EV material that is degraded by chondroitinase ABC and, therefore, included by Sephadex G-50 was calculated. In one case, A2-2, there was insufficient material to digest with chondroitinase ABC.

4. In two cases, A1-3 and A1-5, some material was still excluded from Sephadex G-50 after the three treatments. This material was digested with a mixture of glycosidases from S. pneumoniae and subjected to Sephadex G-50 chromatography. The percentage of the total Group EV material that is degraded by the glucosidase mixture and, therefore, included by Sephadex G-50 was calculated.

5. In one case, the material excluded from Sephadex G-50 after mild alkaline reduction treatment and then leech hyaluronidase was subjected to degradation by nitrous acid (see "Experimental Procedures"). The percentage of the total Group EV material degraded by nitrous acid was calculated.

6. ND indicates treatments not done either because all of the Group EV material was degraded by prior treatments or because the amount of material was too small to permit further analysis.

to appear larger than they actually are. Therefore, in order to obtain a more accurate determination of the molecular weight of the glycosaminoglycan-like material, Group EV material from Band A1 glycoproteins that, even after mild alkaline reduction, remained excluded from Sephadex G-50 using a column buffer of pH 6.5 (0.01 M ammonium acetate/ethanol) was rechromatographed on a Sephadex G-50 column using 0.1 M pyridine acetate buffer, pH 4.0. This material still eluted in the void volume at pH 6.5 but was included in the bed volume at pH 4.0 (Fig. 8). Apparently, the lower pH, being nearer the isoelectric point of uronic acid, minimizes the effect of the negative charge and decreases aggregation (41). At pH 4.0, the material is not included by a Sephadex G-50 column unless the material has first been subject to alkaline reduction (Fig. 8).

The molecular weight of this chondroitinase- and hyaluronidase-sensitive material is estimated to be 1200 to 6000. Assuming a molecular weight of the disaccharides comprising the polysaccharides to be approximately 440, the material should consist of 3 to 13 disaccharide units per carbohydrate chain. Since these molecules are linear, it is possible that there is a considerable overestimation of molecular weight determined by Sephadex G-50 chromatography.

Since glycosaminoglycans are present in the cell culture fluid and in the matrix that the cells attach to in culture (42), it is possible that the observed glycosaminoglycans are simply adsorbed to the glycoproteins and are not covalently bound. Although it is unlikely that any carbohydrate not covalently attached could remain bound through SDS-PAGE and isoelectric focusing in 8.5 M urea, this possibility was further examined by dissociative cesium chloride gradient centrifugation in 4 M guanidinium chloride. It has been shown that 4 M guanidinium chloride minimizes noncovalent interactions and effectively dissociates proteoglycan aggregate structures present in the extracellular matrix (43, 44). Combined A1 glycoproteins not separated further by isoelectric focusing were subjected to analysis by centrifugation (Fig. 9). The glycoproteins migrate to a different location in the density gradient (1.29 p) than does added, standard chondroitin sulfate or hyaluronic acid from human umbilical cord (approximately 1.06 p).

FIG. 8. Sephadex G-50 chromatography. Elution profiles of glycosaminoglycan-like carbohydrate groups in Group EV material from band A1. The sample was originally fractionated by preparative SDS-PAGE to obtain Band A1 glycoproteins (see Fig. 1). These were extensively treated with pronase and then subjected to Sephadex G-50 chromatography using a 0.01 M ammonium acetate/ethanol, pH 6.5, elution buffer. Glycopeptides eluting in the exclusion volume of the Sephadex G-50 column were pooled and lyophilized. A portion was rechromatographed on a column of Sephadex G-50 using an elution buffer of 0.1 M pyridine acetate, pH 4.0 (----). The remainder was subjected to a mild alkaline reduction treatment. Part of the treated sample was again chromatographed on a Sephadex G-50 column, the elution buffer being 0.01 M ammonium acetate/ethanol, pH 6.5 (-----). The remaining portion was subjected to Sephadex G-50 chromatography employing a 0.1 M pyridine/acetate, pH 4.0, elution buffer (----).
The salt density is indicated by a dot-dash line. The salt density is indicated by a dashed line.

Centrifugation lowered the buoyant density of the glycoproteins from 1.29 to 1.21 (Fig. 9a). The ratio of the chondroitinase ABC-digested material included by Sephadex G-50 to the excluded material shows that approximately 5% of the total carbohydrate is susceptible. The fact that chondroitinase-sensitive material remains associated with glycoprotein after centrifugation through a CsCl gradient containing 4 mM guanidinium chloride suggests that the material is covalently bound to the polypeptide portion of the molecule. Treatment of Band A1 glycoproteins from C13/B4 cells with chondroitinase ABC before centrifugation lowered the buoyant density of the glycoproteins from 1.29 to 1.21 (Fig. 9b), again showing that glycosaminoglycan is covalently linked to membrane glycoproteins.

To characterize more precisely the glycosaminoglycan components of the Group EV material, Group EV glycopeptides from A1 glycoproteins were treated exhaustively with chondroitinase ABC and the digestion products were chromatographed on paper. Chondroitinase ABC digestion of hyaluronic acid and chondroitin produces characteristic disaccharides: CS A and CS B -> ΔDi-4S; CS C -> ΔDi-6S; HA -> ΔDi-0HA; and unsulfated chondroitin -> ΔDi-0S. The chromatographic profile of the disaccharides resulting from the chondroitinase ABC digestion of Band A1 material is represented in Fig. 11. The main disaccharides produced are: ΔDi-4S, and ΔDi-0HA with small amounts of ΔDi-6S and ΔDi-0S. The presence of these disaccharide digestion products indicates that the main glycosaminoglycan components of Band A1 glycoproteins are chondroitin sulfate A and/or B and hyaluronic acid, with small amounts of chondroitin sulfate C and unsulfated chondroitin. The relative amounts of the two main disaccharide digestion products, ΔDi-0HA and ΔDi-4S, indicate that in the A1 fraction the overall ratio of hyaluronic acid to chondroitin sulfate A and/or B appears to be approximately...
1:2. This ratio is very nearly the same for C13/B4 cells as for BHK21/C13 cells (Fig. 11). It should be noted that the ratio varies from one purified glycoprotein to another (Table II).

The first peak (Fractions 4 to 8, Fig. 11) represents Group EV material that is not susceptible to degradation by chondroitinase ABC and does not, therefore, migrate. At least 48% and 29% of the Group EV fraction of A1 glycoproteins from C13/B4 and BHK21/C13 cells, respectively, are susceptible to enzymatic degradation and are, therefore, glycosaminoglycans.

DISCUSSION

In our attempt to explain observed differences in the carbohydrate groups of glycoproteins of control and transformed cells, we have isolated and purified homologous membrane glycoproteins. Surface-labeling experiments suggest that the glycoproteins are derived from the cell surface (Fig. 3). Three general classes of carbohydrate groups have been identified: N-glycosidically linked, O-glycosidically linked, and covalently linked glycosaminoglycans of relatively small size. The latter are present on several glycoprotein "hybrids" which appear to be located on the cell surface. A soluble glycoprotein, human thyroglobulin, has also been shown to bear glycosaminoglycan (29). Between 45 and 88% of the Group EV glycopeptide material derived from individual A1, A2, and A3 glycoproteins were found to be small oligosaccharides (M, 800). O-glycosidically linked to serine or threonine (Table II). Mild alkaline reduction releases these small, bound carbohydrate chains from the polypeptide. We have presented evidence that most of the remaining Group EV material appears to be glycosaminoglycan covalently bound to protein as the material is non-dissociable in 4 M guanidine hydrochloride and removed only by appropriate degrading enzymes. The glycosaminoglycan could be converted from a form that is excluded from Sephadex G-50 to an included form at pH 4.0 by mild alkaline reduction (Fig. 8).

Of the total carbohydrate bound to the unfractonated Band A1 glycoproteins, approximately 10% is excluded by Sephadex G-50 (Group EV) after pronase digestion. Since 29% of the Group EV material from Band A1 glycoproteins of BHK21/C13 cells is glycosaminoglycan, one can calculate that 3% of the total carbohydrates from Band A1 glycoproteins of these cells is glycosaminoglycan. Similarly, if 48% of the A1 material of C13/B4 cells is glycosaminoglycan, then 5% of the total A1 carbohydrates is glycosaminoglycan. These findings are consistent with observations of an increase in cell-associated glycosaminoglycans in response to transformation (45-47). Hyaluronic acid may be important in permitting the normal cellular process of release from the substratum that is associated with mitosis and movement (48). Increased amounts of hyaluronic acid that seem to occur with transformation might make it difficult for normal adhesions to form (48). It will be of interest to see whether the hybrid glycosaminoglycans-glycoprotein molecules described interact with fibronectin in some functionally specific manner (49). The presence of bound hyaluronic acid is somewhat surprising since hyaluronic acid is usually found unbound in cell matrix material (50). However, the hyaluronic acid found in the matrix is usually quite large and may be different in nature from the low molecular weight material bound to membrane glycoproteins.

In one of the glycoproteins examined (A2-2), 5% of the Group EV glycopeptides derived from it was degraded by nitrous acid (Table II), which specifically acts on N-sulfated glycosaminoglycans such as heparin or heparan sulfate (26). It is, therefore, possible that glycoprotein A2-2 is a hybrid molecule bearing these glycosaminoglycans, and that hyaluronic acid, chondroitin sulfate, and heparin or heparan sulfate are all components of glycoprotein hybrids.

In two of the purified glycoproteins examined, about 10% of the Group EV material remained in the exclusion volume after alkaline reduction, leech hyaluronidase and chondroitinase ABC treatments (Table II). This material was included by Sephadex G-50 after treatment with a mixture of glycosidases from S. pneumoniae. It may consist of oligosaccharides bound to closely spaced asparagine residues that are not cleaved by pronase, or they could be unusually large oligosaccharide groups similar to those that exist in ascites tumors (52). Since 10 to 20% of the bound carbohydrate of these glycoproteins is Group EV type, and 10% of that is susceptible to glycosidase digestion, this material may account for about 1 to 2% of the total carbohydrate of the two purified glycoproteins examined.

The bulk of the carbohydrate (90%) from purified glycoproteins was included by Sephadex G-50 column chromatography. Almost none of this is susceptible to cleavage by alkaline reduction and is presumed to be N-glycosidically linked. When this material was sequentially fractionated by size, lectin affinity, and DEAE-Sephadex chromatography, no marked quantitative differences were observed between the glycopeptides derived from control and from transformed cell membrane glycoproteins. DEAE-Sephadex profiles show a relative increase of Group A-Con A(+) material (Fig. 5) and a relative decrease of Group B-Con A (+) material in C13/B4 cells (Fig. 7). This shift involves a coordinated alteration of several peaks which suggests a conversion of Group B-Con A (+) carbohydrate structures to corresponding Group A structures that do not bind to Con A. It is also possible that a common precursor is diverted to Group A rather than Group B glycopeptides in transformed cells. The lack of affinity of Group A glycopeptides for Con A has been reported previously (53). The overall balance of the populations of oligosaccharides borne by single glycoproteins of transformed cells is shifted without significantly affecting the characteristics upon which isolation of glycoproteins depends.

The DEAE-Sephadex elution profiles indicate that there are large numbers of oligosaccharides bound to the individual membrane glycoproteins (Figs. 5 to 7). Three other purified membrane glycopeptides (M, 105,000; 32.2; 4.0, and 5.0) from the LiSal aqueous fraction showed elution patterns virtually identical with those seen in Figs. 5 to 7. The remarkable similarity in these elution patterns could be due to the fact that all of the glycopeptides examined are soluble in an aqueous solution of LiSal. Those glycopeptides, soluble in the LiSal-phenol fraction, manifest a different pattern of glycopeptides, and it will be of interest to examine them beyond the Sephadex G-50 stage (14).

Extensive examination of cell surface glycopeptides obtained by trypsinization and pronase treatment of released glycopeptides indicates that the elution patterns from columns of DEAE-Sephadex are complex (54). There are at least 40 different glycopeptides that could be bound to membrane glycoproteins. Preliminary analysis of the purified glycopeptides by gas-liquid chromatography for neutral and amino sugars and by the thiorbarbituric assay for sialic acid shows that they consist of approximately 10% carbohydrate. There is an apparent inconsistency when the number of major peaks and the total glycoprotein carbohydrate are considered (Figs. 5 to 7). The molecular weights of the glycopeptides examined are in the range of 160,000 (A1) and 110,000 (A2), implying that in this case the molecular weight of the carbohydrate associated with these glycopeptides is approximately 11,000 to 16,000. The molecular weight of Groups A and B glycopeptides is approximately 3,000 to 5,000. Twelve oligosaccharides of molecular weight 4,000 have a combined molecular weight of
of glycoproteins has been reviewed. We are dealing with macromolecules of specific size and isoelectric points. Each pair of homologous glycoproteins from control and transformed cells (control and transformed), yielded single, overlapping NH₂-terminal peptides from different glycoproteins were followed by isoelectric focusing. This is consistent with the findings of Spiro (28) who has provided evidence that not all molecules of human thyroglobulin bear glycosaminoglycans, and that some bear more than one copy. Heterogeneity of the carbohydrate component of glycoproteins has been reviewed (55, 56). Homogeneity of the isolated polypeptides is reasonably assured by the observations that we are dealing with macromolecules of specific size and isoelectric points. Each pair of homologous glycoproteins, which came from two different lines of cells (control and transformed), yielded single, overlapping NH₂-terminal groups indicating an identity of macromolecules. The fluorescent NH₂-terminal peptides from different glycoproteins were in different localizations. The purification involving SDS-PAGE followed by isoelectric focusing appears to be dominated by the structure of the polypeptide, although the carbohydrate has some influence. Although homologous glycoproteins from control and transformed cells may differ in the composition and structure of the oligosaccharide structures they bear, their total charges and apparent masses are essentially the same.

Acknowledgment—We are grateful to Ms. Carol Walz for her excellent technical assistance.

REFERENCES
1. Nicolson, G. L. (1975) Biochim. Biophys. Acta 438, 1-72
2. Emmelot, P. (1973) Eur. J. Cancer 9, 319-333
3. Maslow, D. E. (1976) in The Cell Surface in Animal Embryogenesis and Development (Pote, G. and Nicolson, G. L., eds) pp. 697-745, North-Holland Publishing Co., New York
4. Barondes, S. H. (1978) in The Molecular Basis of Cell-Cell Interaction (Lerner, R. A., and Bergman, D., eds) pp. 491-496, Alan R. Liss Co., New York
5. Moscona, A. A., and Hausman, R. E. (1977) in Cell and Tissue Interactions (Lash, J. W., and Burger, M. M., eds) pp. 173-185, Raven Press, New York
6. Lilien, J., and Rutz, R. (1977) in Cell and Tissue Interactions (Lash, J. W., and Burger, M. M., eds) pp. 187-195, Raven Press, New York
7. Buck, C. A., Glick, M. C., and Warren, L. (1971) Science 172, 169-171
8. Warren, L., Buck, C. A., and Tuszynski, G. P. (1978) Biochim. Biophys. Acta 516, 97-127
9. Buck, C. A., Fuhrer, J. P., Solasau, G., and Warren, L. (1974) J. Biol. Chem. 249, 1541-1550
10. Buck, C. A., Glick, M. C., and Warren, L. (1971) Biochemistry 10, 2176-2180
11. Ceccarini, C. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 2687-2690
12. Glick, M. C., Rabinowitz, Z., and Sachs, L. (1973) Biochemistry 12, 4864-4869
13. Smets, L. A., Van Beek, W. P., and van Rooij, H. (1976) Int. J. Cancer 18, 462-468
14. Tuszynski, G. P., Fuhrer, J. P., Buck, C. A., and Warren, L. (1978) J. Biol. Chem. 253, 6992-6999
15. Van Nest, G. A., and Grimes, W. J. (1977) Biochemistry 16, 2902-2908
16. Buck, C. A., Glick, M. C., and Warren, L. (1970) Biochemistry 9, 4567-4576
17. Levine, E. (1972) Exp. Cell Res. 74, 99-109
18. Hubbard, A. L., and Cohn, Z. A. (1973) J. Cell Biol. 55, 390-405
19. Gahmberg, C. G., and Hakomori, S. (1973) J. Biol. Chem. 248, 4311-4317
20. Schaffner, W., and Weissman, C. (1973) Anal. Biochem. 56, 502-514
21. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275
22. Marchesi, V. T., and Andrews, E. P. (1971) Science 174, 1247-1254
23. Sleeman, U. K. (1970) Nature (Lond.) 227, 680-685
24. Arima, T., Sprio, M. J., and Sprio, R. G. (1972) J. Biol. Chem. 247, 1835-1835
25. Saito, H., Yamagata, T., and Suzuki, S. (1968) J. Biol. Chem. 243, 1536-1542
26. Hart, G. W. (1976) J. Biol. Chem. 251, 6513-6521
27. Oegema, T. R., Jr., Brown, M., and Dziewiatkowski, D. (1977) J. Biol. Chem. 252, 6470-6477
28. Sprio, M. J. (1977) J. Biol. Chem. 252, 5424-5430
29. Lehnhardt, W. F., and Winzler, R. J. (1968) J. Chromatog. 34, 471-479
30. Warren, L. (1959) J. Biol. Chem. 234, 1971-1975
31. Holloway, P. W. (1973) Anal. Biochem. 53, 304-308
32. Tichy, H. (1975) Anal. Biochem. 69, 552-557
33. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102-1106
34. Warren, L., Critchley, D., and Macpherson, I. (1972) Nature 235, 275-278
35. Van Beek, W. P., Smets, L. A., and Emmelot, P. (1975) Nature 233, 457-460
36. Warren, L., Zeidman, I., and Buck, C. A. (1975) Cancer Res. 35, 2186-2190
37. Van Beek, W. P., Smets, I. A., and Emmelot, P. (1973) Cancer Res. 33, 2913-2922
38. Sprio, R. G. (1973) Adv. Protein Chem. 27, 349-347
39. Kornfeld, R., and Ferris, C. (1975) J. Biol. Chem. 250, 2614-2619
40. Krausus, T., Finne, J., Karkkainen, J., and Jarnefelt, J. (1974) Biochim. Biophys. Acta 365, 80-92
41. Chiarugi, V. P., Vannucchi, S., and Urbano, P. (1974) Biochim. Biophys. Acta 345, 283-293
42. Culp, L. A. (1978) Current Topics in Membranes and Transport, Vol. 12, pp. 327-386, Academic Press, New York
43. Hascell, V. C., and Sajdera, S. W. (1969) J. Biol. Chem. 244, 2384-2396
44. Hascell, V. C., and Heinegaard, D. (1975) in Extracellular Matrix Influences on Gene Expression (Slavkin, H. C., and Greulich, R. C., eds) pp. 423-433, Academic Press, New York
45. Seiht, C., Duff, R., Rapp, F., and Davidson, E. A. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 54-56
46. Terry, A. H., and Culp, L. A. (1974) Biochemistry 13, 414-425
47. Okiyama, M., Yoshimura, M., Muto, M., Chi, J., Roth, S., and Kaji, A. (1977) Cancer Res. 37, 712-717
48. Kollins, B. J., and Culp, L. A. (1979) Biochemistry 18, 141-148
49. Perkins, M. E., and Hynes, R. O. (1979) Cell 16, 941-952
50. Lindahl, U., and Hook, M. (1978) Annu. Rev. Biochem. 47, 385-417
51. Kyle, E. H. (1966) J. Theoret. Biol. 10, 89-113
52. Walborg, E. F., Davis, E. M., Gilliam, R. B., Smith, D. F., and Nerl, G. (1975) in Cellular Membranes and Tumor Cell Behavior pp. 337-360, The Williams & Wilkins Co., Baltimore, Md.
53. Ogata, S. J., Muramatsu, T., and Kobata, A. (1976) Nature 259, 590-592
54. Glick, M. C. (1979) Biochemistry 18, 2525-2532
55. Montgomery, R. (1972) in Glycoproteins (Gottschalk, A., ed) Part A, 2nd Ed, pp. 518-528, Elsevier Scientific Publishing Co., Amsterdam
56. Sprio, R. G. (1973) Adv. Protein Chem. 27, 393-397