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# Glycoproteins of Cultured Epithelial Cells from Human Colonic Adenocarcinoma and Fetal Intestine*

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**Abstract**—*The molecular weight distribution and Concanavalin A binding properties of labeled glycopeptides and glycoproteins from cell surface membranes, the cytoplasmic fraction and culture media were studied in human fetal and neoplastic colonic cells. Cells were labeled by culturing cells in the presence of* [$^{14}$C]-*or* [$^{3}$H]-*labeled glucosamine or fucose. Both fucose and glucosamine were incorporated into the membrane glycoprotein fraction of the colonic cancer cells to a much lesser extent than into fetal cells. The elution profile obtained from Bio-gel P-*150 *column chromatography indicated that colonic cancer cell membranes contain fucose- and glucosamine-labeled, trypsin labile, high molecular weight glycopeptides not present in fetal cells. Affinity chromatographic studies of membrane glycopeptides using agarose-bound Concanavalin A also indicated that cell surface membranes from fetal and cancerous colonic cells contain not only glycoproteins of different molecular weights, but glycoproteins having different oligosaccharide side chains. These results indicate that there are significant quantitative and qualitative differences in cell surface glycoproteins between fetal and cancerous intestinal cells.*

## INTRODUCTION

CONSIDERABLE alterations in cell surface glycoproteins and glycolipids have been reported to occur in cells transformed *in vitro* by oncogenic viruses [1–10]. However, relatively little information is available on the surface membrane components of spontaneously occurring tumors particularly those consisting of epithelial cells, which account for most human tumors [11]. When membrane fractions prepared from human colonic epithelial cancer tissues and normal adjacent tissues were studied, a significant loss of blood group activity was observed in the tumor tissues concomitant with alterations in the reactivity against some lectins [12]. A marked reduction in the content of some carbohydrate moieties of glycoproteins was also observed in the membrane fraction of tumor tissues [12, 13].

There is suggestive evidence that cancer cells are similar to fetal cells with regard to antigenic expression and isoenzyme patterns [4, 14–17]. Since little is known about the cell surface membrane properties of intestinal epithelial cells from human fetal and colonic adenocarcinoma, and because of the increased interest in oncodevelopmental changes in antigenic and metabolic properties of cancer cells [14–17], we have chosen to study the cell surface components of these human intestinal cells [12, 13].

In the present study we have investigated physical, chemical and some immunochemical properties of the surface membrane glycoproteins from well characterized epithelial cell lines from human colonic adenocarcinoma [18] and fetal intestine [19].

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## MATERIALS AND METHODS

### *Cell cultures*

A human cancerous colonic epithelial cell line (SKCO-1) used in this study was kindly supplied to us by Dr. J. Fogh, Sloan-Kettering Cancer Institute, Rye, New York, who established the malignant properties of

these cells by observing abnormal chromosome patterns (hypertriploid to hypertetraploid) and by the production of tumors by these cells when injected into nude mice [18]. The human fetal intestinal cell line (FHS 74 Int.) was generously given to us by Dr. W. Nelson-Reese, Naval Biomedical Research Laboratories, Oakland, Calif. Further karyotyping and isoenzyme studies kindly performed by Dr. Nelson-Reese indicated that both of these cell lines were not HeLa cells [19]. In our hands the fetal line demonstrated density-dependent inhibition of growth and did not produce tumors when injected into nude mice. In contrast, growth of tumor cells was not inhibited at confluency and the cells produced tumors when injected into nude mice. All cultures were free of mycoplasma when these experiments were performed. Both cell lines were maintained on Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, penicillin (100 units/ml) and streptomycin (100 $\mu$g/ml). The cells were grown in 75 $cm^2$ growth area tissue culture flasks (Falcon Plastics, Oxnard, Calif.), and the medium was changed twice a week. All of the studies were carried out on cells at 60% confluency. The FHS cells were at passage 34; the cancer cells were at passage 38. Another human colonic adenocarcinoma cell line, HT-29, which was derived from a different individual and was at passage 138, gave essentially identical results to those from the SKCO-1 line. The HT-29 line was also generously given to us by Dr. J. Fogh. All tissue culture chemicals and media were purchased from Pacific Biological Co., Berkeley, Calif.

*Radioactive-labeling of cellular glycoconjugates*

In order to label cellular glycoconjugates, fresh medium containing radioactive sugars was added to the cells which were further cultured for 92 hr. For the cancer cells, L-[1-$^{14}$C]-fucose (50 mCi/mmole) and D-[1-$^{14}$C]-glucosamine (8 mCi/mmole) were used while L-[6-$^{3}$H]-fucose (13 Ci/mmole) and D-[6-$^{3}$H]-glucosamine (10 Ci/mmole) were used for the fetal cells. All radiochemicals were purchased from New England Nuclear, Boston, Mass.

*Preparation of radioactive labeled surface membrane glycopeptides*

At 92 hr after the addition of 60 $\mu$mole each of the respective radioactive sugars, the culture media was collected and the cells, which were attached to the flask, were rinsed twice with serum-free DMEM and then twice with Dulbecco's phosphate buffered saline-$Ca^{2+}$ and $Mg^{2+}$ free (DPBS). The cells were then detached from the flasks by adding 3 ml of 0.5 mg/ml of 3 × crystallized trypsin (Worthington Biochemical Corp., Freehold, N.J.) in DPBS and incubating the flasks for 10 min at 25°C. Three milliliters of 0.02 mM phenylmethylsulfonyl-fluoride (Sigma Chemical Co., St. Louis, Mo.) were then added to each flask to inhibit further trypsin activity. A single cell suspension was prepared by quickly aspirating and expelling the suspension using either a 10 ml pipette or a syringe with an 18 gauge hypodermic needle. Aliquots of this cell suspension were examined for cell concentration and viability by trypan blue dye exclusion. Over 90% of the cells were viable at this stage of the procedure. The cell suspension was centrifuged at 2000 rev/min for 10 min. The supernatant fluid containing the trypsin-released membrane glycopeptides was carefully withdrawn, passed through a 0.2 $\mu$m Millipore® membrane filter (Millipore Corporation, Bedford, Mass.), dialyzed extensively against 10 mM ammonium acetate, pH 7.5, until no radioactivity could be detected in the dialysate and then lyophilized.

The sedimented cells were twice resuspended in DPBS and centrifuged to remove traces of trypsin. The washed cells were homogenized using a Polytron homogenizer (Brinkman Instrument, Westbury, N.Y.) and centrifuged at 145,000 *g* for 1 hr. The supernatant fluid containing the cytoplasmic fraction was carefully removed from the pellet to avoid cross-contamination.

The cytoplasmic fraction and culture media were also filtered through a 0.2 $\mu$m Millipore® membrane, dialyzed and lyophilized as described previously for the trypsin-released membrane glycopeptides.

*Bio-gel P-150 column chromatography*

After lyophilization, the surface membrane glycopeptides, the cytoplasmic fraction and the culture media were dissolved separately in 10 mM ammonium acetate, pH 7.5, and centrifuged at 2000 rev/min. Equivalent fractions containing the same amount of radioactivity from the cancerous and fetal cells labeled with the same sugar but with different isotopes, were mixed, applied to a column (1.2 × 90 cm) containing Bio-gel P-150 (Bio-Rad Laboratories, Richmond, Calif.) and eluted at a flow rate of 7 ml/hr. Radioactivity of the

effluent was counted in a Packard Tri-Carb liquid scintillation spectrometer, and the data were corrected for overlapping counts in each channel resulting from use of the double label. The Bio-gel P-150 column was calibrated with the following molecular weight standards: horse heart cytochrome C (Mann Research Laboratories, N.Y.) mol. wt 12,400; bovine pancreas chymotrypsinogen A (Schwartz BioResearch Inc., Orangeburg, N.Y.) mol. wt 25,700; human hemoglobin, mol. wt 62,000; transferrin, mol. wt 82,000, (Sigma Chemical Co.) and 2 × crystallized yeast alcohol dehydrogenase (Sigma Chemical Co.) mol. wt 140,000. For the radioimmunoassay of carcinoembryonic antigen (CEA), the method of Hansen *et al.* [20] was used.

*Bio-gel P-30 column chromatography*

The effluent from the Bio-gel P-150 column chromatography was pooled into A, B and C fractions and each fraction was lyophilized and resuspended in 1 ml of 10 mM ammonium acetate, 1 mM calcium chloride, pH 7.5. Each fraction was incubated at 37°C for 95 hr with pronase (2% of the total protein of the samples) and 1 drop of toluene. An additional amount of pronase was added to the incubation mixture every 24 hr, and the incubation was stopped by freezing. The pronase-digested glycopeptide fractions were chromatographed on a 1.2 × 70 cm column of P-30 (Bio-Rad Labs) in 10 mM ammonium acetate, pH 7.5, at a flow rate of 8 ml/hr. The effluent was counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. The following molecular weight standards were used for the calibration of the column: bacitracin (Sigma Chemical Co.) mol. wt 1000; a pronase-digested glycopeptide prepared from human $\alpha_1$ acid glycoprotein, mol. wt 2600; horse heart cytochrome C (Mann Research Laboratories) mol. wt 12,400; bovine pancreas chymotrypsinogen A (Schwartz BioResearch) mol. wt 25,700.

*Concanavalin A–Sepharose (Con A–Sepharose) affinity chromatography*

The effluent obtained after Bio-Gel P-150 column chromatography of trypsin-released membrane glycopeptides and secreted glycoproteins in the culture media were pooled into fractions A, B and C as shown in the charts. Each fraction was then applied to a column of Con A–Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) with a bed volume of 5 ml, followed by elution with 10 ml of 10 mM ammonium acetate, pH 7.4. Glycopeptides bound to Con A–Sepharose were then eluted with 10 ml of 0.1 M α-methyl-D-mannopyranoside (Sigma Chemical Co.).

The eluates were examined for radioactivity and CEA activity using the methods described above.

## RESULTS

*Gel filtration column chromatography*

Figure 1(a) shows the elution profiles of labeled trypsin-released membrane glycopeptides from cancer and fetal cells using a column of Bio-gel P-150. The eluate was divided into three fractions designated A, B and C, consisting of glycopeptides having molecular weights greater than 150,000, those of intermediate size and a nondialyzable lower molecular weight fraction, respectively. When fucose was used to label the cells, the labeling of the high molecular weight, surface glycopeptide fractions (fraction A) was much greater in the cancer cell than in the fetal cells, while the lower molecular weight fractions (fraction C) appeared to be labeled well by fucose in both fetal and cancer cells.

When the cells were labeled with glucosamine, a different pattern was observed. The elution profiles of the glucosamine-labeled, high molecular weight fraction (fraction A) was similar to that with fucose labeling, showing much greater labeling in the cancer cells than in the fetal cells. Although fraction C from the fetal cells was the predominantly labeled fraction, this fraction was only sparsely labeled in the cancer cells.

Figure 1(b) shows the elution profiles of the pronase digests of fraction A from the trypsin-released membrane glycopeptides separated on a Bio-gel P-30 column. With fucose labeling, both cancer and fetal cells had a similar elution pattern consisting of two main peaks, the first one eluting at the exclusion limit, and a second one with a mean apparent mol. wt of 6000–7000. Although similar, the small molecular weight fraction from the cancer cells eluted slightly ahead of that of the fetal cells. However, with glucosamine, both cells were labeled primarily in the first fraction eluting at the exclusion limit with only a minor second peak. Figure 1(c) shows the elution profile of the pronase digest of fraction C chromatographed on a Bio-gel P-30 column. With both fucose and glucosamine labeling, fraction C from both types of cells

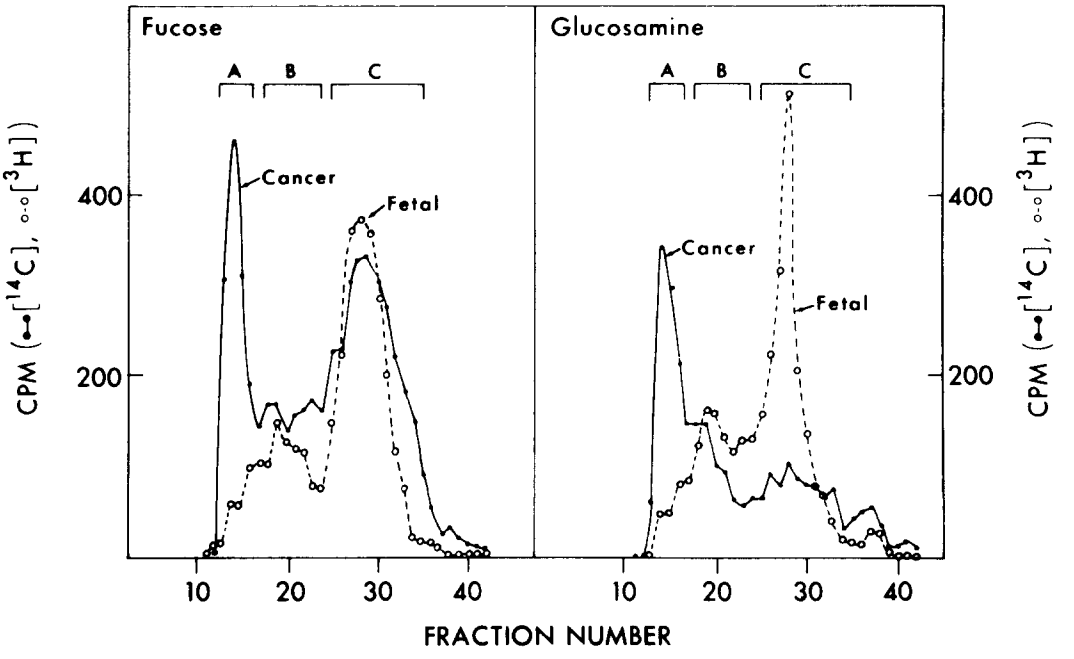


*Fig. 1a. Radioactive elution profiles of trypsin-released surface membrane glycopeptides on a Bio-gel P-150 column. (●), SKCO-1 cells; (○), FHS cells. A, B and C represent respective pooled fractions 11–16, 17–24 and 25–35. Void volume corresponds to fraction No. 12.*

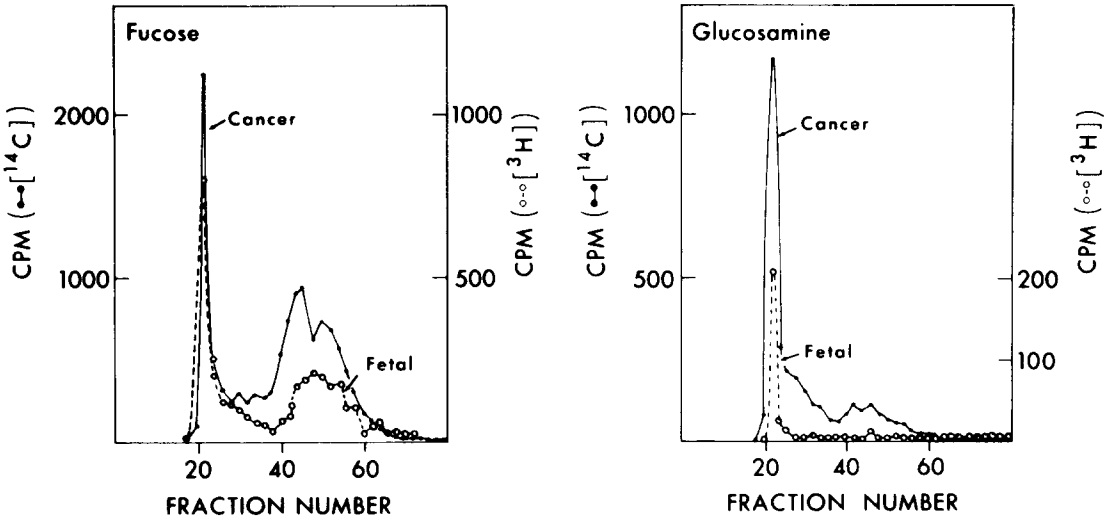


*Fig. 1b. Radioactive elution profile of pronase-digested glycopeptide fraction A described in Chart 1a on a Bio-gel P-30 column. (●), SKCO-1 cells; (○), FHS cells. Void volume corresponds to fraction No. 21.*

was hydrolyzed nearly completely to smaller glycopeptides of mean mol. wt about 4000.

The elution profiles of labeled glycoproteins from the cytoplasmic fractions of cancerous and fetal cells using a Bio-gel P-150 column are shown in Fig. 2(a). With either sugar, most of the radioactivity was associated with the larger molecular weight fraction which was eluted at the exclusion volume (fraction A).

The pronase digest of fraction A from cytoplasmic glycoproteins was then applied to a Bio-gel P-30 column. The elution profiles are illustrated in Fig. 2(b). With either labeled sugar, the cancerous and fetal cells showed similar elution profiles consisting of two peaks, one at the exclusion limit and another one at an apparent mean mol. wt of 6000–7000. Again with fucose labeling, a slight but consistent shifting of the small molecular weight peak to a higher molecular weight region was observed with glycopeptides from cancer cells

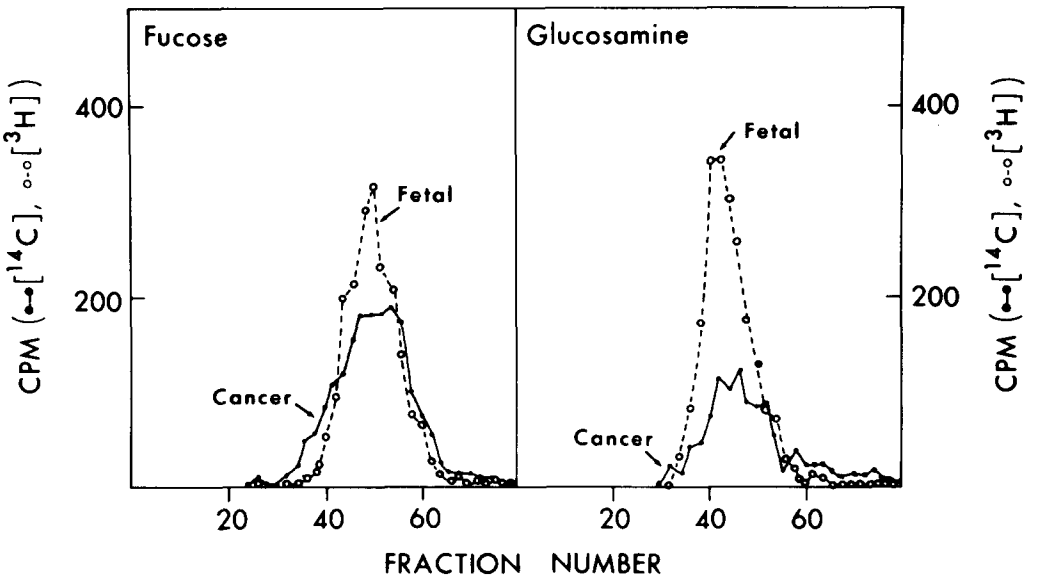


*Fig. 1c. Radioactive elution profiles of pronase-digested glycopeptide fraction C described in Chart 1 a on a Bio-gel P-30 column. (●), SKCO-1 cells; (○), FHS cells. Void volume corresponds to fraction No. 21.*

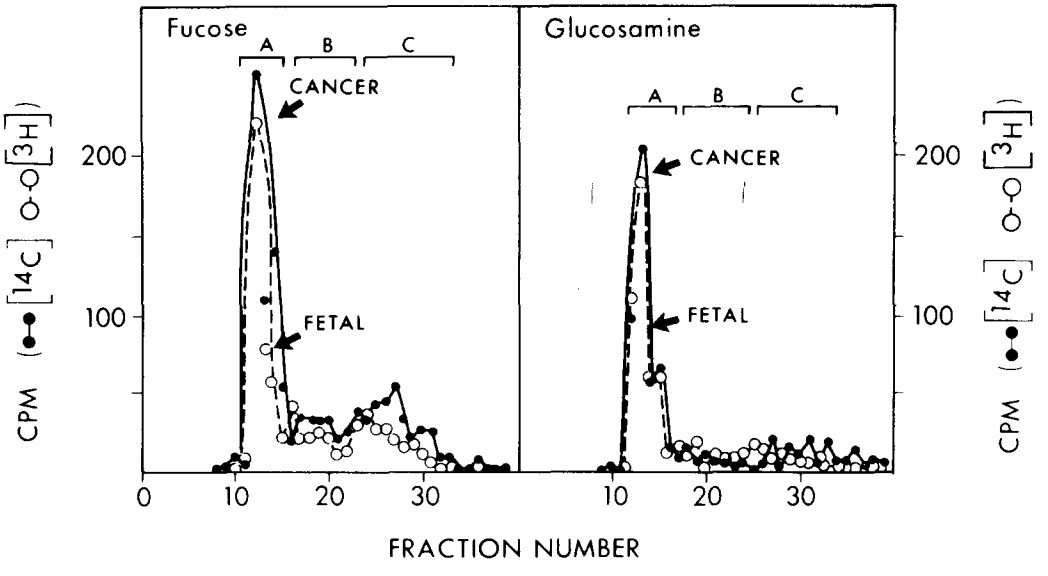


*Fig. 2a. Radioactive elution profiles of soluble cytoplasmic glycoproteins on a Bio-gel P-150 column. (●), SKCO-1 cells; (○), FHS cells. A, B and C represent respective pooled fractions 11–16, 17–24 and 25–35. Void volume corresponds to fraction No. 12.*

compared to those from fetal cells. As in the case of the trypsin-released membrane glycopeptides, glucosamine-labeled cytoplasmic glycoproteins appeared to be more resistant to pronase digestion than those which had been labeled with fucose.

The elution profiles on a Bio-gel P-150 column of the fucose- and glucosamine-labeled glycoproteins from the culture media secreted by both cell lines revealed a major peak at the exclusion limit (fraction A) and a minor peak (fraction B) with an apparent mean mol. wt of about 50,000 [Fig. 3(a)]. The elution profiles of the pronase digested fraction A on a Bio-gel P-30 column are shown in Fig. 3(b). The fucose-labeled fraction A from the secreted glycoproteins of both cancerous and fetal cells appears to be more

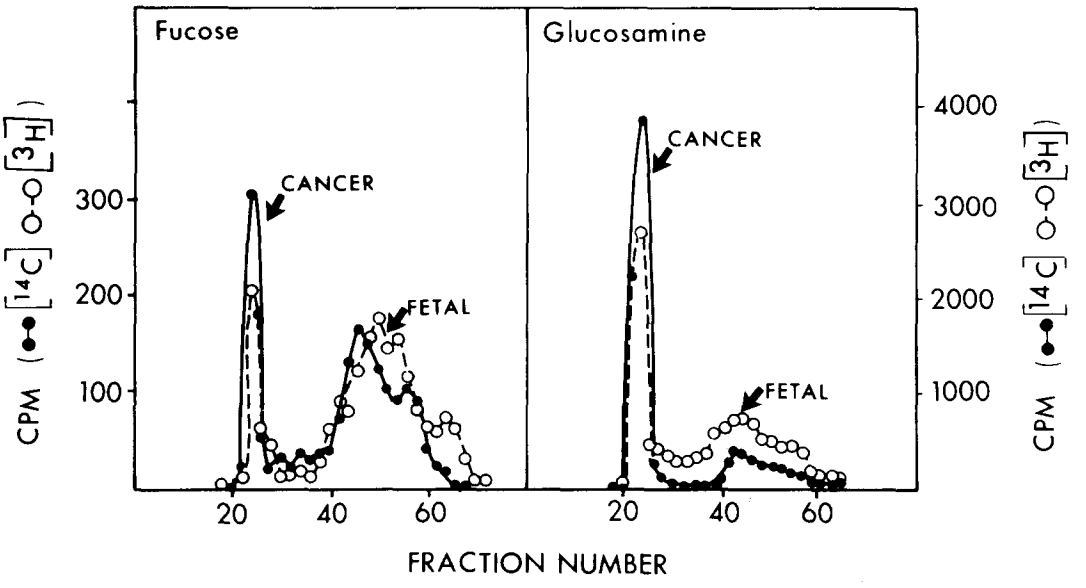


*Fig. 2b. Radioactive elution profiles of pronase-digested cytoplasmic glycoprotein fraction A described in Chart 2a on a Bio-gel P-30 column. (●), SKCO-1 cells; (○), FHS cells. Void volume corresponds to fraction No. 21.*

readily hydrolyzed by pronase than those of the membrane and cytoplasmic fractions. Digestion by pronase reduced the molecular weight of fraction A labeled by either sugar from both cells although the glycoproteins from the cancerous cells appeared to be more resistant to proteolysis.

Using a radioimmunoassay for CEA, over 90% of the CEA was associated with the high molecular weight fraction (fraction A on a Bio-gel P-150 column) of the membrane glycopeptides, cytoplasmic glycoproteins and secreted glycoproteins from cancer cells. Following pronase digestion of the respective high molecular weight fraction, CEA activity remained associated only with the excluded fraction on a Bio-gel P-30 column. No CEA activity was detected in any of the glycopeptide or glycoprotein fractions from the fetal intestinal cells.

*Con A–Sepharose binding properties of various glycopeptides and glycoproteins*

In order to characterize further the glycopeptides and glycoproteins from cancerous and fetal cells, fractions from the surface membrane and secretion obtained following Bio-gel P-150 column chromatography were subjected to affinity chromatography using a Con A–Sepharose column. The cytoplasmic fractions could not be studied due to the low amounts of radioactivity. Table 1 summarizes our results. With fraction A trypsin-released glycopeptides from both fetal and cancerous colonic cells, over half of the fucose- and glucosamine-labeled glycopeptides were adsorbed to the Con A–Sepharose column. However, with fraction C nearly all of the radioactivity from all of the samples passed through the column; only 4–14% of the radioactivity was adsorbed. With glycosamine-labeled secreted glycoproteins, both fractions A and B from fetal and cancerous cells showed little affinity for Con A–Sepharose. However, with fucose-labeled secreted glycoprotein fractions A and B, 62 and 41%, respectively, of the radioactivity secreted by fetal cells were retained on the Con A–Sepharose column while only 18 and 29% of the radioactivity from the cancerous cells were retained.

Ninety percent of the CEA activity associated with fraction A of the secreted glycoproteins and trypsin-released membrane glycopeptides from colonic cancer cells was adsorbed to the Con A–Sepharose column.

*Proportion of non-dialyzable labeled glycopeptides and glycoproteins*

The percentage of non-dialyzable glycopeptides and glycoproteins from fetal and cancerous colonic cells obtained from cell surface glycopeptides released by trypsin, from the cytoplasmic fraction and from culture media, is shown in Table 2. In fetal intestinal cells, 94–98% of the total radioactivity associated with cell surface trypsin-released glycopeptides and cytoplasmic glycoproteins was non-

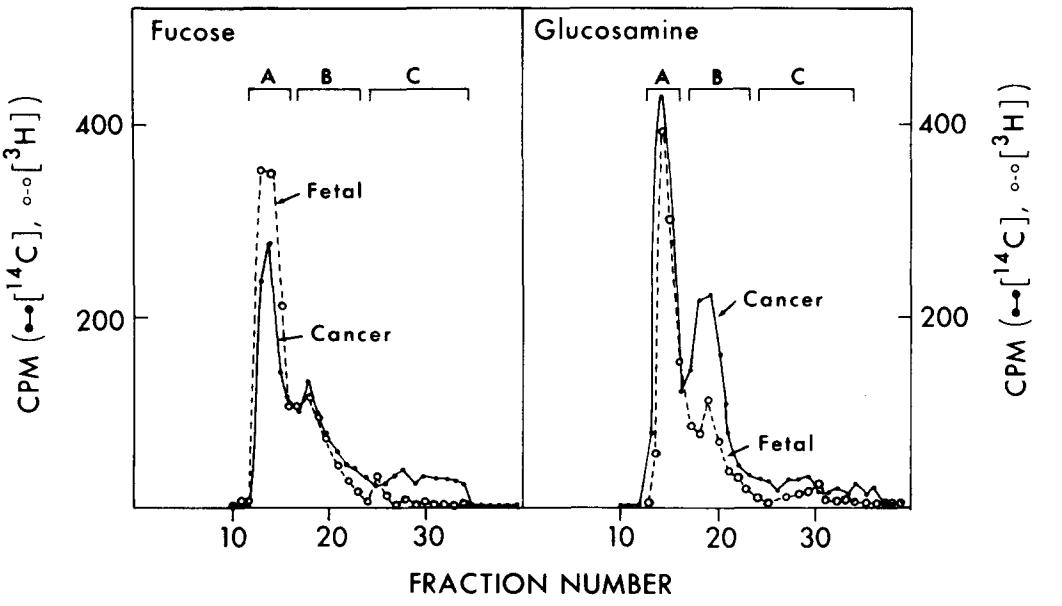


*Fig. 3a. Radioactive elution profiles of secreted glycoproteins on a Bio-gel P-150 column. (●), SKCO-1 cells; (○) FHS cells. A, B and C represent respective pooled fractions 11–16, 17–24 and 25–35. Void volume corresponds to fraction No. 12.*

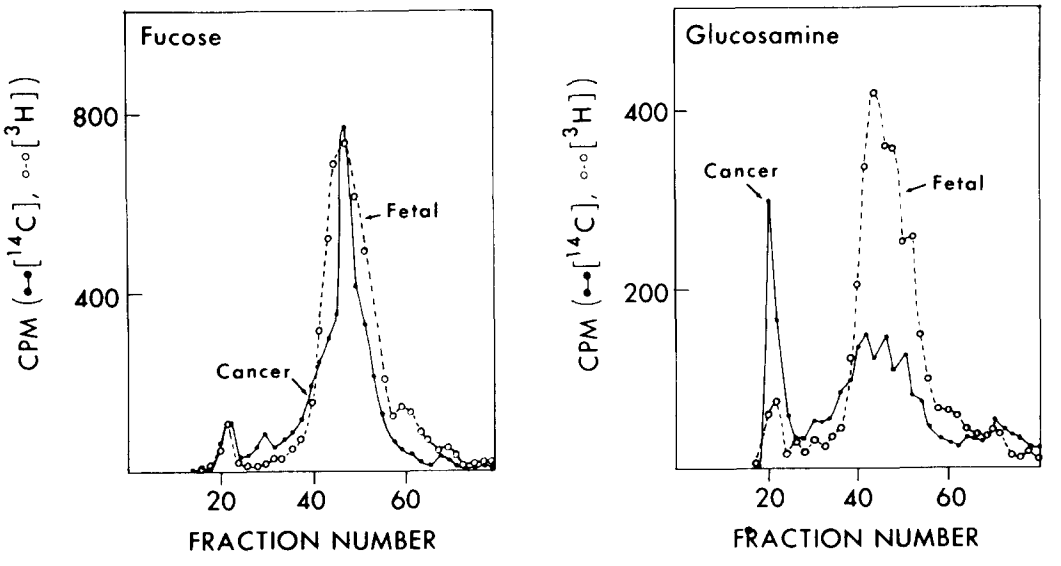


*Fig. 3b. Radioactive elution profiles of pronase-digested secreted glycoprotein fraction A described in Chart 3a on a Bio-gel P-30 column (●), SKCO-1 cells; (○), FHS cells. Void volume corresponds to fraction No. 21.*

dialyzable. In the media from both fetal and cancerous cells, although nearly all of the radioactivity was dialyzable, proportionately more radioactivity appeared to be associated with the non-dialyzable fraction from fetal cells than from cancer cells.

## DISCUSSION

Our data from the present study strongly suggest that there are significant quantitative and qualitative differences in cell surface glycoproteins between fetal and cancerous intestinal cells. For example, both labeled fucose and glycosamine were incorporated into membrane glycoproteins of the colonic cancer cells to a lesser extent than into fetal intestinal cells. This may indicate either a lower synthetic rate, since fucose is often a terminal sugar in oligosaccharide side chains, or there may be relatively incomplete synthesis of membrane glycoproteins in colonic cancer cells compared to fetal intestinal cells. Other

*Table 2. Con A–Sepharose affinity chromatography of trypsin-released membrane glycopeptides and secreted glycoproteins from fetal and cancerous colonic cells*

| | Fucose labeling | | Glucosamine labeling | | CEA |
|---|---|---|---|---|---|
| | Fetal | Cancer | Fetal | Cancer | Cancer |
| | | | % Bound* | | |
| Trypsin-released glycopeptides | | | | | |
| Fraction A | 57 | 48 | 59 | 49 | 91 |
| Fraction B | 7 | 14 | 4 | 9 | —† |
| Secreted glycoproteins | | | | | |
| Fraction A | 62 | 18 | 4 | 9 | 90 |
| Fraction B | 41 | 29 | 0 | 6 | —† |

*In all cases, the retained counts were eluted with 0.1 M α-methyl-D-mannopyranoside with recovery of greater than 89%.
†No CEA activity was detected.

*Table 2. Non-dialyzable radioactivity in different fractions of fetal and cancerous cells labeled with radioactive fucose or glucosamine*

| | Fetal cells | | Cancer cells | |
|---|---|---|---|---|
| | Fucose | Glucosamine | Fucose | Glucosamine |
| | | % | | |
| Trypsin-released glycopeptides | 95.4 | 98.0 | 34.0 | 26.7 |
| Cytoplasmic fraction | 95.4 | 94.4 | 30.0 | 24.0 |
| Media | 2.1 | 5.3 | 0.1 | 1.0 |

The results are means of three experiments.

reasons for these differences are also possible, such as differential susceptibility to trypsin cleavage by the respective cell surfaces, differences in precursor pools, or merely qualitative differences among the surface glycoproteins.

Incomplete synthesis of oligosaccharide side chains of glycolipids has been reported to occur not only in virally transformed cells, but also in malignant gastrointestinal tumor tissues [21]. It has also been reported from this laboratory that membrane glycoproteins of human colonic cancer tissues contained much less carbohydrate than those prepared from the adjacent normal mucosa [12, 13]. However, similar studies have not been carried out in fetal intestinal cells or tissues.

Qualitative differences between the membrane glycoproteins of fetal and cancerous colonic cells are suggested from our dialysis data, the results from the double labeling experiments, as well as from the Con A affinity chromatography study. Our dialysis study clearly indicates that the trypsin-released, surface membrane glycopeptides from colonic cancer cells consisted of a considerable amount of small molecular size glycopeptides of less than 12,000, as well as higher molecular weight glycopeptides. Fetal intestinal cell surface membrane glycopeptides, on the other hand, were primarily of large molecular weight. This may indicate either the presence in the colonic cancer cell

surface of smaller glycoproteins or of a greater number of peptide bonds within the membrane glycoprotein molecule susceptible to trypsin or that there is an increased activity of endogenous cell surface membrane proteases in colonic cancer cells.

The use of a double label method in labeling the surface membrane glycoproteins allows us to make an accurate comparison between the fetal and cancerous membrane glycoproteins since manipulative variations are internally controlled by processing the fetal and cancerous samples together [10]. Our results indicate that colonic cancer cell membranes contain fucose-labeled and glucosamine-labeled, trypsin-labile, high molecular weight glycopeptides not present in fetal intestinal cells. The labeled glycopeptides released from fetal cells were predominantly of lower molecular weight. It must be pointed out that these studies have been carried out on samples after extensive dialysis and, therefore, all the glycopeptide fractions examined had mol. wt greater than 12,000.

These results are consistent with those of Walborg and his associates who reported that hepatoma cells contained large molecular weight, papain-releasable, cell surface membrane glycopeptides which were nearly absent from the surface membrane of normal rat liver cells [22, 23]. Our observation that colonic cancer cell membrane glycopeptides with mol. wt over 150,000 incorporated both fucose and glycosamine equally well, while those of mol. wt about 12,000 were labeled predominantly with fucose in colonic cancer cells, suggests that the low molecular weight glycopeptide fraction is not simply a trypsin digestion product of large molecular weight glycoproteins, but may represent either a distinct molecular species of membrane glycoprotein or a distinct region of the same glycoprotein molecule having different types of oligosaccharide side chains.

Even after prolonged pronase digestion up to 4 days, the larger mol. wt (over 150,000) membrane glycopeptides from fetal and cancerous colonic cells were resistant to pronase digestion. Furthermore, glucosamine-labeled large molecular weight membrane glycopeptides from both types of cells appear to be more resistant to pronase digestion than the fucose-labeled glycopeptides [Fig. 1(b)].

Although fucose is probably associated only with glycoproteins, it is possible that some of the glucosamine label may be in glycosaminoglycans which would be resistant to pronase digestion. However, examination of the pronase glycopeptides from the SKCO-1 cells showed that less than 20% of the glucosamine-labeled large molecular weight fraction was associated with glycosaminoglycans [24]. Furthermore, results obtained from studies on another human adenocarcinoma cell line, HT-29, were essentially identical to those reported here for the SKCO-1 line. In studies of the HT-29 line, less than 2% of the labeled glucosamine in the large molecular weight peak was associated with glycosaminoglycans [24]. In contrast, smaller mol. wt membrane glycopeptides (12,000) from both types of cells could be digested completely to smaller size with mean mol. wt of 4000. A slight but consistent difference in elution profile of the pronase digested fucose-labeled membrane glycopeptides could be observed between fetal and colonic cancer cells as has been reported by Warren and his co-workers who found a greater proportion of high molecular weight membrane sialoglycopeptides in virally transformed malignant fibroblasts following pronase digestion [10, 11].

Unlike surface membrane glycopeptides, elution profiles from Bio-gel P-150 chromatography from the cytoplasmic fractions of fetal and colonic cancer cells and those secreted into the media were similar. The elution profiles on a Bio-gel P-30 column of the pronase digests of large mol. wt (over 150,000), fucose-labeled cytoplasmic glycoproteins from fetal and colonic cancer cells also showed a slight but consistent difference similar to that observed with the membrane fractions.

In addition, these glycopeptides demonstrated considerable resistance to pronase digestion like the corresponding molecular weight fraction of the membrane glycopeptides. In contrast, the large mol. wt (over 150,000), secreted glycoproteins from both types of cells were susceptible to pronase digestion except for the glucosamine-labeled colonic cancer glycoproteins which were considerably resistant to proteolysis. These results (1) suggest there is a differential susceptibility of fucose-labeled and glucosamine-labeled, secreted glycoproteins to pronase digestion, (2) indicate a heterogeneity among secreted glycoproteins and (3) demonstrate a qualitative difference in secreted glycoproteins between fetal and cancerous colonic cells.

The results of affinity chromatographic studies using Con A–Sepharose also indicate that cell surface membranes from fetal and cancerous colonic cells not only may contain

glycoproteins of different molecular weights, but also may have oligosaccharide side chains with different structures. In the case of secreted glycoproteins, these qualitative differences in constituent sugars appear to be greater between fetal and cancerous cells when labeled with fucose than when cells were grown in the presence of radioactive glucosamine.

The association of significant CEA activity with the trypsin-released high molecular weight surface membrane fractions of carefully washed cancer cells indicates that this antigen is an integral part of the surface membrane of the colonic cancer cells [25, 26]. The detection of significant CEA activity in the high molecular weight fraction of the culture media from SKCO-1 cells is consistent with the previous report of the presence of CEA activity in the culture media of colonic cancer cells [27, 28] and may indicate either the shedding or turnover of cell surface membrane antigens into the culture media.

Both trypsin-released cell surface and secreted CEA exhibited a similar degree of heterogeneity in carbohydrate side chains since they were similar in the proportion of CEA activity adsorbed to Con A–Sepharose. Heterogeneity of CEA molecules prepared from colonic cancer tissues has previously been demonstrated by Con A—Sepharose column chromatography [29]. Although CEA activity was detectable in the homogenate as well as in the media from human fetal intestinal cells, the amount was very low compared to that from colonic cancer cells. This is reminiscent of the data of Gold and Freedman who reported much higher concentrations of CEA in colonic adenocarcinoma tissue than in fetal intestinal tissues between the second and sixth month of gestation [17].

Further purification, chemical and immunochemical characterization of the glycoproteins from surface membranes of human fetal and cancerous colonic cells is necessary for a better understanding of the relationship between the appearance of oncofetal antigens on the surface of some human intestinal epithelial tumor cells and the process of tumorigenesis.

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