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Anionic glycopeptides and glycosaminoglycans synthesized by embryonic neural tube and neural crest

(cell surface/differentiation/neurogenesis)

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ABSTRACT **Anionic glycopeptides and glycosaminoglycans synthesized by embryonic neural tube (epithelium) and neural crest (mesenchymal outgrowth) developing *in vitro* were examined. The profile of surface glycopeptides is relatively simple (two major ones for neural crest; four major ones for neural tube). There is one major glycopeptide found in the medium which is present only in trace amounts on the cells. Both hyaluronate and chondroitin sulfate are synthesized. Hyaluronate is predominantly cell associated; chondroitin sulfate is found predominantly in the medium. It is suggested that independent sorting of these relatively few glycopeptides can result in qualitative surface differences. Morphological state (epithelial or mesenchymal) and differentiated state may be related to these differences as well as to quantitative differences in surface glycosaminoglycans.**

Embryogenesis involves such processes as mesenchymal aggregation (condensation) and epithelial-mesenchymal transformation (somite breakdown, neural crest formation). These events seemingly involve alterations of cell surface adhesiveness. Under experimental conditions *in vitro* (as well as in the intact embryo) cells do not admix randomly, but rather seem to recognize specifically other cells (for discussion, see ref. 1). Such cell-surface mediated recognition appears to be quite specific. Consequently, it is of importance to determine whether qualitative differences exist between surfaces of different cell types and whether morphological changes such as epithelial-mesenchymal transformation are accompanied by changes in the cell surface. For a recent discussion of cell surfaces, see ref. 2. We have, in this study, examined one aspect of this question directly by comparing the anionic cell surface glycopeptides synthesized by embryonic neural tube and the mesenchymal neural crest cells that migrate from its dorsum. A previous report (3) had suggested that neural crest synthesizes glycosaminoglycans and incorporates [^{3}H]fucose.

MATERIALS AND METHODS

Preparation of Cultures. Neural tubes from Japanese quail (*Corturnix coturnis japonica*) embryos [stages equivalent to Hamburger-Hamilton (4) stages 14–15] were cultured as described earlier (5).

Labeling Cultures. After 16 hr, cultures were labeled with 0.5 ml of medium containing 500 μCi/ml of D-[6-^{3}H]glucosamine (10 Ci/mmol, New England Nuclear) for a 6-hr period. The labeling medium was saved and the cultures were washed four times. Neural tubes were detached from the petri plates, pooled, and treated either with 0.25% trypsin in saline G for 30 min at 24° or with activated papain at 68° overnight. The tryptic digest was separated from the intact cells by centrifugation. The mesenchymal crest cells left behind after removal of the neural tubes and the medium were treated as above. Digests were stored frozen until characterized.

Separation of Labeled Molecules. A minimum of two analyses were performed for each labeled sample. Aliquots of digested material were applied to 0.9 × 15 cm columns of DE-52 cellulose (Whatman). Unincorporated label as well as neutral and positively charged molecules were eluted with starting buffer consisting of 2 mM Tris·HCl pH 7.2. Elution was then continued with a linear 0–0.15 M LiCl gradient (150 ml). For those samples digested with papain, elution was continued with a linear 0.15–1.0 M LiCl gradient to elute glycosaminoglycan polysaccharides. Authentic samples were used to calibrate the columns. A final elution, using 4 M LiCl, was done to remove highly charged molecules such as heparin.

Aliquots from each radioactive tryptic glycopeptide peak were pooled and applied to 0.9 × 30 cm Bio-Gel P-4 columns and eluted with 0.1 M NaCl. The included volume (V_i) was calibrated with tritiated water (New England Nuclear). Radioactivity was determined with a Searle MK III liquid scintillation spectrometer.

RESULTS

Morphology of the Culture System. Mesenchymal cells migrate from the dorsal region of the explanted neural tube within a few hours, and a large population of dorsal neural tube-derived mesenchyme is present 22 hr after explanting (Fig. 1).

Tryptic Glycopeptides from Mesenchyme. There are two dominant glucosamine-labeled anionic glycopeptides recovered from the cell surfaces by trypsinization. These molecules elute from DE-52 in two sharp peaks at LiCl concentrations of 0.02 M and 0.055 M (Fig. 2). A small peak, or shoulder, eluting at 0.03 M LiCl immediately after peak A, was invariably present. Peak B contains 6.7 times the radioactivity present in peak A. Further characterization of the labeled materials in peaks A and B by means of adsorption chromatography on Bio-Gel P-4 shows that by this criterion each peak appears relatively homogeneous but elutes at a different position (Fig. 3).

Papain Digest of Mesenchyme. The *total* labeled glycopeptides synthesized during the labeling period were examined after the cells were digested with papain. Two dominant anionic glycopeptides are recovered from DE-52 (Fig. 4), which elute at the same LiCl concentrations as those obtained by mild tryptic digestion, 0.02 M and 0.055 M LiCl. The ratio of radioactivity in peak B to that in peak A is 4.4.

The elution gradient was changed after 0.15 M LiCl to a linear 0.15–1.0 M LiCl gradient in order to elute glycosaminoglycan carbohydrates. Most of the remaining radioactivity appeared in the hyaluronate fractions (about 0.2 M LiCl), whereas much less eluted at about 0.6 M LiCl (chondroitin sulfate).

Tryptic Glycopeptides from Intact Neural Tube. The ep-

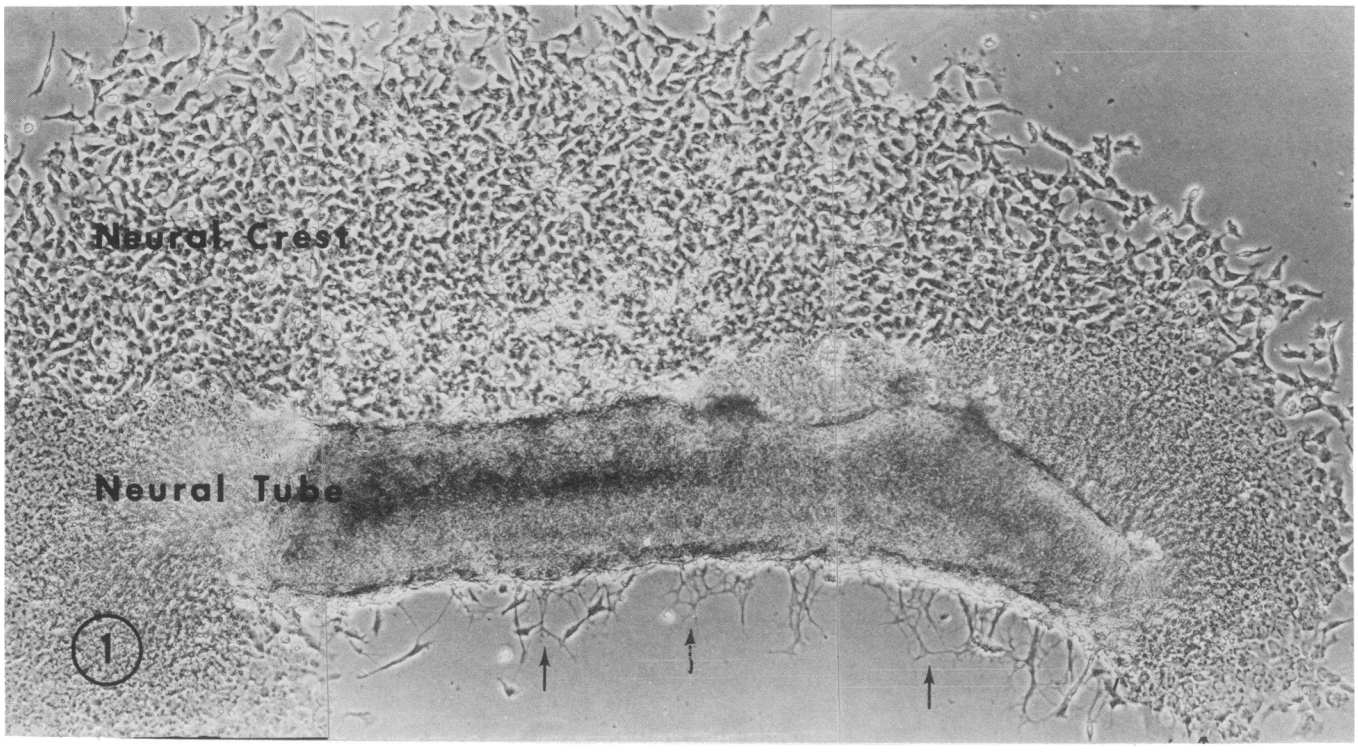

FIG. 1. A phase contrast composite photomicrograph of a living neural tube explanted to a collagen-coated petri plate for 22 hr. Neural crest cells have migrated as mesenchyme from the dorsal surface of the neural tube. The basal surface of the neural tube is marked by outgrowth of nerve cell processes (arrows). ×164.

ithelial neural tube, trypsinized after the emigration of the mesenchyme, also yields two principal labeled glycopeptides (Fig. 5) eluting at the same LiCl concentration as those obtained from the mesenchyme (Fig. 2). The ratio of counts (peak B/peak A) is 6.0.

Peak A from neural tube, although eluting from DE-52 at the same ionic strength as peak A from mesenchyme, is separable into three discrete fractions on P-4 (Fig. 6). Material in peak B is recovered from Bio-Gel P-4 as a principal peak containing 60% of the applied radioactivity, with the remainder eluting in later fractions (Fig. 6).

Papain Digest of Neural Tubes. Two major peaks are recovered from DE-52, each eluting at the same LiCl concentration as those recovered from mesenchyme. The minor peaks are present also (Fig. 7). The ratio of radioactivity (peak B/peak A) is 4.8.

Glycosaminoglycans are also present. Most of the radioactivity appears in the hyaluronate fractions but there is an ad-

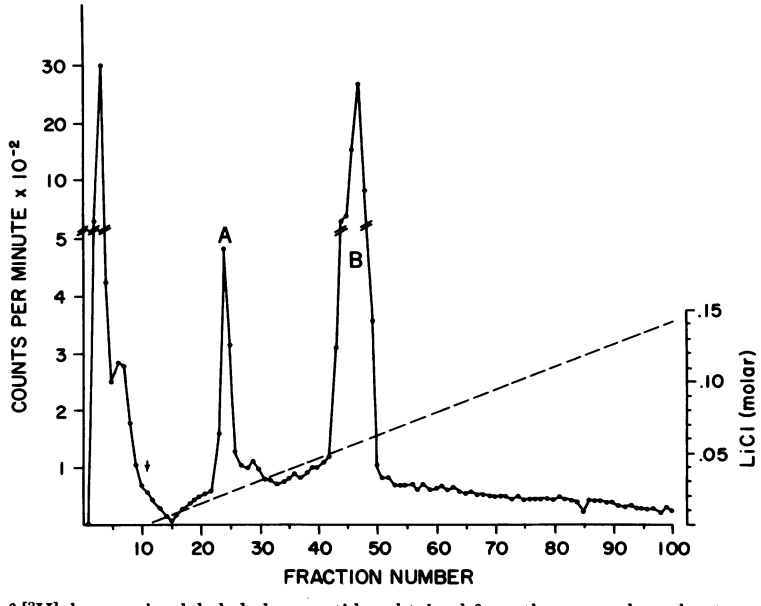

FIG. 2. DE-52 separation of [^{3}H]glucosamine-labeled glycopeptides obtained from the mesenchymal outgrowth after trypsinization. Material eluting in the buffer wash (fractions 1–11) represents unincorporated label as well as possible nonanionic macromolecules. The arrow marks the start of the gradient. The principal anionic glycopeptides elute in two major peaks. Peak A elutes at 0.02 M LiCl; B at 0.05–0.06 M LiCl. A smaller peak (fraction 29) is characteristically present and elutes at 0.03 M LiCl.

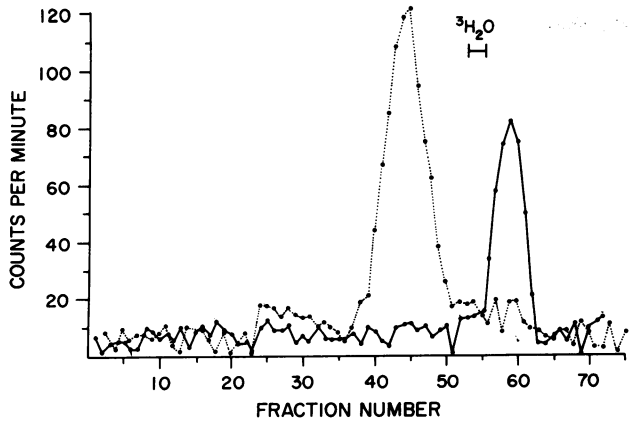

FIG. 3. Bio-Gel P-4 chromatography of peak A (● — ●) and peak B (○ ···· ○) shown in Fig. 2.

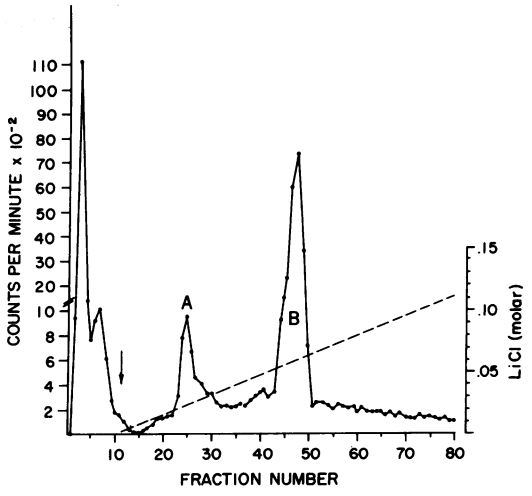

FIG. 5. DE-52 separation of [^{3}H]glucosamine-labeled tryptic surface glycopeptides obtained from intact neural tube. Peaks A and B have the same elution properties as those from the mesenchyme (see Fig. 2).

ditional amount of label eluting in the position characteristic of chondroitin sulfate (Fig. 7).

Tryptic Digest of Incubation Medium. Ion exchange chromatography shows the presence of a single principal glycopeptide peak (Fig. 8) eluting at a LiCl concentration (0.03 M) slightly greater than that required to elute peak A of mesenchyme and neural tube (0.02 M). Although small, this difference was always detected. Despite this close similarity between the medium glycopeptide and cell-associated peak A, further analysis on Bio-Gel P-4 (Fig. 9) indicates that they are not homologous.

Papain Digest of Medium. Total anionic molecules in the medium were examined after papain digestion. Most (76%) of the radioactivity associated with glycopeptides is recovered from DE-52 in a single peak eluting at 0.03 M LiCl (Fig. 10). A lesser amount of label is recovered in fractions 45–55. Label eluting at ionic strengths characteristic of hyaluronate and chondroitin sulfate is recoverable from the medium (Fig. 10).

DISCUSSION

The spectrum of newly synthesized glucosamine-labeled anionic surface glycopeptides appears remarkably simple. Two dominant classes, separated on the basis of charge, are present on both intact neural tube and its mesenchymal outgrowth, the neural crest. An earlier study, also using ion exchange chromatography, indicated similarly simple profiles of surface glycopeptides obtained from a variety of cell types (6). In the present study, further characterization of the labeled moieties enhances this impression of simplicity. The two labeled peaks obtained by ion exchange chromatography from neural tube (epithelial) were resolved further into four principal components on Bio-Gel P-4, whereas the two homologous peaks from neural crest (mesenchymatous) were resolved further into only two principal components. This apparent deletion of two glycopeptide fragments from the crest cell surface was the only qualitative difference detected in the present study. Digestion of the total labeled tissue by papain failed to recover significant additional material with different elution characteristics from those shown on DE-52. This indicates that the labeled moieties released by tryptic digestion did not merely represent selected digestion products of only a part of the surface components. The similarities between the papain and tryptic products also

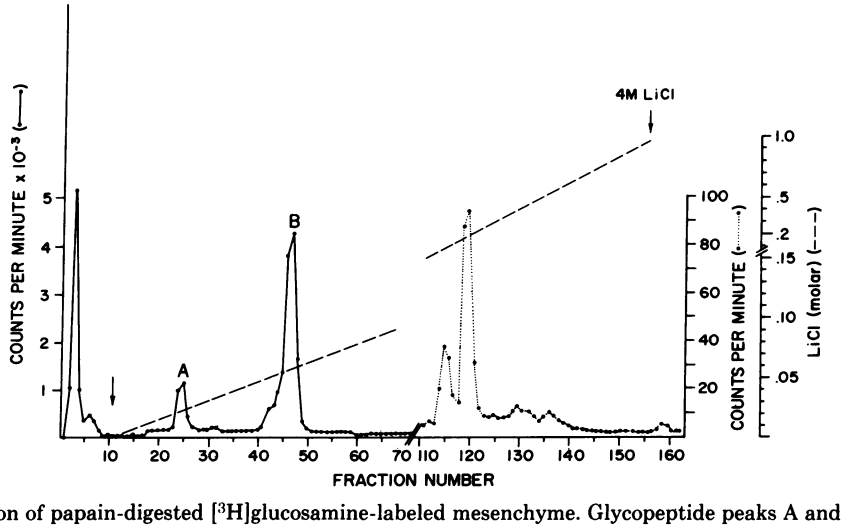

FIG. 4. DE-52 separation of papain-digested [^{3}H]glucosamine-labeled mesenchyme. Glycopeptide peaks A and B have the same elution characteristics as the tryptic glycopeptides shown in Fig. 2. Continued elution with a linear 0.15–1.0 M LiCl gradient elutes material at 0.18 M and 0.55 M LiCl, which corresponds to the positions of authentic hyaluronate and chondroitin sulfate, respectively. A final 4 M LiCl wash elutes a small amount of label, presumably in heparin.

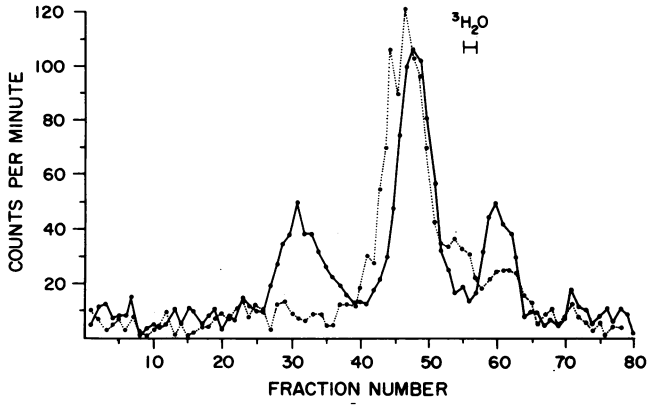

FIG. 6. Bio-Gel P-4 chromatography of peak A (● — ●) and peak B (○ · · · · ○) shown in Fig. 5. Neural tube peak A has a component similar to mesenchyme peak A (eluting around fraction 60) but has two additional peaks absent in mesenchyme peak B, with a major component around fractions 40–50 and two minor components eluting later.

suggests that these fragments represent principally carbohydrate.

The behavior of the labeled proteolysis products on Bio-Gel columns in this study is at variance with that reported earlier (7–12) for a variety of cell surface and extracellular glycopeptides. In these earlier studies surface glycopeptides were separable on Sephadex G-50 or Bio-Gel P-10 apparently according to size. Sephadex G-50 was found inadequate for the present study because labeled material recovered from DE-52 is recovered from Sephadex G-50 in fractions coinciding with V_i (unpublished observation). The reason for this disparity is unclear, but it may result from the fact that the glycopeptides are separated from glycosaminoglycans (with which they may interact) by DE-52, whereas in previous studies they were not. The present study does not determine the true size of the glycopeptides because their elution from Bio-Gel P-4 is affected by sorption phenomena, as indicated by the elution of some fractions after V_i (determined by ^3H_2O).

Neural crest cells represent a population of migrating cells of ectodermal origin that leave the neural epithelium as isolated cells and migrate to specific distant sites within the embryo (see ref. 13). The culture conditions used in the present study permit this cell type transformation to occur *in vitro*. The mesenchymal cells have been identified as neural crest cells (5), and the remaining neural tube retains its epithelial organization. This suggests that the cultures are good analogues of the normal

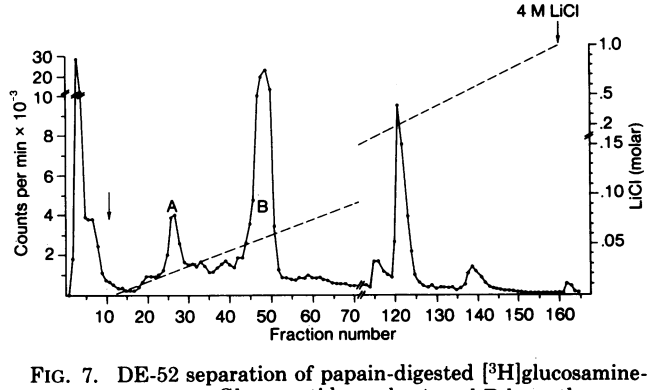

FIG. 7. DE-52 separation of papain-digested [^{3}H]glucosamine-labeled neural tube. Glycopeptide peaks A and B have the same elution characteristics as those in Figs. 2, 4, and 5. Continued elution results in recovery of hyaluronate and chondroitin sulfate, as in Fig. 4. A small amount of label is recovered with 4 M LiCl.

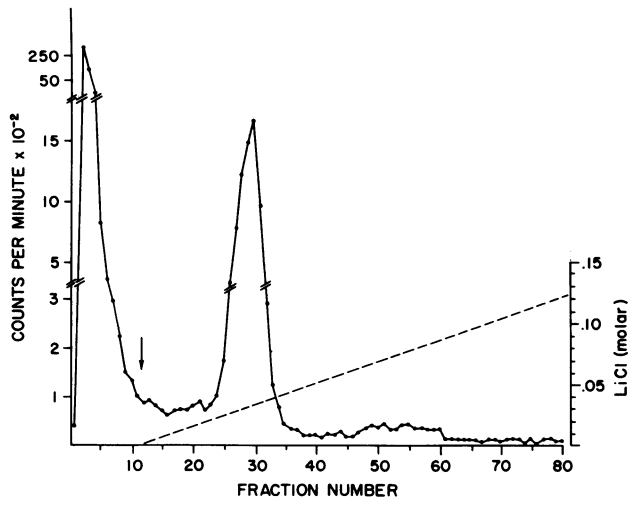

FIG. 8. DE-52 separation of tryptic digest of incubation medium. A single prominent peak is recovered at about 0.03 M LiCl, corresponding to the small shoulder seen after peak A in Figs. 2 and 4.

event *in vivo*. It may be assumed that the ontogenically normal event of crest cell emigration reflects a decrease in adhesive forces between both prospective crest and neural epithelium and between crest cells themselves. Such changes in surface properties may result from several factors. Parent glycoproteins may differ qualitatively as a result of the differences in glycopeptides detected in this study as well as by quantitatively different representations of individual glycopeptides. Independent sorting of relatively few (on the order of five) carbohydrate moieties could create a vast number of differences. At the present time, however, we must remain cautious about equating the glycopeptides synthesized by tissue *in vitro* to those synthesized *in situ*.

Glycosaminoglycans are synthesized at the same time as glycoproteins. Amounts of hyaluronate and chondroitin sulfate synthesized cannot be determined from the present study because neither precursor pool sizes (*N*-acetylglucosamine and *N*-acetylgalactosamine) or end-product specific activity are readily measurable because of the small amounts of end-products synthesized and the presumed nonsteady state of the system. However, relative *distribution* of each glycosaminoglycan can be ascertained, assuming uniform labeling of each species. Relatively little chondroitin sulfate is associated with the cells; the bulk of the labeled material appears in the medium. Hyaluronate is, on the other hand, associated with cells. In

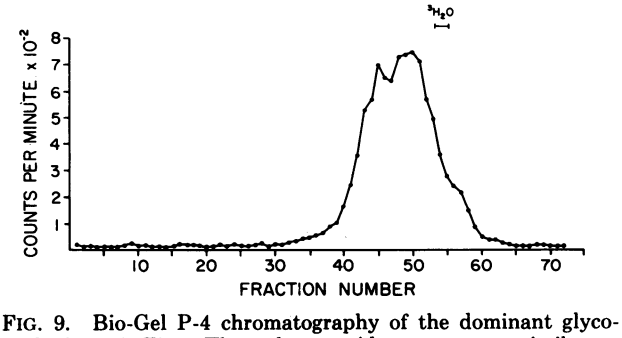

FIG. 9. Bio-Gel P-4 chromatography of the dominant glycopeptide shown in Fig. 8. These glycopeptides appear more similar, on P-4, to those of cell-surface peak B despite their charge similarity (as determined by ion exchange chromatography, Fig. 8) to peak A.

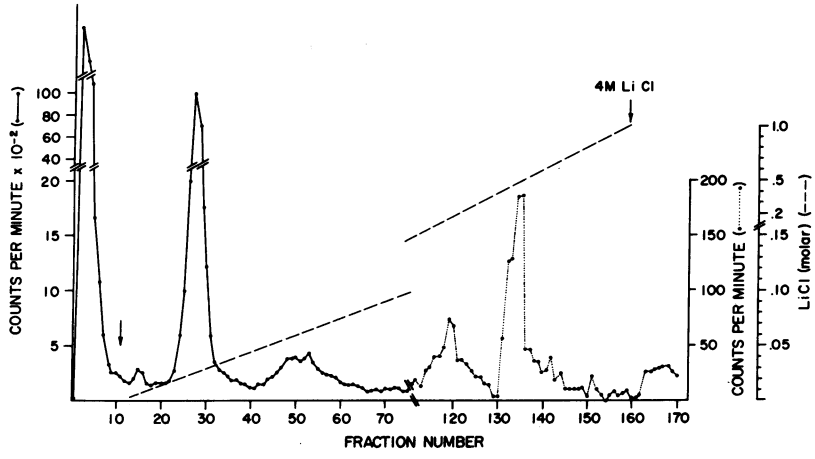

FIG. 10. DE-52 separation of papain-digested incubation medium. The dominant glycopeptide peak is similar to that in Fig. 8. The minor peak, fractions 48–60, corresponds to the small amount of material seen in Fig. 8. A larger aliquot was loaded onto this column than that used for Fig. 8. Both hyaluronate and chondroitin sulfate were recovered from the medium and again, a small amount of label was eluted by 4 M LiCl.

other embryonic systems embryonic glycoproteins interact with matrix glycosaminoglycans (14). The resulting complexes could be expected to have properties different from either the parent glycosaminoglycans or the parent glycoprotein.

The major labeled glycopeptide recovered from the medium is scarcely detectable on the cell surface, yet appreciable quantities are found in the medium. This suggests that it is turning over rapidly, or that the small amount recovered from the surface represents material in transit at the time of harvesting. Despite its charge similarity to cell-associated peak A, its behavior on Bio-Gel P-4 is similar to that of the peak B glycopeptides. These results suggest that not all cell surface components are "shed" into the medium.

If indeed the molecules detected in the present study are instrumental in changing adhesiveness, hence cell morphology, then it is tempting to predict that other ontogenically normal epithelial-mesenchymal transformations (such as sclerotome migration from the epithelial somite) will be accompanied by characteristic differences in glycopeptide synthesis similar to those found during neural crest formation. It is equally plausible that subtle changes in surface glycopeptides are also related causally to phenotypic expression ("differentiation") during development. One would then expect to find, within each differentiating cell line, an ontogeny of surface glycopeptides that reflects its degree of functional specialization.

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