

Putative Inhibitory Effects of Chrysotile, Crocidolite, and Amosite Mineral Fibers on the More Complex Surface Membrane Glycolipids and Glycoproteins

by Howard A. I. Newman,* Yeheskel A. Saat,* and Ronald W. Hart*

Syrian hamster embryo cells were treated with galactose oxidase, followed by reduction with tritiated sodium borohydride at pH 7.4. The labeling patterns of galactosyl and *N*-acetyl galactosaminyl residues on the cell surface were altered in comparing scraped vs. unscraped and buffer vs. media-soaked cells treated with galactose oxidase. From these preliminary studies, the procedure to be used in most of the asbestos treatment studies was to treat cells *in situ*, in buffer with galactose oxidase, and then to label treated scraped cells with NaB³H₄. After 20 hr interaction between chrysotile asbestos and Syrian hamster cell cultures, an alteration in surface labeling of glycolipids and glycoproteins was observed. Tritiated disialogangliosides (G_{2b}) and the higher molecular weight labeled glycoproteins were significantly reduced by asbestos treatment.

Similar chrysotile asbestos-treated cultures were grown in monolayers in MEM (Eagles) with 10% fetal bovine serum for 2, 24, 48, and 72 hr and then surface-labeled with galactose oxidase-. NaB³H₄ in phosphate buffer. Little or no difference was observed between surface-labeled lipid or protein distribution in untreated cells and those treated with asbestos for 2 hr. Asbestos-induced polar and neutral glycolipid pattern changes were observed at 24, 48, and 72 hr. Disialo- and trisialogangliosides (the more complex gangliosides) were decreased 85%, whereas globoside GL-4 was decreased by 60% at 72 hr. An overall decrease of labeled glycoproteins was observed at 24-48 hr. By 72 hr there was a complete loss of labeled protein bands with 80,000 dalton molecular mass. Since the changes in glycoproteins and glycolipids occur only after extended exposure of the cells to asbestos, the present studies support the concept that a metabolic rather than immediate masking effect is involved.

Comparisons of treatment of Syrian hamster embryo cells with various asbestos fibers for 48 hr in the order of decreasing reduction in complex gangliosides were crocidolite > chrysotile (intermediate) > amosite. Effects of the above fibers on high molecular weight glycoproteins labeling followed the same order. The labeling pattern is reminiscent of the increased simplification of glycolipids and glycoproteins found in transformed cells. In the case of asbestos which appears to have no independent mutagenic capability, it is more likely that the membrane changes induced by asbestos serve to allow other mutagens to pass into the cell so as to act on the nuclear structure.

Introduction

Glycolipids simplification and high molecular mass glycoprotein loss have been correlated with cell transformation in culture (1-8) and in tumorigenesis (8) with viruses and chemical carcinogens. Only one recent observation has been made about

glycolipid and glycoprotein simplification with "physical" carcinogens such as asbestos (9). The present study involves making such a set of observations *in vitro* with Syrian embryo hamster cells, in culture, incubated with a number of different asbestos particulates. The initial observation to be made specifically involves the determination of proportional distribution of components on the cell surface through the use of a galactose oxidase-NaB³H₄ labeling technique with chrysotile asbestos at vari-

*Departments of Pathology and Radiology, College of Medicine, The Ohio State University, Columbus, Ohio 43210.

ous time intervals in an attempt to ascertain the mechanism of the modified labeling distribution among gangliosides and glycoproteins after chrysolite interaction and to establish an optimal time period for the asbestos effects on Syrian hamster cells.

Since the initial interaction of asbestos may be with the carbohydrate moieties in the glycocalyx of the plasma membrane, we examined the glycolipids and glycoproteins looking for changes that could occur on the cell surface as a result of exposure to asbestos fibers.

Material and Methods

Surface Labeling of Cultured Cells *in Situ* in the Presence of Culture Media or Phosphate-Buffered Saline

Syrian Hamster embryo cells at passage 2 were cultured on 14 glass Petri dishes in modified Eagles' media (MEM) containing 10% fetal bovine serum in a 5% CO₂ atmosphere. Prior to confluency, cell morphology was observed under phase contrast microscopy, and only those cultures which had few granules and were mostly of fibroblastic configuration were accepted for further study. The cell preparations, matched with control cultures, were then incubated for 20 hr with asbestos fiber (10 µg/ml) by adding the asbestos and then allowing the fibers to settle onto the exposed cell surfaces.

For labeling in the presence of phosphate-buffered saline (PBS), the medium was decanted, cells washed three times with PBS, and the incubation volume adjusted to 5 ml with the same buffer. Galactose oxidase (Sigma Biochemical Type III (125 units/mg) or Grand Island Biological Co. (100 units/mg) (200-250 µg) and 2 mM phenylmethyl sulfonyl fluoride (Sigma) were added to give a final concentration of 25 units per dish, and the plates were incubated in Precision Scientific Co. P⁵ Model 2 under CO₂-O₂ atmosphere for 3 hr at 37°C. After incubation, the dish was washed twice with PBS and the excess incubation mixture was aspirated. Tritiated sodium borohydride, 1 mCi of NaB³H₄, with specific activity 9Ci/mole (New England Nuclear; stored in 0.01N NaOH solution at -40°C) was added and allowed to stand with occasional shaking for 30 min at 37°C. Finally, the cells were detached from the plates with a policeman with wide rubber blade. The reaction mixtures obtained from the various plates were washed five times with PBS (pH 7.4) and centrifuged at 300g for 10 min each time. The pelleted cells were suspended in 200 µl PBS, and aliquots were taken for the glycolipids and glycoproteins determinations.

Surface Labeling of Scraped Syrian Hamster Cells

The growth media was decanted and the cells washed twice with PBS, then scraped with a wide rubber blade attached to a policeman and finally centrifuged at 1500 rpm for 10 min. The cell pellet was suspended in 0.5 ml of PBS with or without 2mM phenylmethylsulfonylfluoride (Sigma), a protease inhibitor. Galactose oxidase (10 units) dissolved in 100 µl PBS was then added, and the cells were washed twice with PBS (pH 7.4) and resuspended in 0.5 ml of the same buffer. NaB³H₄ (0.5 mCi) was added, and the reaction mixture allowed to stand at room temperature for 30 min with occasional gentle shaking. The cells were washed five times with PBS and suspended in 200 µl of PBS.

SDS-Polyacrylamide Gel Electrophoresis

Electrophoresis was performed according to the method of Weber and Osborn (10). Gel buffer contained 7.8 g KH₂PO₄·H₂O·38.6 g K₂KPO₄·7H₂O, and 2 g of SDS per liter. For electrophoresis with 10% acrylamide solution, 22.2 g of acrylamide and 0.6 g of methylene bisacrylamide were dissolved in water to give 100 ml solution which was kept at 4°C in dark bottles.

The glass gel tubes were 10 cm long with an inner diameter of 6 mm. These tubes were soaked in cleaning solution, rinsed, and dried before use. For a typical run of 12 gels, 12 ml of gel buffer were deaerated under N₂ and mixed with 10.8 ml of acrylamide solution; 1.2 ml freshly prepared ammonium persulfate solution (15 mg/ml) and 0.036 ml of *N,N,N',N'*-tetramethylethylenediamine (TEMED) were added. After mixing each tube was filled to 7 cm with the solution. Before the gel hardened, a few drops of water were layered on top of the gel solution. After about 10 min an interface could be seen, indicating that the gel had solidified.

The cells (10⁶) were digested in PBS (100 µl) containing 1% SDS and 5% β-mercaptoethanol and heated in a water bath at 85°C for 10 min. Electrophoresis was performed after adding to the digested cells 3 µl of tracking dye (0.05% Bromphenol Blue) and 1-2 drops of glycerol. β-Galactosidase, urease, albumin, peroxidase, and lysozyme with molecular weights, 130,000, 83,000, 68,000, 44,050, and 13,930, respectively, were run as standards in separate gel tubes. The gels which contained the standard proteins were run together with the cell glycoproteins in the same electrophoresis apparatus. The two compartments of the electrophoresis cell were filled with gel buffer diluted 1:1 with water. Electrophoresis was performed at a constant current of 8mA per gel, and was stopped when the marker

dye migrated to about 1 cm from the lower end of the gel.

After electrophoresis, the gels were removed from the tubes, and those which contained the protein standards were stained with Commassie Blue overnight and then destained with glacial acetic acid, ethanol, water (10:25:65 by volume). The gels which contained the digested cells were sliced with a razor blade gel slicer, to produce slices 1 mm in width, and the protein in the slices was extracted for 48 hr in toluene scintillation fluid containing PPO, POPOP, and Triton X100 (Thrift, Kew Scientific, Inc.). The radioactivity was measured by liquid scintillation spectrometry.

Extraction of Syrian Hamster Cell Glycolipids

An aliquot (100 μ l) of surface-labeled cells (10^6) was soaked with 3 ml of chloroform:methanol overnight at room temperature to give a final ratio of 30 volumes of chloroform:methanol (2:1, v/v) to 1 volume of cells. It was then centrifuged at 1500 rpm for 10 min, and the precipitate was washed twice with chloroform:methanol (2:2, v/v).

Isolation of Glycolipids

Following the extraction of lipid from the cells, polar glycolipids (gangliosides) were separated from neutral glycolipids by the procedure of Folch et al. (11). The individual glycolipids were separated by a modification of the method of Laine et al. (12). After Folch partition, the total upper layer was reduced in volume to 1-2 ml and dialyzed at 4°C against distilled water for 24 hr. The dialyzed upper phase was evaporated to dryness under N_2 , and the residue extracted with a small volume of chloroform:methanol (2:1, v/v). This fraction represents a major part of the gangliosides and contains some neutral glycolipids in minor quantities. The analysis of gangliosides was performed by thin-layer chromatography (TLC) (Kontes/quantum, precoated TLC plates) and developed in a solvent system (tetrahydrofuran:0.5% aqueous KCl, 7:1, v/v). Standards of gangliosides were scraped with a razor blade and counted for radioactivity.

The lower phase which contains the neutral glycolipids, sulfatides, neutral lipid, and phospholipid was evaporated to dryness. The residue was dissolved in absolute pyridine:acetic anhydride, (2:1, v/v) and left overnight at room temperature. The reaction mixture was then evaporated to dryness and the residue dissolved in hexane:dichloroethane (DCE), (2:1, v/v) and finally applied to a column of Florisil 50-100 mesh. The column was eluted with 5 bed volumes of each of (a) hexane:DCE

(1:4, v/v), (b) DCE:acetone (1:1, v/v), and (c) DCE:methanol:water (2:8:1, v/v). The acetylated glycolipid was eluted with the DCE-methanol-water solvent system. The fraction so eluted was evaporated to dryness and the residue dissolved in chloroform:methanol (2:1, v/v), containing 0.5% sodium methoxide in methanol (1/5 volume of the chloroform:methanol). The reaction mixture was allowed to stand for 30 min at room temperature and then neutralized with acetic acid in methanol. All the glycolipids were deacetylated and evaporated to dryness. The residue was dissolved in chloroform:methanol (2:1, v/v), and an aliquot was applied to TLC under the same conditions previously described (except that the solvent system for development was tetrahydrofuran:0.5% aqueous KCl, 20:1, v/v and then 7:1, v/v to 25% of the first solvent front).

For all procedures a Student *t* test was performed on data from true replicates.

Results

Baselines for Cell Surface Glycolipids and Glycoproteins

A baseline for surface membrane labeling patterns of polar and neutral glycolipids in Syrian hamster embryo cells was established (Table 1). These labeling patterns represent the available galactose-containing glycolipids on the plasma membrane surface. The cells were labeled as previously described under these conditions: (a) cells incubated with media containing fetal bovine serum, (b) cells incubated with media without fetal bovine serum, and (c) cells incubated with buffer. No significant differences in surface labeling distribution of polar and neutral glycolipids were observed when the labeling pattern of cells incubated with buffer was compared to that of cells incubated with total media, except in the case of trisialoganglioside (G_{T1}), where an increase of 1.8 fold was observed under the same conditions (Table 1).

As a percent of the control (unscraped cells in buffer) scraped cells ganglioside G_{M1} was 177; G_{M2} , 138; G_{D1a} , 27.5; and G_{T1} , 49.8. Globoside, a neutral glycolipid, also had reduced labeling. Therefore, profound differences were found between scraped and nonscraped glycolipid labeling patterns.

The differences observed in labeling patterns of glycoproteins in the presence of media as compared to buffer led us to examine the possibility of glycolipids exchange between cell surface and media gangliosides. However, no marked differences were observed in surface labeling patterns of cells incubated with total media or with media without fetal bovine serum (Table 1).

Table 1. Relative percentage distribution of surface-labeled polar and neutral glycolipids of Syrian hamster embryonic cells treated with chrysotile asbestos.

	Labeling, % ^a				
	Media + FBS ^b	Media ^c	Buffer ^b	Chrysotile asbestos	
				Buffer ^c	Media + PBS ^c
Polar glycolipids:					
Monosialoganglioside (G _{M2})	27.1 ± 1.70	28.4 ± 1.84	15.7 ± 1.59	15.9 ± 0.42	12.8 ± 0.91
Monosialoganglioside (G _{M1})	28.2 ± 3.52	28.0 ± 2.19	37.0 ± 2.18	6.4 ± 1.27	62.2 ± 0.35
Disialoganglioside (G _{D1a})	23.0 ± 3.54	19.6 ± 0.56	16.7 ± 1.85	4.4 ± 0.14 ^d	4.0 ± 0.14 ^e
Trisialoganglioside (G _{T1})	17.6 ± 0.72	24.0 ± 0.84	33.3 ± 3.04 ^f	15.0 ± 0.70	21.1 ± 1.13
Neutral glycolipids:					
"Glucocerebroside"	48.3 ± 16.7	43.7 ± 5.09	43.2 ± 3.12	25.8 ± 0.84 ^d	24.6 ± 0.72
Cerebroside	15.0 ± 1.97	17.0 ± 4.03	22.8 ± 0.80	19.0 ± 0.14	20.6 ± 0.38
Globoside GL-4	36.7 ± 15.0	36.4 ± 1.06	36.0 ± 1.08	55.7 ± 0.28 ^d	55.4 ± 1.13

^aBased on TLC comparison with known glycolipid standards.

^bMean ± standard deviation, *n* = 3.

^cMean ± standard deviation, *n* = 2.

^dStatistically significant, *p* < 0.05 (buffer vs. buffer + asbestos).

^eStatistically significant, *p* < 0.05 (media + FBS vs. media + FBS + asbestos).

^fStatistically significant, *p* < 0.05 (media + FBS vs. buffer).

Surface Glycolipids of Cells Treated with Chrysotile Asbestos

Asbestos showed no preferential binding of specific protein components of fetal bovine serum when known amounts of asbestos were incubated with various quantities of FBS for 30 min and then centrifuged at 1500 rpm for 20 min. The supernatant was submitted to agarose electrophoresis and stained for both protein and lipoprotein. Glycolipids from buffer-incubated cells after 20 hr asbestos treatment (Table 1) showed a significant decrease in both di-

sialoganglioside (G_{D1a}) and "glucocerebroside" and an increase in globoside GL-4. On the other hand, a decrease in G_{D1a} only was observed in media-incubated cells previously treated with asbestos. However, no marked differences were observed in surface-labeling patterns of cells incubated with either buffer or media after treatment with asbestos (Table 1).

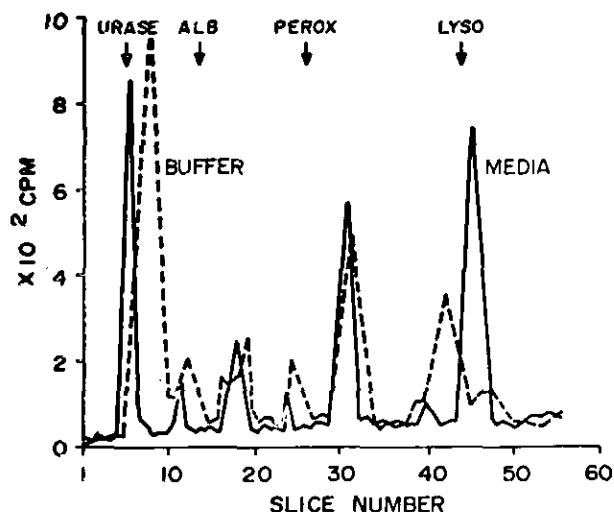


FIGURE 1. Comparisons of ³H-labeling patterns of electrophoretically separated glycoproteins from (- -) buffer-treated and (—) media-treated SHE cells.

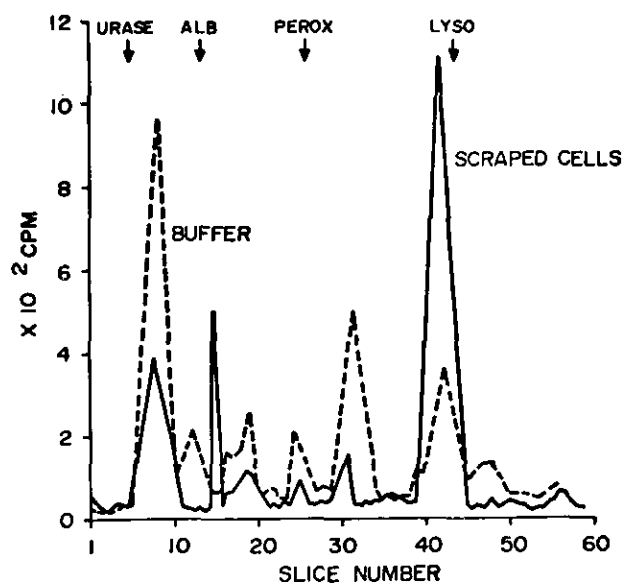


FIGURE 2. Comparisons of ³H-labeling patterns of electrophoretically separated glycoproteins from (- -) buffer-treated and (—) scraped SHE cells.

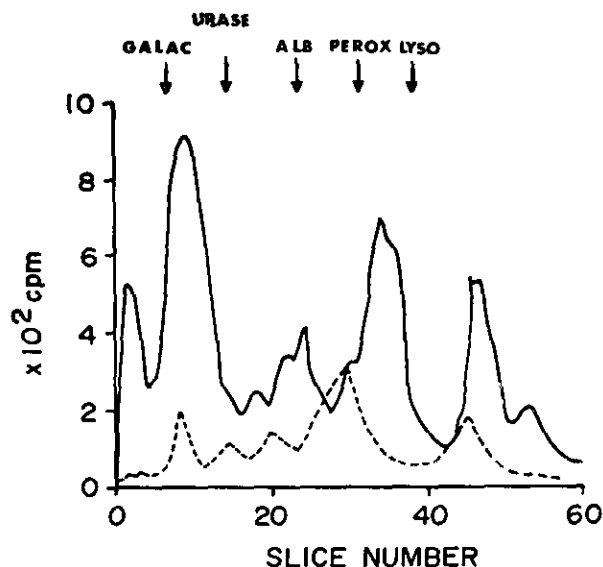


FIGURE 3. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from (---) mixed chrysotile-treated (2 hr) and (—) media-treated SHE cells.

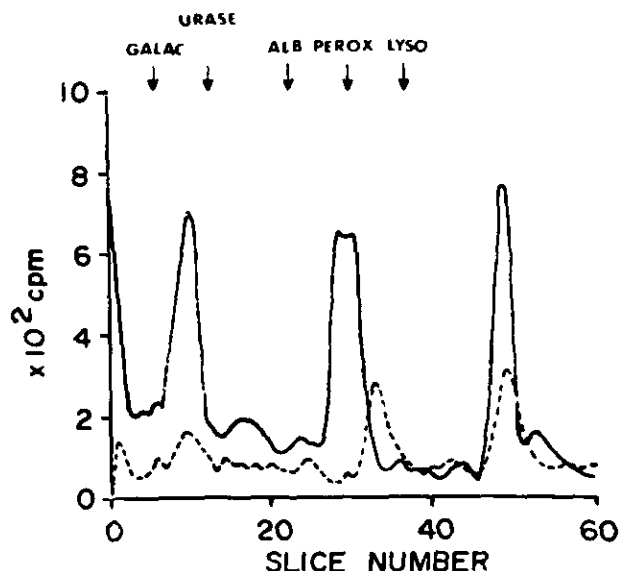


FIGURE 4. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from (---) mixed chrysotile-treated (2 hr) and (—) media-treated SHE cells.

SDS Electrophoresis

The electrophoretic patterns of labeled glycoproteins obtained with after surface labeling in buffer and media (Fig. 1) showed to some extent a similar pattern with a higher labeling of protein molecular weight (83,000) in cells incubated with buffer than those incubated with media. In contrast, there was a lower labeling of protein with molecular weight 14,000 in cells incubated with buffer than those incubated with media. Scraped cell glycoprotein patterns

reflected the changes in glycolipid patterns with a lesser proportion of label in high molecular species with a concomitant increase in the labeling of the lowest molecular mass species (Fig. 2). The electrophoretic patterns of cells treated with asbestos and labeled in buffer or media on gel with half the amount of the crosslinker showed a decreased labeling in all protein bands. The asbestos also modified the protein pattern so that high molecular weight components were not significantly labeled in asbestos-treated cells (Figs. 3 and 4).

Table 2. Relative distribution of surface-labeled polar and neutral glycolipids of syrian hamster embryonic cells treated with chrysotile asbestos.

Compounds	Labeling, % ^{a,b}				
	Untreated cells	Cells treated with chrysotile asbestos			
		2 hr	24 hr	48 hr	72 hr
Polar glycolipids					
Monosialoganglioside (G_{M1})	24.9 ± 2.33	25.2 ± 2.05	26.0 ± 2.47	37.5 ± 1.62	48.1 ± 2.05
Monosialoganglioside (G_{M2})	30.3 ± 3.74	29.8 ± 1.76	48.1 ± 2.89	51.2 ± 0.91	43.1 ± 1.20
Disialoganglioside (G_{D1a})	20.7 ± 1.97	20.2 ± 2.96	6.96 ± 1.89	3.92 ± 1.12	4.43 ± 1.68
Trisialoganglioside (G_{T1})	24.0 ± 3.39	24.7 ± 3.25	18.8 ± 1.48	7.30 ± 1.86	4.29 ± 0.86
Neutral glycolipids:					
"Glucocerebroside"	32.8 ± 1.69	30.5 ± 1.20	21.2 ± 1.76	26.4 ± 0.56	29.6 ± 1.06
Cerebroside	31.1 ± 1.56	36.0 ± 1.20	35.4 ± 4.24	42.4 ± 1.69	56.5 ± 1.62
Globoside GL-4	35.9 ± 0.42	33.4 ± 2.40	43.3 ± 2.47	31.2 ± 2.26	13.8 ± 0.56

^aBased on TLC comparison with known glycolipid standards.

^bMean ± standard deviation, $n = 2$.

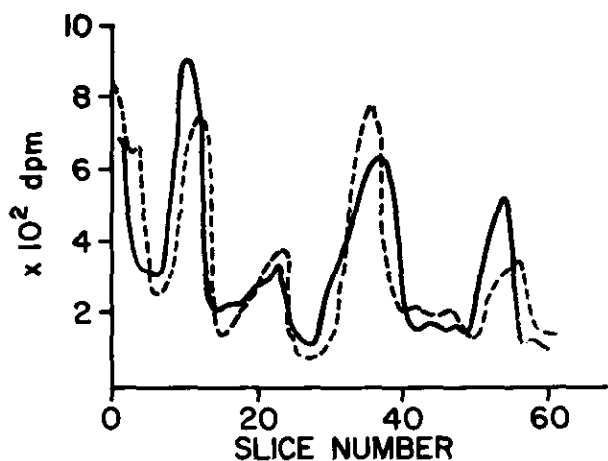


FIGURE 5. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from (---) mixed chrysotile-treated and (—) buffer-treated SHE cells.

Surface Glycolipids and Glycoproteins of Cells Treated with Asbestos

Syrian hamster embryo cell cultures were incubated with $10\ \mu\text{g}$ asbestos/ml media for 2, 24, 48, and 72 hr and then surface-labeled with galactose oxidase- NaB^3H_4 . There was little or no change in glycolipid or glycoprotein labeling patterns at 2 hr (Table 2 and Fig. 5), but chrysotile asbestos-induced polar and neutral glycolipid pattern changes were observed at 24, 48, and 72 hr. Disialo- and trisialo-gangliosides (more complex gangliosides) were decreased by 85%, whereas globoside GL-4 was decreased by 60% at 72 hr (Table 2). An overall decrease in labeled glycoproteins was observed at 24-48 hr (Fig. 6). Electrophoretic separation of

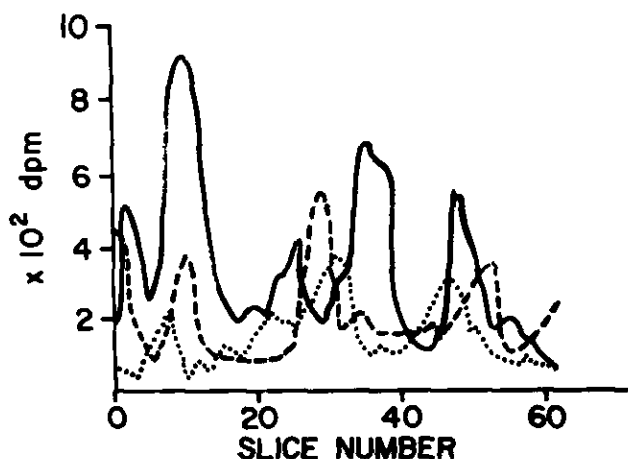


FIGURE 6. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from mixed chrysotile-treated (· · ·) 24 hr, (---) 48 hr and (—) buffer-treated SHE cells.

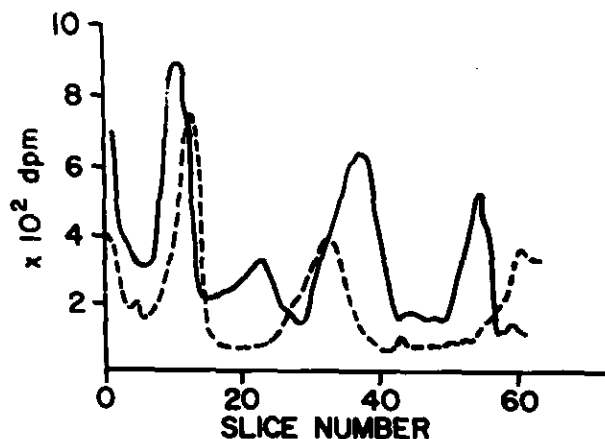


FIGURE 7. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from (---) mixed chrysotile-treated (72 hr) and (—) buffer-treated SHE cells.

glycoproteins showed that by 72 hr there was a complete loss of labeled protein bands with molecular mass 80,000 daltons. (Fig. 7). Higher molecular masses (120,000-150,000 daltons) of labeled glycoprotein species were decreased at 24 and 48 hr and appeared to increase at 72 hr. The incubation of amosite, crocidolite, and intermediate-sized chrysotile asbestos fibers ($10\ \mu\text{g}/\text{ml}$) with Syrian hamster embryo cells for 48 hr (a time at which near maximum effects for mixed chrysotile fiber were observed) had the following effects on surface galactose and galactosamine containing substances. The complex ganglioside proportionate decreases as percentages of the control (untreated cells) were in the order of decreasing effect for crocidolite (Table 3): G_{M1} , 190; G_{M2} , 112; G_{D1a} , 21.9;

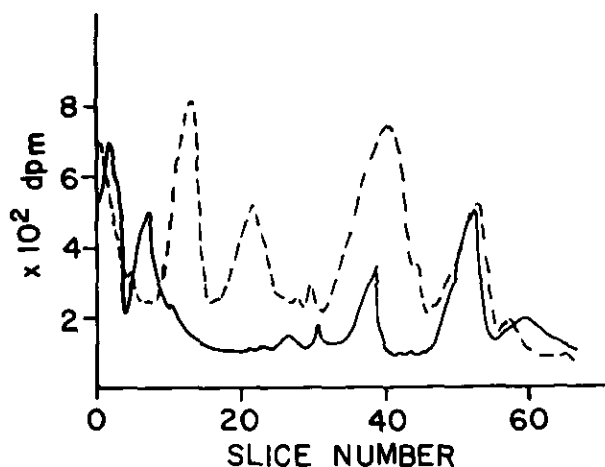


FIGURE 8. Comparisons of ^3H -labeling patterns of electrophoretically separated glycoproteins from (---) crocidolite-treated (48 hr) and (—) buffer-treated SHE cells.

Table 3. Relative distribution of surface-labeled glycolipids of Syrian hamster embryonic cells treated with different asbestos fibers for 48 hr.

Compounds	Labeling, % ^{a,b}			
	Untreated cells (control)	Cells treated with asbestos		
		Amosite	Chrysotile (intermediate)	Crocidolite
Polar glycolipids:				
Monosialoganglioside (G _{M1})	27.2 ± 2.35	34.4 ± 6.50	38.9 ± 4.21 ^c	51.8 ± 4.62 ^c
Monosialoganglioside (G _{M2})	36.0 ± 3.60	35.8 ± 1.93	48.5 ± 5.90 ^c	40.2 ± 3.37 ^c
Disialoganglioside (G _{D1a})	19.4 ± 2.95	12.7 ± 1.55 ^c	4.69 ± 1.01 ^c	4.25 ± 1.26 ^c
Trisialoganglioside (G _{T1})	17.4 ± 3.33	17.1 ± 3.21	7.97 ± 1.30 ^c	3.82 ± 0.83 ^c
Neutral glycolipids:				
"Glucocerebroside"	28.3 ± 2.41	25.9 ± 2.96	25.1 ± 1.41	21.9 ± 2.12 ^c
Cerebroside	31.2 ± 1.66	46.3 ± 3.66 ^c	48.5 ± 4.27 ^c	58.6 ± 0.60 ^c
Globoside GL-4	39.9 ± 2.40	27.8 ± 3.60 ^c	26.4 ± 3.74 ^c	19.5 ± 1.82 ^c

^aBased on TLC comparison with known glycolipid standards.

^bMean ± standard deviation, *n* = 3.

^cStatistically significant *p* < 0.05 (amosite vs. control).

^dStatistically significant *p* < 0.05 (chrysotile intermediate vs. control)

G_{T1}, 22.0 percent compared to chrysotile (intermediate) G_{M1}, 143; G_{M2}, 135; G_{D1a}, 24.2; G_{T1}, 45.8% and to amosite G_{M1}, 126; G_{M2}, 99.4; G_{D1a}, 65.5; G_{T1}, 98.3%. This order of graded response to asbestos fibers was also found in GL-4 reductions in the neutral glycolipids.

Decreases in the proportion of high molecular weight glycoproteins related to asbestos treatment were in the same order as glycolipid, crocidolite > chrysotile (intermediate) > amosite (Fig. 8-10). The intermediate molecular mass labeled glycoproteins (80,000 daltons) were absent from the crocidolite-treated cell culture surfaces.

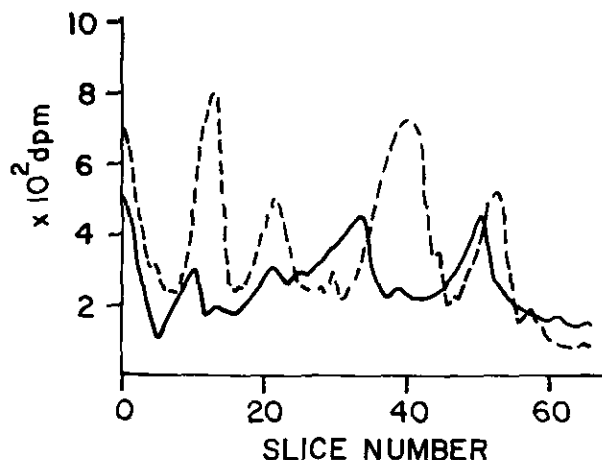


FIGURE 9. Comparisons of ³H-labeling patterns of electrophoretically separated glycoproteins from (- -) intermediate chrysotile-treated (48 hr) and (—) buffer-treated SHE cells.

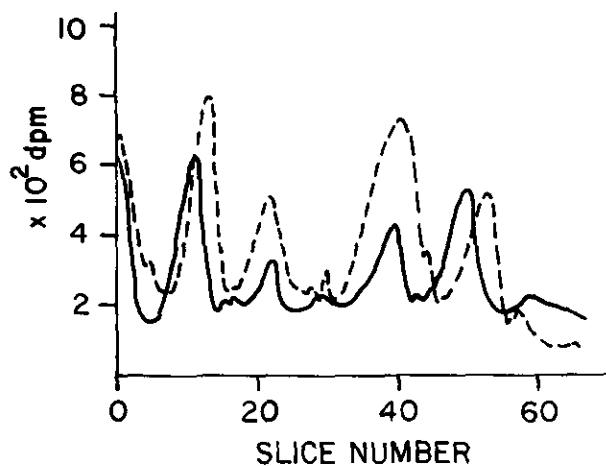


FIGURE 10. Comparisons of ³H-labeling patterns of electrophoretically separated glycoproteins from (- -) amosite-treated (48 hr) and (—) buffer-treated SHE cells.

Discussion

The simplification of the molecular structure of glycolipids and glycoproteins appears to be a manifestation of metabolic changes within cells brought about by interaction of asbestos with intracellular elements rather than through direct masking or removal of cell surface components since the removal or masking of surface components would be an immediately manifest reaction and would certainly be consummated in a 2-hr time span, a period of time in which no significant alteration in glycolipid or glycoprotein pattern could be discerned. That these

changes are brought about by alterations in the metabolism of the Golgi and/or the lysosomes requires additional study. Evidence by Harington et al. (13) with macrophages points to enhanced lysosomal enzyme release with the consequent modification of cell membranes through the production of lysophosphatides at the expense of diacyl phospholipids which are predominantly situated in cellular membranes. In addition, peritoneal macrophages prepared by intraperitoneal injection of asbestos 4-5 days prior to cell harvesting caused marked increases in cell surface areas as determined by scanning electron microscopy and results in greatly increased cell receptors for IgG and the third component of complement (14, 15). If the macrophage receptors are ganglioside in nature, surface labeling of these cells should show a modification of surface distribution due to asbestos treatment.

The differential effect of amphibolic double chained serpentine (crocidolite-chrysotile) and single-chained amosite may not be fortuitous but instead may relate to the molecular configuration of these particles vis à vis their interaction with sub-cellular elements.

Our treatment of embryonic Syrian hamster cells with asbestos shifted the percentage labeling from G_{D1a} to G_{M2} and increased the label in GL-4. Overall, however, the labeling of gangliosides and, therefore, possibly the amount of exposed ganglioside was reduced with asbestos treatment. This effect may be related to either masking of the labeling sites or a reduction in the amount of ganglioside present on the cell surface. In contrast, when lectins bind to the cell surface (5) or when cells become transformed (16), there is an increased labeling observed. The changed distribution of surface gangliosides after asbestos treatment is consistent with such distributions after cell transformation, but since in the presence of asbestos the surface labeling is reduced, this finding is unlikely to be a reflection of early changes en route to transformation. It is more likely to be related to asbestos interactions with membrane coenzymes such as retinol phosphate which may be responsible for surface glycosylation of gangliosides and ganglioside precursors (17, 18) and for glycoproteins.

After asbestos treatment a considerable shift in distribution of label toward lower molecular weight cell surface glycoproteins was recorded, but there was also an overall reduction of labeling. This shift in distribution is similar to that observed in transformed cells. A glycoprotein, galactoprotein a (possibly the same as LETS), with a molecular weight of 200,000 daltons was found to be deleted in highly tumorigenic NIL py cells (19). Lectin treatment also suppressed labeling of surface galactoprotein a in

normal cells (6), but had little effect on lower molecular weight surface galactoprotein. This could be a step in the direction of transformation by these cells or more likely a direct inhibition of glycoprotein glycosylation at the membrane surface through the removal of retinol phosphate (17).

Electron microscopy of fibroblast cells after asbestos treatment demonstrated only a few fibers present at cell membranes (20). This finding is consistent with our preliminary ganglioside binding studies on asbestos. Our finding showed that a mixture of ^{14}C -gangliosides, which could in part represent the cell surface conditions, bind loosely to asbestos; it is possible, therefore, the asbestos initially coats the cells, but, on washing, some of the asbestos is removed.

Since asbestos induced early changes in the plasma membrane as evidenced in transformed cells and since asbestos has not been demonstrated to be a mutagen (21), the role of asbestos in the carcinogenic process may be to modify the cell surface to give carcinogens and viruses greater access to the cell nucleus, thus, to promote cell transformation.

REFERENCES

1. Mora, P. T., Brady, R. O., Bradley, R. M., and MacFarland, V. W. Gangliosides in DNA virus-transformed tumorigenic mouse cell line. *Proc. Natl. Acad. Sci. (U.S.)* 62: 1290.
2. Yogeeswaran, G., Sheinen, R., Wherrett, J. R., and Murray, R. K. Studies on the glycosphingolipids of normal and virally transformed 3T3 mouse fibroblasts. *J. Biol. Chem.* 247: 5146 (1972).
3. Robbins, P. W., and MacPherson, I. A. Glycolipid synthesis in normal and transformed animal cells. *Proc. Roy. Soc. (London)* B177:49 (1971).
4. Sakiyama, H., Gross, S. K., and Robbins, P. W. Glycolipid synthesis in normal and virus-transformed hamster cell lines. *Proc. Natl. Acad. Sci. (U.S.)* 69: 872 (1972).
5. Hakomori, S. Cell density-dependent changes of glycolipid concentrations in fibroblasts, and loss of this response in virus-transformed cells. *Proc. Natl. Acad. Sci. (U.S.)* 67: 1741 (1970).
6. Hakomori S.-I. Structures and organization of cell-surface glycolipids depending on cell-growth and malignant transformation. *Biochim. Biophys. Acta* 417: 55 (1975).
7. Brady, R. O., Borek, C., and Bradley, R. M. Composition and synthesis of gangliosides in rat hepatocyte and hepatoma cell lines. *J. Biol. Chem.* 244: 6552 (1969).
8. Cheema, P., Yogeeswaran, G., Morris, H. D., and Murray, R. Ganglioside patterns of three Morris minimal deviation hepatomas. *FEBS Letters*, 11: 181 (1970).
9. Saat, Y. A., Newman, H. A. I., Hart, R. W., and Allison, D. K. The effects of asbestos on plasma membrane: Surface glycolipids and glycoproteins of Syrian hamster embryo cells. *J. Environ. Pathol. Toxicol.*, in press.
10. Weber, K., and Osborn, M. The reliability of molecular weight determinations by dodecylsulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244: 4406 (1969).
11. Folch, J., Lees, M., and Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* 226: 497 (1957).
12. Laine, R. A., Stellner, K., and Hakomori, S.-I. Isolation and

- characterization of membrane glycosphingolipids. In: *Methods of Membrane Biology*, E. Korn, Ed., Plenum Press, New York, 1974, pp. 205-244.
13. Harington, J. S., Allison, A. C., and Badami, D. V. Mineral fibers, chemical, physicochemical and biological properties. *Adv. Pharmacol. Chemotherap.* 12: 291 (1975).
 14. Miller, K., and Kagen, E. *In vivo* effects of asbestos on macrophages-scanning electron microscope study. *J. Retic. Soc.* 20: 159 (1976).
 15. Miller, K., and Kagen, E. Membrane studies on macrophages following mineral dust exposure. *S. Afr. Med. J.* 50: 1009 (1976).
 16. Gahmberg, C. G., and Hakomori, S. Surface carbohydrates of hamster fibroblasts. 2. Interaction of NIL cell surfaces with Ricinus-communis lectin and concanavalin A as revealed by surface galactosyl label. *J. Biol. Chem.* 250: 2447 (1975).
 17. Yogeewaran, G., Laine, R. A., and Hakomori, S. Mechanism of cell contact-dependent glycolipid synthesis. Further studies with glycolipid-glass complex. *Biochem. Biophys. Res. Commun.* 59: 591 (1974).
 18. Laine, R. A., Yogeewaran, G., and Hakomori, S. Glycosphingolipids covalently linked to agarose gel or glass beads. Use of compounds for purification of antibodies directed against globoside and hematoside. *J. Biol. Chem.* 249: 4460 (1974).
 19. Gahmberg, C. G., and Hakomori, S. Altered growth-behavior of malignant cells associated with changes in externally labeled glycoprotein and glycolipid. *Proc. Natl. Acad. Sci. (U.S.)* 70: 3329 (1973).
 20. Blakeslee, J., Jr., personal communication, 1979.
 21. Chamberlain, M., and Tarmy, E. M. Asbestos and glass fibres in bacterial mutation tests. *Mutat. Res.* 43: 159 (1977).