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MEMBRANES OF ANIMAL CELLS

VII. CARBOHYDRATES OF SURFACE MEMBRANES AND WHOLE CELLS

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SUMMARY

The carbohydrate content of mouse fibroblasts (L cells) and surface membranes was examined. The amount of sialic acid in intact cells was found to vary. A portion of this variation could be related statistically to change in protein content of the cell. Some of the variation correlated with the growth rate of the culture, slower growing cultures containing higher amounts of sialic acid.

The surface membranes were rich in most monosaccharides relative to the rest of the cell. Although considerable variation in the sialic acid and other monosaccharides was observed, some of the monosaccharides appeared to have a fairly constant molar ratio, one to the other. The results suggest that the glycoproteins and/or glycolipids of the intact L cell and surface membrane are quite variable.

Studies with neuraminidase indicated that 25–35 % of the sialic acid of the surface membranes was resistant to cleavage by the enzyme under the conditions employed. The amount of sialic acid released from the whole cells indicated that neuraminidase probably entered the cells during the incubation period.

INTRODUCTION

There is a rapidly growing literature to support the belief that glycoproteins and glycolipids are important constituents of animal cells and that they have important functions at the cell surface. They comprise the blood group substances[1], tissue antigens[2] and have been shown to be receptor sites for some viruses[3]. A glycolipid (Forssmann antigen) has been demonstrated by immunological techniques in cells transformed by a virus but not in the same cell line which has not been transformed[4]. Differences have been found in the glycolipid content of cells before and after viral transformation[5,6]. The carbohydrate content of membrane preparations of virus-transformed mouse fibroblasts has been shown to be decreased from normal[7].

Glycoproteins have been demonstrated by a number of techniques and with a variety of membrane fractions to be components of the cell surface (see COOK[8] for review). However, with the exception of the red blood stroma, only a few studies have been done with isolated whole surface membranes[9,10]. Glycolipids have also been

Abbreviation: FMA, fluorescein mercuric acetate.

examined in fractions of membranes by various workers[11–13] and in isolated whole surface membranes[14].

As part of our studies to determine the function of these glycoproteins and glycolipids on the cell surface we have examined the carbohydrate composition of intact mouse fibroblasts and whole surface membranes. The variations in the amount of carbohydrate present in the membrane and whole cell in certain conditions will be discussed. Sialic acid was studied in most detail.

METHODS

Growth of cells

Mouse fibroblasts (L cells) were grown and harvested as described previously[15]. Aureomycin (50 μg/ml of media) was added to the culture once a week. The cultures were all negative for Mycoplasma. The washed L cells were suspended in 0.16 M NaCl ($5 \cdot 10^7$ cells/ml) and analyzed directly or used to prepare surface membranes.

Preparation of surface membranes

Surface membranes were prepared by the Zn^{2+} and fluorescein mercuric acetate (FMA) procedures as described previously[16]. The whole surface membranes obtained by these procedures were counted in a hemocytometer.

Analytical procedures

All analyses were performed in duplicate and in some cases in quadruplicate. Sialic acid was determined by the thiobarbituric acid method[17] after elution from a column of Dowex-1 (acetate)[18]. *N*-Acetylneuraminic acid (Calbiochem) was used as a standard. Sialic acid was removed from the L cells or surface membranes by hydrolysis with 0.025 M H_2SO_4 at 80° for 1 h or with neuraminidase (*Vibrio cholerae*, Calbiochem). Total hexosamines were determined by the method of BOAS[19]. Neutral carbohydrates were analyzed as alditol acetates by gas–liquid chromatography as described by LEHNHARDT AND WINZLER[20]. The column used was 3 % ECNSS on Gas-Chrom P (Applied Sciences Laboratories). The internal standard was 2-deoxy-glucose. Alditol acetates used as references were synthesized and crystallized by the method of ABDEL-AKHER *et al.*[21]. The surface membranes were dialyzed 48 h against water for removal of sucrose prior to hydrolysis with Dowex-50 (H^+) in 0.02 M HCl for 40 h at 100°. Proteins were determined by the method of LOWRY *et al.*[22] using bovine serum albumin as the standard. DNA was determined by the method of BURTON[23].

Neuraminidase treatment

Whole cells or surface membranes ($1 \cdot 10^7$) were suspended in 0.7 ml of 0.16 M NaCl. Neuraminidase (7.5 units) and $CaCl_2$ to 0.75 mM were added and the fractions were incubated for 1 h at 37°. Further incubation did not release significantly more sialic acid.

RESULTS

Variability in the amount of sialic acid in the whole cell

The amount of sialic acid found in the L cell varied from $6.4 \cdot 10^{-10}$–$17 \cdot 10^{-10}$ μmoles. This variation was studied over a period of several weeks by analyzing the

cells each day for sialic acid and protein content. These values are plotted in Fig. 1 where each point represents duplicate analyses for sialic acid each day, expressed as sialic acid per $5 \cdot 10^8$ cells (Fig. 1A) or per mg of protein (Fig. 1B).

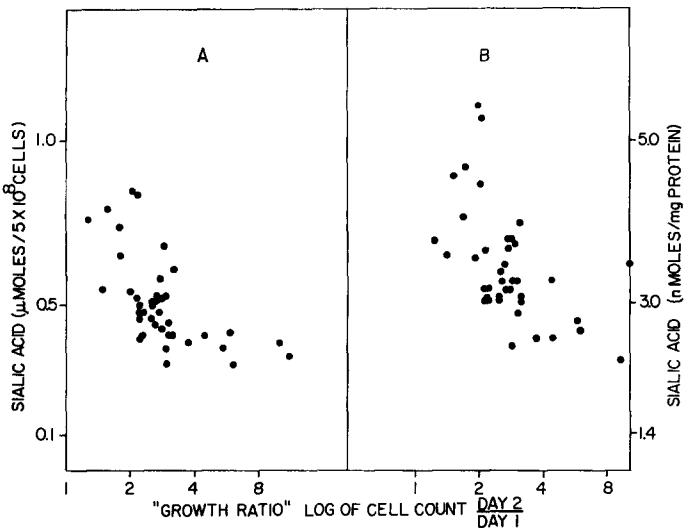

Fig. 1. Variation in sialic acid content of L cells. The L cells were diluted to $2 \cdot 10^5$–$3 \cdot 10^5$ cells/ml (Day 1) and harvested (Day 2) at the cell count indicated by the cell count ratio. A ratio of 2 indicated the cells had doubled in number. The exact numbers were determined by a Coulter counter and by hemocytometer count. Sialic acid and protein contents of the cells were determined as described in the text and are plotted against the log of the cell count ratio. A = μmoles of sialic acid per $5 \cdot 10^8$ cells; B = nmoles of sialic acid per mg of cell protein. The slope of the regression of the points in A, calculated with 95% confidence limits, is -0.43 ± 0.26. The regression is not linear as the sialic acid content per $5 \cdot 10^8$ cells levels off at high cell count ratios.

It can be shown statistically that some of this variability is related to change in the size of the cell if change in protein content of the cell is used to represent change in cell size[24]. In fact, the percentage of the variability which is related to cell size can be determined statistically by representing the amount of sialic acid per cell by the expression sialic acid/cell = (sialic acid/protein) × (protein/cell). If all the variation in sialic acid per cell is due to size variation, then sialic acid per cell should be related to protein per cell with a correlation coefficient (r) of 1 and to sialic acid per protein with r equal to 0. Using the values of the sialic acid and protein contents of the L cell from the experiments expressed in Fig. 1, the value for r calculated for the former relationship is 0.73 and for the latter, 0.70; therefore only a portion of the change in sialic acid is due to cell size. The percentage that each term of the expression contributes to the change in sialic acid per cell is the square of the correlation coefficient if the terms are independent of each other. The correlation coefficient between sialic acid per protein and protein per cell ($r = 0.18$) is not significantly different from zero, demonstrating that the two are, in fact, independent. Thus the percentages can be calculated and are 53 and 49 %, respectively.

In attempting to define parameters for the variation in sialic acid not related

to change in cell size, the amount of sialic acid was related to the log of the growth ratio. The growth ratio represents an index of the growth rate of the culture, the cell count per ml of culture medium of the starting culture (Day 1) divided into the cell count at the time of harvest (Day 2). A ratio of 2 means that the number of cells has doubled one time in a non-synchronous culture. The amount of sialic acid per $5 \cdot 10^8$ cells (Fig. 1A) or the amount of sialic acid per mg of protein (Fig. 1B) is plotted against the log of the growth ratio. When the L cells divided at a slower rate the sialic acid content was significantly higher than in the cells which divided at a faster rate. The correlation coefficient between log of the cell count ratio and sialic acid content per cell for the data represented in Fig. 1 is —0.60 which is statistically different from 0 at the 1 % level. When sialic acid is expressed per mg of cell protein, r is —0.52 which is also statistically different from 0 at the 1 % level. The correlation coefficient between log of the cell count ratio and protein content per cell for these experiments is —0.40. Again since the protein per cell can represent a size change[24], the correlation coefficients reveal that only a portion of the variation in sialic acid per cell which is related to the growth ratio is correlated to a change in cell size. That is, if the variation were due completely to change in cell size, the correlation between sialic acid per mg of protein and log of the growth ratio would not be significantly different from 0.

Sialic acid and hexosamine content of growing and nongrowing cells

The sialic acid and hexosamine contents of L cells which were growing were compared to cells which were not growing[10] in order to see if these criteria could account for some of the variations of sialic acid which were observed. The nongrowing cells cultured for 2 days did not appear to vary enough from the growing cells (Table I) to account for the fluctuation as generally observed and reported in Fig. 1. The mean value and standard deviation for the sialic acid content of growing cells harvested from six different cultures was $6.7 \cdot 10^{-10} \pm 0.6 \cdot 10^{-10}$ μmoles/cell and $5.6 \cdot 10^{-10} \pm 0.7 \cdot 10^{-10}$ μmoles/cell for nongrowing cells harvested from six companion cultures. This represented an average decrease of 16 % for the nongrowing cells. An increase of 14 % was observed for the hexosamines. The values for DNA and protein are included in Table I. The average values for the total protein of the cell show a decrease of 30 % for the nongrowing cells.

TABLE I

SIALIC ACID AND HEXOSAMINE CONTENT OF THE GROWING AND NONGROWING CELL

Growing: L cells were harvested at a concentration of $3.5 \cdot 10^5$–$4.5 \cdot 10^5$ cells/ml of culture medium. Nongrowing: L cells from each starting culture as the growing experiments were concentrated to $2 \cdot 10^6$ cells/ml and cultured for 2 days. At the time of harvest, the cell counts were from $2.0 \cdot 10^6$–$3.5 \cdot 10^6$ cells/ml of culture medium. The methods used were as described in the text and the values represent mean values and the standard deviation of the mean. Cells from the following number of cultures contributed to each mean: growing, sialic acid and protein, 6; hexosamines and DNA, 4; nongrowing, sialic acid and protein, 6; hexosamines and DNA, 4.

L cells	*Sialic acid*	*Hexosamines*	*DNA*	*Protein*
	($\mu moles \times 10^{-10}/cell$)		($mg \times 10^{-8}/cell$)	($mg \times 10^{-7}/cell$)
Growing	6.7 ± 0.6	50.7 ± 11.5	1.12 ± 0.05	2.50 ± 0.39
Nongrowing	5.6 ± 0.7	58.7 ± 9.8	1.09 ± 0.10	1.75 ± 0.20

TABLE II

COMPARISON OF MEAN VALUES OF THE CARBOHYDRATE CONTENT OF SURFACE MEMBRANES AND WHOLE CELLS

The methods used were as described in the text. The values represented mean values and the standard deviation for each carbohydrate. The following number of membrane preparations and whole cells contributed to each mean. Surface membranes prepared by the Zn^{2+} method: sialic acid, 5; hexosamines, 4; other values, 7; surface membranes prepared by the FMA procedure: sialic acid and hexosamines, 3; other values, 8; whole cells: sialic acid and hexosamines, 5; other values, 4.

L cell	*Sialic acid*	*Hexosamines*	*Fucose*	*Mannose*	*Galactose*
			(*nmoles/mg protein*)		
Surface membranes					
Zn^{2+} method	15.0 ± 2.2	53.5 ± 12.6	7.6 ± 7.1	23.7 ± 6.6	59.4 ± 8.2
FMA method	24.3 ± 1.2	35.7 ± 11.7	4.7 ± 3.6	40.0 ± 12.3	83.6 ± 23.3
Whole cells	3.4 ± 0.4	19.8 ± 6.1	2.4 ± 1.2	20.3 ± 6.0	12.5 ± 2.2

TABLE III

THE CARBOHYDRATE CONTENT OF SURFACE MEMBRANES AND WHOLE CELLS

All values (mean and standard deviation) are derived from the experiments reported in Table II. The numbers in parentheses represent the percentage of the total carbohydrate of the cell found in the surface membrane.

L cell	*Sialic acid*	*Hexosamines*	*Fucose*	*Mannose*	*Galactose*
		(*μmoles* × 10^{-10} *per surface membrane or cell*)			
Surface membranes					
Zn^{2+}	6.5 ± 1.4 (73)	25.9 ± 5.5 (44)	2.3 ± 2.2 (36)	8.5 ± 2.5 (16)	21.6 ± 2.9 (66)
FMA	3.4 ± 0.4 (38)	5.0 ± 1.9 (9)	0.7 ± 0.6 (11)	5.7 ± 1.8 (11)	11.9 ± 3.3 (36)
Whole cells	8.9 ± 1.7 (100)	58.3 ± 17.3 (100)	6.3 ± 3.8 (100)	52.8 ± 12 (100)	32.8 ± 4.6 (100)

Variability in the amount of sialic acid in the surface membrane

The amount of sialic acid present in the surface membranes prepared by the Zn^{2+} procedure varied from $2.5 \cdot 10^{-10}$–$8.8 \cdot 10^{-10}$ μmoles. As the amount in the whole cell varied also (Fig. 1), sialic acid was determined on the whole cells each time surface membranes were prepared for sialic acid studies. In eighteen different experiments the percentage of the total sialic acid of the whole cell found in the surface membrane varied from 40 to 85 %. The mean values and standard deviation for sialic acid of these experiments was $8.9 \cdot 10^{-10} \pm 3.1 \cdot 10^{-10}$ μmoles per cell and $5.7 \cdot 10^{-10} \pm 1.3 \cdot 10^{-10}$ μmoles/surface membrane. No significant correlation was found when the sialic acid content of the membranes from these experiments was related statistically to the sialic acid content in the rest of the cell (r was equal to —0.37 which was not significantly different from 0).

Recovery of sialic acid

Since the amount of sialic acid in the surface membranes prepared by the Zn^{2+} procedure varied from the extreme ranges of 40–85 % of the sialic acid of the intact L cell it was necessary to determine whether or not the procedure for isolating the membranes interfered with the recovery of sialic acid. Fractions obtained from sucrose gradients during the first part of the procedures for the isolation of the surface membranes[16] were analyzed. The total amounts of sialic acid and protein recovered were 75 and 78 %, respectively. A loss of only 25 % of both components might be expected in view of the large number of fractions involved in the analyses and the difficulty in recovering small amounts of sticky fractions. In less complicated experiments, that is, treating the L cells with 0.001 M $ZnCl_2$, homogenizing and centrifuging at 6000 × g for 10 min to obtain a pellet and supernatant solution, the recovery of protein and sialic acid was 98 %. This did not represent a stage of membrane purification but did rule out the interference of the homogenizing procedures and Zn^{2+} with the analyses for sialic acid and proteins.

Comparison of the carbohydrate content of surface membranes and whole cells

Table II shows a comparison of the carbohydrates in the intact L cell and surface membranes prepared by the Zn^{2+} and FMA procedures. The numbers are average values expressed per mg of protein of whole cell or surface membrane. The sialic acid and hexosamine values which contributed to the mean values for the whole cells and membranes prepared by the Zn^{2+} procedure represented five separate experiments. The number of times the cells divided per 24 h in these five cultures was similar and therefore the extreme variations in the sialic acid content of the whole cell (Fig. 1) are not apparent here.

The carbohydrate content of the surface membrane and whole cell can be related since the Zn^{2+} and FMA methods yield whole membranes. In Table III these average values are expressed per membrane and per cell. The percentages of the carbohydrates of the whole cell which were present in the surface membranes are given in parentheses. In the Zn^{2+} membranes, fucose represented 36 % of the total cell fucose. Galactose and mannose represented 66 and 16 %, respectively, of the total cell values. The content of sialic acid and hexosamines was 73 and 44 %, respectively.

When surface membranes were prepared by the FMA procedures the percentages of total cell contents of sialic acid and hexosamines in the membranes fell to 38 and

9 % respectively (Table III). Fucose and galactose represented 11 and 36 %, respectively, of the total. The percentage of mannose (11 %) was similar to the mannose content of the Zn^{2+}-prepared membranes (16 %). In general, the FMA membranes contained less of the total carbohydrates of the cell than did the membranes prepared by the Zn^{2+} procedure.

TABLE IV

COMPARISON OF THE MOLAR RATIOS OF SIALIC ACID TO OTHER MONOSACCHARIDES IN SURFACE MEMBRANES PREPARED BY THE Zn^{2+} METHOD

The numbers given in parentheses represent the concentration of the monosaccharides per surface membrane (μmoles $\times$ 10^{-10}). The molar ratios are derived from these numbers. Each experiment represents a different culture of L cells. For Expts. 1–4, the sialic acid content of the surface membranes represented 65, 41, 66 and 82 %, respectively, of the total cell sialic acid.

Monosaccharide	*Expt. 1*	*Expt. 2*	*Expt. 3*	*Expt. 4*
Sialic acid	1 (5.3)	1 (4.5	1 (6.2)	1 (8.8)
Hexosamines	—	—	3.0 (18.4)	3.0 (26.0)
Mannose	1.9 (10.0)	1.5 (6.9)	—	—
Galactose	4.9 (26.0)	4.7 (21.0)	—	—
Fucose	0.2 (0.9)	1.4 (6.5)	—	—

Table IV shows the molar ratios of sialic acid to the other monosaccharides in the surface membranes isolated from L cells grown in four separate cultures. Of the four experiments, two represented extremes in the variation of the percentage of sialic acid of the total cell found in the surface membranes. In Expts. 1 and 2, even though different amounts of the sialic acid of the whole cell were found in the surface membranes (65 and 41 %), the molar ratio of sialic acid to mannose and galactose was fairly constant in the surface membranes. However, the molar ratio of sialic acid to fucose appeared to vary independently of the other monosaccharides. In Expts. 3 and 4, again representing variations in the percentage of total cell sialic acid found in the surface membrane (66 and 82 %), the amount of hexosamines was found in a constant molar ratio of 3.0 to the sialic acid in the surface membranes.

Sensitivity to neuraminidase

When whole L cells were treated with neuraminidase for 1 h as described in METHODS, 45–60 % of the total cell sialic acid was removed. Further incubation did not significantly increase the amount of sialic acid cleaved. Fig. 2 illustrates an experiment which demonstrates the sensitivity of the sialic acid in the intact L cell and surface membrane to neuraminidase. In this experiment 61 % of the total cell sialic acid was removed. The surface membranes were isolated and purified by the Zn^{2+} procedure. These membranes still contained 16 % of the total cell sialic acid. Further treatment of these membranes with neuraminidase did not remove additional sialic acid (Fig. 2). The membranes isolated from the same washed whole cells but not treated with neuraminidase contained 42 % of the sialic acid of the whole cell. Treatment of the isolated membranes with neuraminidase removed 65 % of the sialic acid of the surface membranes. Thus approx. 35 % of the sialic acid of the membrane was not susceptible to neuraminidase. This represented 15 % of the sialic acid of the whole cell. In other experiments 25–30 % of the sialic acid of the membranes isolated

by the Zn^{2+} procedure was not susceptible to neuraminidase and represented approx. 10 % of the sialic acid of the whole cell. Surface membranes isolated by the FMA procedure also showed that approx. 20 % of the sialic acid was not susceptible to neuraminidase. In the experiment reported in Fig. 2, $3.6 \cdot 10^{-10}$ μmoles of sialic acid were released by neuraminidase from each cell, although each membrane contained only $2.5 \cdot 10^{-10}$ μmoles of sialic acid of which 35 % was not susceptible to neuraminidase. This was observed in three experiments and indicated that more sialic acid was re-

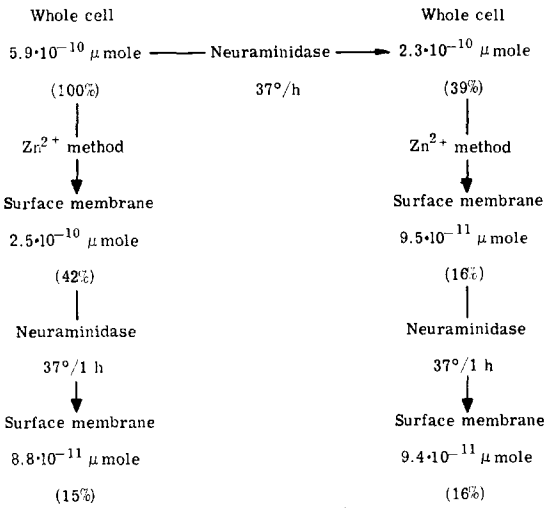

Fig. 2. Release of the sialic acid from the surface membrane and whole cell by neuraminidase. Sialic acid was expressed as μmoles/cell or μmoles/surface membrane. The numbers in parentheses represent the percentage of the total cell sialic acid. All procedures were as described in the text.

leased from the whole cell than was present on the cell surface. Cell lysis could not explain this finding because when L cells were deliberately lysed and treated with neuraminidase for 1 h a maximum of 66 % of the total cell sialic acid was released. In control cells with less than 10 % lysis as determined by cell counts, 56 % of the cell sialic acid was released after 1 h incubation with neuraminidase. Our data suggests that neuraminidase can enter the L cell under these incubation conditions.

DISCUSSION

These studies have shown that intact L cells grown in suspension culture vary considerably in sialic acid content. Approx. 50 % of the variation was shown to correlate statistically to the change in protein content of the cell. Variations in protein content per cell have been shown by COHEN AND STUDZINSKI[24] to correlate with changes in cell size for the HeLa cell. The size distribution, as determined by the Coulter size distribution plotter, and the protein content per cell have been shown to correlate also for the L cells (unpublished observations). Therefore the protein content could reflect the cell size in the experiments reported here and thus approx. 50 % of the variation in sialic acid content of the L cell could represent a change in cell size. The remaining variation represents changes in sialic acid content relative to protein,

about one-third of which was calculated to be related to the growth rate of the culture. Cells in slowly growing cultures had higher sialic acid values than those of more rapidly growing cultures (Fig. 1). In attempting to define additional parameters which might cause this variation, growing and nongrowing cells were compared. No marked difference was found in the amounts of sialic acid per cell in these two growth conditions. The nongrowing cells were produced in these experiments by suspending cells at very high concentrations and culturing for 2 days. It has been shown that the sialic acid content of the nongrowing L cell does not differ significantly if the saturated populations of cells are artificially produced by concentrating or brought about through natural growth in culture (unpublished observations).

In other experiments, with KB cells, synchronized with a double thymidine block, sialic acid was shown not to decrease at the same rate as the protein during the mitotic cycle[25]. It is quite possible that these two variations are related, however, additional experiments are needed to determine this. The decrease in sialic acid content observed in the more actively dividing cells could be due to a lack of precursors for sialic acid synthesis brought about by lack of nutrients from the medium or lack of cytidine precursors in the cell.

The variation in sialic acid of the whole cell was reflected in the individual membrane preparations made by the Zn^{2+} method (Table IV). In these experiments, galactose, mannose and the hexosamines appeared to vary with the sialic acid content (Table IV) indicating a possible fluctuation in the amount of membrane-bound glycoproteins rather than in the sialic acid level alone. However, small fluctuations of sialic acid or the monosaccharides could not be ascertained by these methods. It will have to be determined whether these changes in sialic acid represent changes in the glycoprotein pattern only or if they also represent changes in the glycolipids of the cell. In contrast to sialic acid, fucose appeared to vary independently of the other monosaccharides. This extreme variation of fucose could not be accounted for by loss during the hydrolysis procedure.

The average amounts of sialic acid, hexosamine, galactose and fucose were considerably less in the surface membranes prepared by the FMA procedure than the membranes prepared by the Zn^{2+} procedure (Table III). These differences in the amount of carbohydrate could reflect difference in the structures that were isolated by the two procedures. The surface membranes isolated by the Zn^{2+} procedure contain more of the total cell protein than the FMA-prepared membranes (10 *vs.* 4.7 %). Therefore when the carbohydrates are expressed per mg of protein (Table II) the FMA membranes contain higher concentrations of the carbohydrates with the exception of hexosamines and fucose. Since the membranes prepared by the Zn^{2+} procedure were not contaminated with significant amounts of other cell material such as endoplasmic reticulum, nuclei and mitochondria[26] the increased amounts of carbohydrates per membrane could not come from these other cell organelles. It is possible that there is a loss of glycoproteins from the membranes prepared by the FMA procedure. Mannose was the only monosaccharide examined which was present in the membranes in the same amount, that is, approx. 11–16 % of the total cell mannose was found in both membrane preparations (Table III). Mannose could be part of a background structure while some of the other glycoproteins are more loosely attached to the surface membrane. The small percentage of the total amount of mannose of the cell found in the surface membrane suggests that most of the mannose-containing macro-

molecules are found in the cell internally. Indeed, LI *et al.*[27] have reported high concentrations of mannose in fractions of mitochondria, microsomes and lysosomes from liver and Walker tumor cells.

Membrane preparations from mouse liver and hepatoma cells have been reported by EMMELOT AND BOS[28] to contain 28 nmoles of sialic acid per mg of protein. Of this amount, 63–70 % was released by neuraminidase. MOLNAR[29] has reported 27 nmoles of sialic acid per mg of protein in plasma membrane preparations from Ehrlich ascites cells. The sialic acid content per whole membrane could not be calculated since fragments of surface membranes were isolated by their procedures. In the procedures reported here, the preparations of surface membranes were isolated as whole ghosts from which the internal structures and cytoplasm were removed. Under these conditions we never found 100 % of the total cell sialic acid on the surface. Experiments were reported which eliminated the possibility of artifacts due to the Zn^{2+} method. In addition, in a study of the distribution of the sialic acid and hexosamine content of the L cell, both carbohydrates were found in significant amounts in some of the other subcellular fractions[30].

NORDLING AND MAYHEW[31] have provided evidence that neuraminidase can enter cells. Our studies showing that more sialic acid from the whole L cell is released by incubation with neuraminidase than is found in the surface membrane (Fig. 2) supports this conclusion. The fact that similar amounts of sialic acid were released from lysed cells as from whole cells (determined by microscopic examination and cell counts) indicates that cell lysis during incubation was not responsible for the increased cleavage of sialic acid.

WEINSTEIN *et al.*[14] have shown that 27 % of the total sialic acid of the L cell is in glycolipid form and that 21–24 % of the sialic acid of the surface membrane is in glycolipids. These glycolipids in the cell or surface membrane are probably not accessible to neuraminidase. In the present study, 25–35 % of the sialic acid of the surface membranes was not susceptible to neuraminidase under the conditions of incubation (Fig. 2). Thus, most, if not all, of the sialic acid remaining in the surface membranes after incubation with neuraminidase could be glycolipid. It should be added that the glycolipids of the surface membrane (hematoside and disialoganglioside) are susceptible to neuraminidase in the extracted state[14].

The experiments reported here indicate that the glycoproteins and/or glycolipids of the surface membrane and intact L cell are more variable than anticipated. In view of these fluctuations one should exercise caution in interpreting glycoprotein and glycolipid patterns, particularly when rigidly controlled conditions of culture are not always attainable.

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