BBA 75630

# MEMBRANES OF ANIMAL CELLS

## VIII. DISTRIBUTION OF SIALIC ACID, HEXOSAMINES AND SIALIDASE IN THE L CELL

MARY CATHERINE GLICK, CAROLE A. COMSTOCK,
MARTIN A. COHEN AND LEONARD WARREN

*Department of Therapeutic Research, University of Pennsylvania School of Medicine, Philadelphia, Pa. (U.S.A.)*

(Received October 13th, 1970)

SUMMARY

The distribution of sialic acid and hexosamines was studied in purified organelles obtained from L cells. The major portion of the sialic acid of the intact cell is found in the surface membranes (66 %). Only small amounts of sialic acid are found in the other purified fractions with the exception of the lysosomes which contained approx. 16 %. The hexosamines are largely distributed between the surface membranes (33 %) and soluble fraction (25 %). Microsomes and mitochondria contain 14 and 11 %, respectively, of the hexosamines of the intact cell and the nuclei contain 4 %. The molar ratio of hexosamines to sialic acid of these fractions indicate differences in glycoprotein and/or glycolipid contents of the cell organelles.

Some sialidase (*N*-acetylneuraminate glycohydrolase, EC 3.2.1.18) activity is present in all of the cell fractions which were examined. However, upon further purification only the lysosomes show an increased specific activity of the enzyme.

A procedure is reported for obtaining purified nuclei with the outer nuclear membrane morphologically intact.

## INTRODUCTION

Previous studies have shown that surface membranes obtained from L cells contain an average of 60 % of the sialic acid and 40 % of the hexosamines of the cell. However, there is considerable variation in the level of sialic acid in the surface membrane as well as in the whole cell[1]. To examine this variability it became necessary to determine the location of sialic acid and hexosamines not present in the surface structure. It was also necessary to determine whether the activity of sialidase (*N*-acetylneuraminate glycohydrolase, EC 3.2.1.18) throughout the cell could contribute to the observed variations. L cells were fractionated into surface membranes, mitochondria, nuclei, microsomes, lysosomes and soluble material and the contents of sialic acid and hexosamines were determined in each fraction. The enzyme activity of sialidase was determined in the cell homogenate and some of the cell fractions. The results are reported in this paper.

METHODS

*Cell culture*

L cells were grown and harvested as reported previously[2].

*Preparation of cell fractions*

*Surface membranes.* Surface membranes were isolated from L cells by the $Zn^{2+}$ procedure[2]. The whole surface membranes were counted in a hemocytometer.

*Microsomes and soluble material.* Microsomes and soluble protein fractions were obtained from the same L cells from which the surface membranes were isolated[3]. These preparations have been designated "$Zn^{2+}$" microsomes and "$Zn^{2+}$" soluble fractions. In addition, microsomes were obtained from whole L cells by techniques commonly used as reported[3]. The soluble fraction was the supernatant material (110000 × *g*) of the microsomal fraction.

*Nuclei.* Nuclei were prepared from the same whole cells from which the surface membranes and microsomes were isolated. The first gradient in the purification of the surface membranes contained a pellet of whole cells and nuclei[2]. This pellet from a homogenate of $10^8$ cells was resuspended in 2 ml of a solution of 35 % sucrose. Sucrose solutions were made according to *Handbook of Chemistry and Physics*[4]. Acetic acid (1 M) was added to bring the solution to 0.1 M and the thoroughly mixed suspension was held at 2° for 45 min. An equal volume of 35 % sucrose was added and the suspension of cells and nuclei was transferred to a Dounce homogenizer (Kontes Glass Co., Vineland, N.J.) with a B pestle. The nuclei were freed of surrounding cytoplasm with approx. 7–10 strokes of the pestle. The homogenization was followed in a phase contrast microscope as were the subsequent purification steps. At this point the homogenate contained much cytoplasmic debris and many fragments of surface membranes. The nuclei were purified by centrifugation through sucrose solutions. For the first step, the preparation was diluted with 0.5 vol. of 45 % sucrose and then with 1 vol. of 60 % sucrose and centrifuged at 3200 × *g* in the $HB_4$ rotor of the $RC_2$ Sorvall centrifuge for 12 min. The pellet of nuclei was resuspended in 45 % sucrose and then in 60 % sucrose in the same volumes as used in the preceding step. The mixture was again centrifuged. This step was repeated 3 or 4 times until the nuclei were no longer contaminated with particles visible under the phase contrast microscope. The yield of nuclei from the original number of whole cells was approx. 40 %.

For examination in the electron microscope, the isolated nuclei were fixed in 2.5 % glutaraldehyde for 15 h at 4° and treated as described[5]. The electron micrographs showed the nuclei to contain their outer membranes and to be free of cell contamination (Fig. 1).

*Mitochondria.* The mitochondria were prepared as reported by NASS[6]. In some cases the final centrifugation in the Spinco ultracentrifuge was repeated and these fractions were designated as "purified" mitochondria. When stated, subsequent centrifugation through discontinuous gradients of sucrose solutions as described by VIGNAIS AND NACHBAUR[7] was performed.

*Lysosomes.* The method as described by SAWANT *et al.*[8] was used to prepare "purified" lysosomes from the L cells. A good proportion of the lysosomes remained with the mitochondrial fractions so the yield was very low. For example, 1 mg of lysosomal protein was obtained from $2 \cdot 10^9$ cells which had a total protein content of

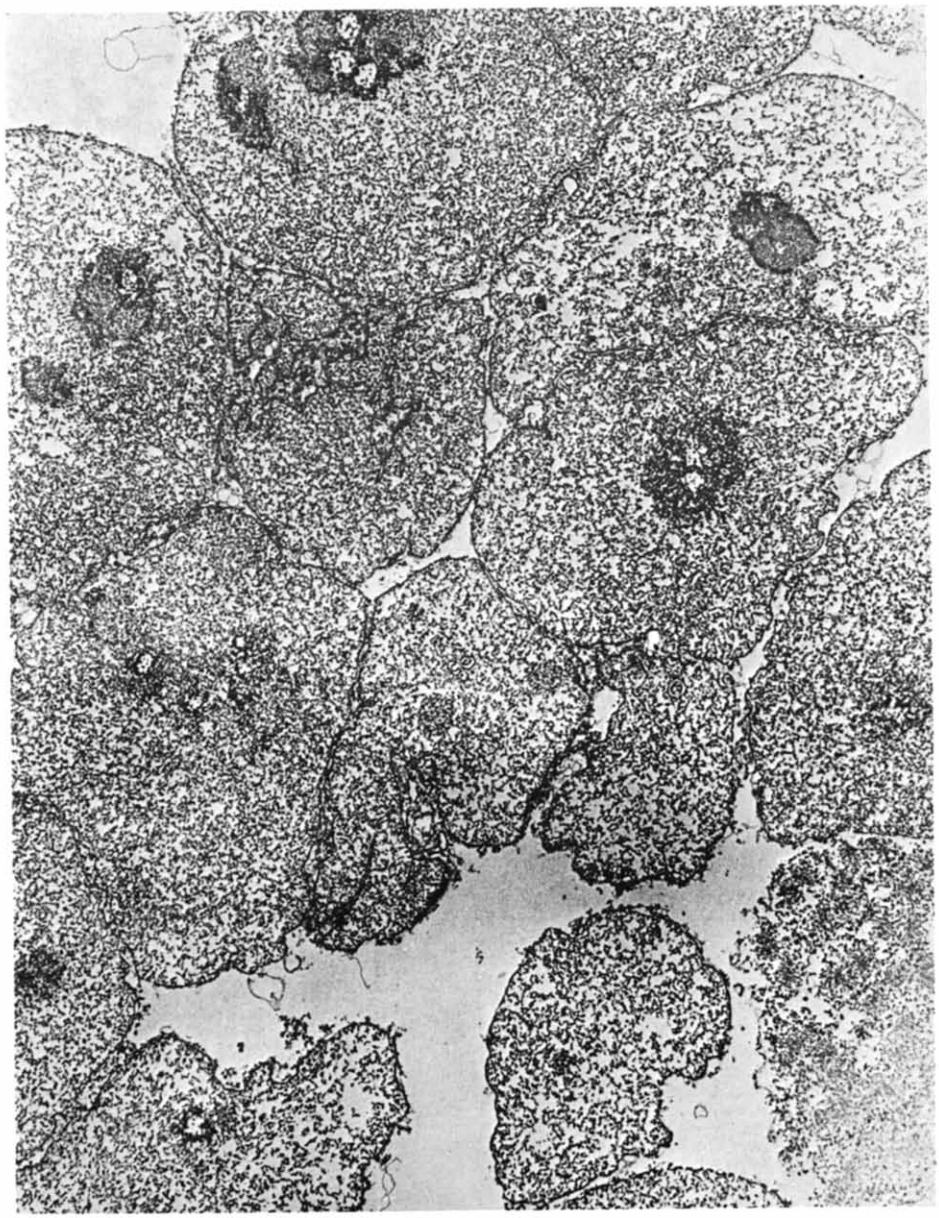

Fig. 1. Electron micrograph of nuclei isolated from L cells. × 3900.

520 mg. When stated, lysosomes were less purified to obtain higher yields. In these preparations centrifugation over discontinuous gradients of solutions of 0.6 and 0.7 M sucrose was not carried out[8].

*Chemical analyses*

Sialic acid was determined by the thiobarbituric acid assay[9]. Hexosamines were determined by the method of BOAS[10]. Proteins were determined by the method of

LOWRY *et al.*[11]. The details for the L cells have been described[1]. DNA was determined on whole cells and nuclei by the method of BURTON[12] and served as an adjunct to direct counting in a hemocytometer using a phase contrast microscope. The whole cell or nucleus contained 1.2 $(\pm 0.2) \cdot 10^{-8}$ mg of DNA.

*Enzymic analyses*

Sialidase was measured by the release of sialic acid from fetuin (Nutritional Biochemical Corp., Cleveland, Ohio). Each cell fraction was frozen and thawed before assaying. Aliquots of the fraction and fetuin (4.0 mg, containing 0.8 $\mu$mole of sialic acid) were suspended in 0.4 ml of 0.05 M sodium acetate buffer, pH 4.2, and incubated at 37° for 20 h. The incubated mixtures were placed on columns (0.5 cm × 8 cm) of Dowex 1-X 8 (acetate). Free sialic acid was eluted with 0.6 M sodium acetate buffer, pH 4.8, and determined by the thiobarbituric acid assay[9]. Samples of the fetuin and the enzyme fractions alone were incubated with each assay and subjected to the same procedure. A correction was made for any free sialic acid in these assays.

The whole homogenate of L cells was assayed for sialidase with endogenous glycoproteins as substrates. L cells were homogenized in a Dounce homogenizer at a concentration of $1.3 \cdot 10^8$ cells per ml of 0.05 M sodium acetate buffer, pH 4.2, and incubated at 37°. Aliquots of 1 ml were removed at the start of the incubation and at specified times throughout the incubation. These aliquots were centrifuged at 10400 × *g* for 5 min in a Sorvall RC-2 centrifuge. A portion (0.4 ml) of the supernatant material was assayed directly for free sialic acid[9].

Acid phosphatase (orthophosphoric monoesterphosphohydrolase, EC 3.1.3.2) was measured by the release of *p*-nitrophenol from *p*-nitrophenol phosphate (Sigma Chemical Co., St. Louis, Mo.) as described by ARSENIS AND TOUSTER[13].

## RESULTS

*Distribution of sialic acid and hexosamines in the cell fractions*

Table I shows the distribution of sialic acid, hexosamines and protein in the surface membrane, nucleus and mitochondrion as compared to the whole cell. The values reported here show that the surface membrane contained 66 % of the total cell sialic acid and 33 % of the total cell hexosamine. These values were those obtained from one culture of L cells. Average values for sialic acid and hexosamines of the whole cell and surface membrane of the L cell have been reported[1]. Nuclei isolated by the procedure described in METHODS contained less than 0.6 % of the sialic acid of the whole cells. It is possible that acetic acid hydrolyzed the sialic acid from the nuclei although analysis of the 110000 × *g* supernatant fraction from such a preparation contained less than 1 % of the sialic acid of the whole cell. The nucleus contained 4 % of the total hexosamines of the L cell.

The L cell has been reported to contain approx. 200 mitochondria with a protein content of $8 \cdot 10^{-11}$ mg per mitochondrion[6]. From these numbers the amount of sialic acid and hexosamines which contributed to the total cell values were calculated. Sialic acid found in the mitochondria represented 4 % of the whole cell value and hexosamines represented 11 % (Table I).

From Table I the amount of protein of the whole cell not accounted for by

TABLE I

SIALIC ACID, HEXOSAMINES AND PROTEIN DISTRIBUTION IN THE L CELL

The numbers in parentheses represent the percentage of the total cell value. The preparation of the cell fractions and the assays are described in the text. The amount of protein in the microsomal and soluble fractions and the values for sialic acid and hexosamine in these fractions per mg of protein as given in Table II were used to calculate the percentage of the total cell sialic acid and hexosamines. A similar calculation was made for the lysosomal fraction with the exception that it was estimated that the lysosomes represent 5% of the total cell protein. The preparations of whole cells and surface membranes were from the same culture of L cells. All analyses were done in duplicate and the values obtained corresponded within 5%. For the nuclei, the values of sialic acid and hexosamines represented preparations of nuclei from 8 cultures of L cells which were combined for one analysis each. This was repeated 3 times. For the mitochondria ("purified" mitochondria as described in METHODS) the sialic acid value is a mean of 2 preparations which were analyzed separately and 3 preparations which were combined for one analysis. The latter was also used for the hexosamine analysis. For the microsomal fraction, the sialic acid value is a mean of 5 preparations from L cells analyzed separately and 6 preparations which were combined from one analysis. The latter was also used for the hexosamine analysis. For the soluble fraction, the sialic acid value is a mean of 3 preparations, analyzed separately and 6 preparations which were combined for one analysis. The latter was also used for the hexosamine analysis. For the lysosomes, the sialic acid value represented two preparations which were combined.

| *Cell fraction* | *Distribution per cell fraction* | | |
|---|---|---|---|
| | *Sialic acid* ($\mu moles \times 10^{10}$) | *Hexosamines* ($\mu moles \times 10^{10}$) | *Protein* ($mg \times 10^{8}$) |
| Whole cell | 9.4 (100) | 56.0 (100) | 28.7 (100) |
| Individual organelles | | | |
| Surface membrane ($Zn^{2+}$) | 6.2 (66) | 18.4 (33) | 3.1 (11) |
| Nucleus | <0.05 (<0.6) | 2.4 (4) | 8.3 (29) |
| Mitochondrion | 0.002 (0.02) | 0.025 (0.04) | 0.008 (0.03) |
| Total organelles | | | |
| Mitochondria | 0.4 (4) | 6.2 (11) | 1.6 (6) |
| Microsomes ($Zn^{2+}$) | — (7) | — (14) | 2.6 (9) |
| Soluble ($Zn^{2+}$) | — (8) | — (25) | 7.9 (27) |
| Lysosomes | — (16) | — | — (~5) |

the isolated organelles was approx. 50%. This amount was distributed largely between the microsomal, lysosomal and soluble fractions. The soluble fraction of the L cell contained 27% of the total cell protein and the microsomal fraction contained 9%. From this percentage of the total cell protein and the amount of sialic acid and hexosamine found per mg of protein (Table II) the percentage of the total sialic acid and hexosamines of the whole cell that each of these fractions contributed was calculated. These percentages are given in Table I. The lysosomal fraction appears to be the only fraction which contributed significantly to the total cell content of sialic acid. In contrast, significant amounts of the hexosamines were present in the mitochondrial, microsomal and soluble fractions and represented 11, 14 and 25%, respectively, of the whole cell.

In Table II the sialic acid and hexosamine concentrations of the cell fractions are compared. The concentration of the sialic acid per mg of lysosomal protein (11 nmoles) approached the concentration of the surface membranes (20 nmoles). The other purified cell fractions contained considerably less sialic acid per mg of protein. The surface membranes also had a higher concentration of hexosamines than the other cell fractions.

TABLE II

THE CONCENTRATION OF SIALIC ACID AND HEXOSAMINES IN THE L CELL

The fractions were as described in the legend to Table I with the exception of mitochondria (Stn), microsomes (Stn) and soluble (Stn) which were prepared by standard techniques as described in METHODS. For mitochondria (Stn), the mean value for sialic acid represented 3 different preparations which were analyzed separately and 4 preparations which were combined for 1 analysis. The latter preparation was also used for hexosamine analysis. For microsomes (Stn) the value for sialic acid and hexosamines represented two preparations which were combined for 1 analysis each. For soluble (Stn) the value for sialic acid represented two preparations which were combined for 1 analysis. The standard error of the mean is given when applicable.

| *Cell fraction* | *Sialic acid (nmoles/mg protein)* | *Hexosamines (nmoles/mg protein)* | *Molar ratio Hexosamines/ Sialic acid* |
|---|---|---|---|
| Whole cell | 3.3 | 19.5 | 5.9 |
| Surface membranes ($Zn^{2+}$) | 20.0 | 59.3 | 3.0 |
| Nuclei | $>0.05$ | 1.7 | $>34$ |
| Mitochondria (purified) | $2.8 \pm 0.2$ | 29.8 | 13 |
| Microsomes ($Zn^{2+}$) | $2.4 \pm 0.5$ | 30.0 | 12.5 |
| Soluble ($Zn^{2+}$) | $1.0 \pm 0.4$ | 18.0 | 18 |
| Lysosomes | 11.0 | — | — |
| Mitochondria (Stn) | $5.1 \pm 0.2$ | 40.0 | 8 |
| Microsomes (Stn) | 12.2 | 43.0 | 3.5 |
| Soluble (Stn) | 1.2 | — | — |

*Sialic acid content of mitochondria, microsomes and soluble fractions prepared by different procedures*

Mitochondria which were prepared from L cells by a standard procedure (Stn) contained a higher concentration of sialic acid per mg of protein (5 nmoles) and hexosamine (40 nmoles) than the purified mitochondria which contained 2.8 nmoles of sialic acid and 30 nmoles of hexosamine per mg of protein (Table II). Repeated centrifugation through sucrose solutions[7] to obtain additional purification reduced the amount of sialic acid to 1.3 nmoles per mg of protein in the mitochondrial fractions. Centrifugation at $110000 \times g$ of the supernatant solutions obtained during the purification procedures showed that sialic acid was still in particulate form, as the pellets contained a high concentration of sialic acid per mg of protein (13 nmoles).

Included also in Table II is a comparison of microsomes prepared by two techniques. When microsomes ($Zn^{2+}$) were prepared from the same whole cell homogenate from which the surface membranes were isolated the concentration of sialic acid expressed per mg of protein was approximately a fifth of the concentration of sialic acid in the standard microsomal preparations (Stn) which were prepared directly from a cell homogenate. The concentration of hexosamines (per mg of protein) was reduced by approx. 30 %. The purification procedures were essentially the same but the microsomes prepared by standard techniques contained surface membrane fragments and therefore had a higher concentration of sialic acid per mg of protein. In contrast to the microsomal fractions, the soluble fractions prepared by both procedures contained similar concentrations of sialic acid per mg of protein (Table II).

*Molar ratio of hexosamine to sialic acid*

Table II gives the molar ratio of hexosamines to sialic acid for the various cell fractions. In general, it appears from the molar ratios, that the internal cell com-

ponents were richer in hexosamines than in sialic acid when compared to the surface membranes.

*Sialidase activity of the cell fractions*

*Whole homogenate.* L cells were homogenized as described in METHODS and the homogenate was incubated at 37° for 90 min. The whole cell glycoproteins served as substrates and under these conditions approx. 10 % of the sialic acid of the homogenized whole cells was cleaved. The pH optimum of the whole cell sialidase was pH 4.2. At pH 5.9 which was the pH of the cell homogenate for the preparation of surface membranes by the $Zn^{2+}$ procedure[2] less than 5 % of the total cell sialic acid was cleaved. The release of sialic acid was not detectable when incubation was at pH 5.9 for 12 h at 5°, *i.e.* conditions which simulated the preparation of surface membranes by the $Zn^{2+}$ procedure.

No *N*-acetylneuraminic acid aldolase activity was detected in the homogenate as sialic acid when added to the homogenate was recovered after 90-min or 16-h incubations at 37°.

*Lysosomes.* Lysosomes prepared from L cells and not completely purified (see METHODS) released 0.09 μmole of sialic acid per mg of lysosomal protein with fetuin as the substrate. Optimal enzyme activity was obtained with 4 mg of fetuin. Fig. 2A shows the activity of this less purified lysosomal preparation with increasing protein concentration. The activity was inhibited by 60 % in the presence of 10 mM $CaCl_2$ and 100 % in the presence of 50 mM $CaCl_2$. Lysosomes which were further purified as described in METHODS showed increased sialidase activity when expressed per mg of protein. The release of sialic acid at three different protein concentrations is shown in Fig. 2B.

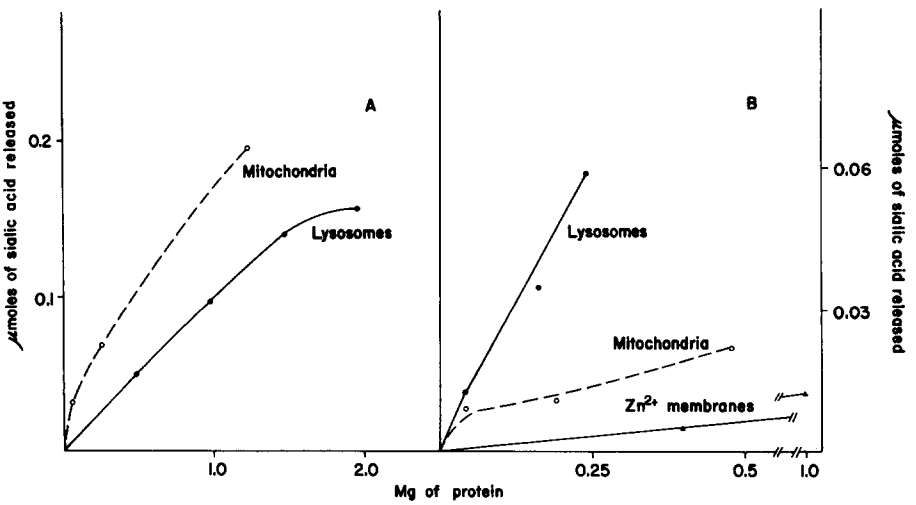


Fig. 2. Sialidase activity of fractions prepared from L cells. A. Lysosomes (●—●) and mitochondria (○---○) were prepared as described in METHODS as standard or less purified preparations. B. Lysosomes (●—●) and mitochondria (○---○) were prepared as described in METHODS as "purified" fractions. Surface membranes (▲—▲) were prepared by the $Zn^{2+}$ procedure. Sialidase was measured by the release of sialic acid from fetuin. All methods are described in the text. Note scale changes between A and B.

*Mitochondria.* Since a portion of the sialidase activity of the cell homogenate was discarded with the mitochondrial fraction further attempts were made to separate mitochondria and lysosomes and to determine if the mitochondria had sialidase activity. The less purified mitochondria showed sialidase activity comparable to the enzyme activity of the less purified lysosomes (Fig. 2A). When the mitochondrial fraction was further purified as described in METHODS the "purified" mitochondria (Fig. 2B) still retained approx. 20 % of the sialidase activity of the less purified mitochondrial preparations (Fig. 2).

The "purified" mitochondrial preparation was shown to have the activity of acid phosphatase. This was demonstrated by the linear appearance of *p*-nitrophenol from *p*-nitrophenol phosphate with increasing concentrations of mitochondrial protein. 1.0 mg of mitochondrial protein released 0.57 $\mu$mole of *p*-nitrophenol after 1-h incubation. At the same time 1 mg of homogenate protein released 0.14 $\mu$mole while the less purified lysosomes cleaved 0.41 $\mu$mole.

*Surface membranes.* When surface membranes, prepared by the $Zn^{2+}$ procedure, were incubated with fetuin as substrate under optimal conditions for sialidase activity, 0.012 $\mu$mole of sialic acid were released per mg of membrane protein (Fig. 2B). In contrast, when the surface membranes (2 mg of protein) were incubated with the endogenous glycoproteins as substrate under conditions of membrane isolation no release of sialic acid was detected. The amounts used were sufficient to measure the release of less than 5 % of the sialic acid of the surface membrane.

## DISCUSSION

Studies on the distribution of specific substances or enzyme activities in cell organelles are dependent on the ability to obtain purified cell fractions. Only after extensive purification and monitoring in the electron microscope can an organelle be ascertained to be reasonably homogeneous. At this point one can argue that a particular substance or activity actually belonged with the cell organelle but was removed during the purification procedure. In this study we have purified extensively fractions of the L cell and analyzed them for sialic acid and hexosamines.

We have reported previously[1] the average sialic acid content of whole cells and surface membranes and the extreme variation in the amount of sialic acid present in different cultures of L cells. The experiments showed that 40–80 % of the total sialic acid of the cell was found in the surface membrane. The preparation of surface membranes reported here represents a midpoint of these values, that is, 66 % of the total sialic acid (Table I). The variation in the sialic acid content of the whole cell has been discussed, and partially related to the number of times the cell divides in a particular culture[1]. In addition, experiments have shown that the content of sialic acid of KB cells increased above the normal levels prior to and in early mitosis, indicating a variation in glycoproteins and/or glycolipids throughout the division cycle of a cell[14]. The number of analyses performed on the other cell organelles was not sufficient to determine with certainty if sialic acid also fluctuated in these fractions. In addition some of the analyses which were reported for the other cell fractions were done on combined fractions of different cell cultures in order to obtain enough material for sialic acid and hexosamine determinations.

Nuclei prepared by the method reported here contained no detectable sialic

acid when carefully freed of contaminating membrane material (Fig. 1). Preparations which contained some membranous material as seen under the phase contrast microscope, also contained a small amount of sialic acid. The fact that the contaminating material still contained bound sialic acid argues against chemical removal of the sialic acid from the nuclei with the 0.1 M acetic acid solution at 5°. Others[15–17] have reported the presence of sialic acid in nuclear preparations, while KASHNIG AND KASPER[18] have found negligible amounts in membrane preparations of rat liver nuclei. It was a surprising finding that the nuclei from the L cells with a double membrane contained less than 0.6 % of the sialic acid and about 4 % of the hexosamines of the whole cell. This could indicate a significant difference in glycoprotein and glycolipid composition of the nuclear membrane when compared to other membranes of the cell. Indeed, in membrane preparations from rat liver nuclei, glucosamine was the only amino sugar present[18]. Sialyltransferase activities have been reported in cytoplasmic membrane fractions[19] so nuclei may not have many sialic acid-containing glycoproteins. In spite of the fact that nuclei contain so little bound sialic acid, nuclear preparations may be the sole site of the formation of cytidine 5'-monophosphosialic acid[20].

Table II shows that the soluble fraction from microsomes which were prepared by a standard procedure (Stn) contained a similar concentration of sialic acid per mg of protein (1.2 nmoles) when compared with the soluble fraction ($Zn^{2+}$) from microsomes prepared by the $Zn^{2+}$ procedure (1.0 nmole). This indicated that the $Zn^{2+}$ microsomes had a reduced amount of sialic acid as a result of removal of particulates during the purification procedure (probably in the form of contaminating lysosomes and membrane fragments) rather than a loss of soluble material directly from the microsomes.

The high concentration of sialic acid per mg of protein of the lysosomal fractions (11 nmoles/mg of protein) approaching that of the surface membranes (20 nmoles/mg of protein) could indicate that the lysosomal membrane is at least partially derived from the surface membrane[21]. THINÈS-SEMPOUX[22] has observed similarities in the phospholipids and cholesterol of plasma membranes and lysosomes of rat liver. A high level of carbohydrate in the lysosomes presumably as glycoprotein, has been suggested by the histochemical evidence of RAMBOURG[23].

The problem of determining the presence of low levels of a substance or the activity of an enzyme is particularly apparent in this study with the mitochondrial preparations. As the mitochondria were further purified, the concentrations of sialic acid per mg of protein decreased. It is possible that with the purification procedures the mitochondria lose their outer membranes which could contain sialic acid. Alternately, the less purified fractions of mitochondria containing larger amounts of sialic acid (5 nmoles per mg of protein) could be contaminated with lysosomes and fragments of surface membranes. The extensively purified fractions still contained a small amount of sialic acid (1.3 nmoles per mg of protein). The finding that these preparations contained acid phosphatase and sialidase and the fact that the sialic acid which was removed by purification was in particulate form makes it likely that there is some contamination with lysosomes. These findings make it impossible to draw conclusions as to the presence or absence of sialic acid in the mitochondria of the L cell. Using different cells, MOLNAR[16] and WU *et al.*[17] have reported the sialic acid and hexosamine content of mitochondrial fractions. Neither of these preparations were extensively purified. In studying the enzyme activities of a lysosomal fraction

of Ehrlich ascites tumor cells, HORVAT AND TOUSTER[24] encountered a similar difficulty in separating the mitochondrial and lysosomal fractions, but eventually accomplished the separation with detergent[25].

All of the purified cell fractions examined appeared to have a low level of sialidase activity but of these purified fractions only the lysosomes contained a higher specific activity than the less purified fraction (Fig. 2). Sialidase activity was not detected under conditions which simulated the isolation of surface membranes by the $Zn^{2+}$ procedure and probably does not contribute significantly to the variation in the amount of sialic acid found in the surface membranes.

Variation in the glycoprotein and/or glycolipid constituents of the cell organelles is indicated by the difference in the molar ratios of hexosamines to sialic acid (Table II). In general, the internal membrane systems appeared to have proportionately more hexosamines than sialic acid when compared to the surface membranes. The fact that the glycolipids of the surface membranes are different from the glycolipids of the whole cell has been reported[26]. Disialogangliosides and hematosides were found in the surface membranes from L cells while the whole cells contained, in addition to the above, monosialogangliosides and neutral glycolipid.

It appears from the experiments reported here and from the other literature cited that the glycoprotein and glycolipid components of membranes probably vary between different organelles within the same cell and indeed it appears that there is also variation within the same organelle. The reasons for this variation and the extent of the variation may become apparent when individual glycoproteins and glycolipids are isolated.

ACKNOWLEDGEMENTS

This research was supported by U.S. Public Health Service Grants 5-P01-AI07005-04 and 5-T01-AI00357-02 and Molecular Biology 5-P01-GM-00694-08 and American Cancer Society Grants No. PRA-68 and PRP-28.

We wish to thank Dr. Herbert Blough, Department of Microbiology for the electron micrograph of the nuclei. The excellent technical assistance of Mrs. Roberta Koser and Miss Annemarie Klein is gratefully acknowledged.

REFERENCES

1 M. C. GLICK, C. A. COMSTOCK AND L. WARREN, *Biochim. Biophys. Acta*, 219 (1970) 290.
2 L. WARREN AND M. C. GLICK, in K. HABEL AND N. P. SALZMAN, *Fundamental Techniques in Virology*, Academic Press, New York, 1969, p. 66.
3 M. C. GLICK AND L. WARREN, *Proc. Natl. Acad. Sci. U.S.*, 63 (1969) 563.
4 *Handbook of Chemistry and Physics*, 27th ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1943.
5 H. A. BLOUGH, J. M. TIFFANY, G. GORDAN AND M. FIALA, *Virology*, 38 (1969) 694.
6 M. M. K. NASS, *J. Mol. Biol.*, 42 (1969) 521.
7 P. M. VIGNAIS AND J. NACHBAUR, *Biochem. Biophys. Res. Commun.*, 33 (1968) 307.
8 P. L. SAWANT, S. SHIBKO, U. S. KUMTO AND A. L. TAPPEL, *Biochim. Biophys. Acta*, 85 (1964) 82.
9 L. WARREN, *J. Biol. Chem.*, 234 (1959) 1971.
10 N. F. BOAS, *J. Biol. Chem.*, 204 (1953) 353.
11 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
12 K. BURTON, *Biochem. J.*, 62 (1956) 315.
13 C. ARSENIS AND O. TOUSTER, *J. Biol. Chem.*, 243 (1968) 5702.
14 M. C. GLICK, E. W. GERNER AND L. WARREN, *J. Cell Physiol.*, 77 (1971) 1.

15 P. I. MARCUS, J. M. SALB AND V. G. SCHWARTZ, *Nature*, 208 (1965) 1122.
16 J. MOLNAR, *Biochemistry*, 6 (1967) 3064.
17 H. C. WU, E. MEEZAN, P. H. BLACK AND P. W. ROBBINS, *Biochemistry*, 8 (1969) 2509.
18 D. M. KASHNIG AND C. B. KASPER, *J. Biol. Chem.*, 244 (1969) 3786.
19 H. SCHACHTER, I. JABBAL, R. L. HUDGIN, L. PINTERIC, E. J. MCGUIRE AND S. ROSEMAN, *J. Biol. Chem.*, 245 (1970) 1090.
20 E. L. KEAN, *J. Biol. Chem.*, 245 (1970) 2301.
21 C. DE DUVE AND R. WATTIAUX, *Ann. Rev. Physiol.*, 28 (1966) 435.
22 D. THINÈS-SEMPOUX, *Biochem. J.*, 105 (1967) 20p.
23 A. RAMBOURG, *J. Microscopie*, 8 (1969) 325.
24 A. HORVAT AND O. TOUSTER, *Biochim. Biophys. Acta*, 148 (1967) 725.
25 A. HORVAT, J. BAXANDALL AND O. TOUSTER, *J. Cell Biol.*, 42 (1969) 469.
26 D. W. WEINSTEIN, J. B. MARSH, M. C. GLICK AND L. WARREN, *J. Biol. Chem.*, 245 (1970) 3928.