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Enzymatic Block in the Synthesis of Gangliosides in DNA Virus-Transformed Tumorigenic Mouse Cell Lines

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Abstract. The ganglioside pattern of both SV40- and polyoma virus-transformed mouse cell lines differs from that of the parent cell lines or of cell lines that have transformed spontaneously in tissue culture. This is manifested by a dramatic decrease of gangliosides with an oligosaccharide chain larger than sialyllactose. Present investigations indicate that this change probably cannot be attributed to excessive catabolism of gangliosides, but is caused by impaired synthesis of tri- and tetrahexosyl gangliosides in the virus-transformed cell lines. We present evidence for the block of a required step for the biosynthesis of these ganglioside homologs. The block involves the enzyme catalyzing the transfer of *N*-acetylgalactosamine from uridine diphosphate *N*-acetylgalactosamine to hematosides (*N*-glycolylneuraminyl or *N*-acetylneuraminylgalactosylglucosyl ceramide). This well-defined enzymatic change opens the way for studies of the biochemical mechanism of the alteration of cell membranes which occurs after transformation by the tumorigenic DNA viruses polyoma and SV40.

Ever since it was recognized that the closely similar small DNA viruses, polyoma and simian virus 40 (SV40), which induce tumors in rodents, also have many similarities when heritably altering the growth properties of cells in culture, it was a primary aim to find a well-defined common biochemical change which characterizes cell transformation in culture. We have reported recently[1,2] that mouse cell lines which have been transformed by SV40 or polyoma virus exhibit marked changes of ganglioside composition compared with the respective parent cell line or cell lines which have transformed spontaneously in tissue culture. This alteration is characterized by a profound decrease in the quantity of the higher ganglioside homologs *N*-acetylneuraminyl-galactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide (G_{D1a}) and galactosyl-*N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide (G_{M1}). In the more recent study,[2] a decrease was also observed in the quantity of *N*-acetylgalactosaminyl-[*N*-acetylneuraminyl]-galactosylglucosylceramide (G_{M2}) in SV40- and polyoma virus-transformed Swiss mouse 3T3 cell line compared with the parent cell line. The major gangliosides in the DNA virus-transformed cell lines are *N*-acetylneuraminylgalactosylglucosylceramide and *N*-glycolylneuraminylgalactosylglucosylceramide

(hematosides; $G_{M3}NAc$ and $G_{M3}NGlyc$ respectively). The concentration of these compounds in the virally transformed cell lines is generally similar to or slightly higher than that of the parental cell lines.

When the biosynthesis of gangliosides in intact cells was examined by the addition of labeled precursors of these glycolipids to the tissue culture medium, striking differences were found in the labeling pattern of gangliosides in the control and SV40-transformed cell lines. The distribution of radioactivity amongst the various gangliosides was completely consistent with the previous analytical observations. With either *N*-[^{3}H]acetyl-D-mannosamine or *N*-[^{3}H]acetyl-D-glucosamine as precursors, most of the radioactivity in recovered gangliosides was found in G_{D1a} and G_{M1} in the control cell lines. However, in SV40-transformed cells grown under identical conditions, only the G_{M3} derivatives contained significant radioactivity.[2]

At least two alternative phenomena must be considered in order to account for the differences in the composition and pattern of labeling of gangliosides in the DNA virus-transformed cell lines. The first of these possibilities is an increase in the catabolism of the higher ganglioside homologs; the second is an impairment of the biosynthesis of these gangliosides in the virally transformed cell lines.

The data obtained in the present experiments support the latter postulate. Furthermore, we have localized this biochemical change as a block of at least one enzymatic reaction required for the lengthening of the oligosaccharide chain of gangliosides.

Materials and Methods. Cell lines: The established mouse cell lines employed have been described in detail.[1,2] The seeds of the AL/N mouse cell lines were donated by Dr. K. K. Takemoto. N AL/N line is a "normal" epithelial clonal cell line from inbred AL/N mouse embryo kidney.[3] These cells in early passages exhibit low "saturation density" in tissue culture[1] and no tumorigenicity.[3] T AL/N cells are tumorigenic cells which came about by "spontaneous" transformation in tissue culture after prolonged passage; they have about twice the saturation density of the parent N AL/N cells.[1] SVS AL/N cells were obtained[3] by transformation of the N AL/N cells in tissue culture by a small plaque-forming variant of SV40. They have three times the saturation density of the N AL/N cells.[1] They are tumorigenic in the syngeneic mouse when the host is rendered immunologically incompetent.[3,4] They carry SV40-specific T antigen[3] and also the transplantation rejection antigen[4] and a rescuable virus genome.[5] Py AL/N cells were obtained from N AL/N cells after transformation with the polyoma virus. They carry the polyoma-specific T antigen (K. K. Takemoto, personal communication) and in their tissue-culture growth and *in vivo* characteristics generally are similar to the SVS AL/N line.[2]

The following Swiss cell lines were donated by Dr. R. E. Pollack. The Swiss 3T3 line (clone 421) is an aneuploid fibroblast cell line from randomly bred Swiss mouse embryo. These cells were cultivated under conditions designed to maintain their very low saturation density (or high sensitivity to "contact inhibition of cell division") in culture.[6] The SV40-transformed derivative line (Swiss SV 101 clone) has a saturation density approximately 11 times that of 3T3 cells.[7] The polyoma virus-transformed derivative cell line (Swiss Py 11) is a similar clonal isolate. Both virus-transformed Swiss cell lines carry the virus-specific T antigen (R. E. Pollack, personal communication).

All cell lines were monitored for mycoplasma infection. We excluded the possibility that the biochemical results in this communication were affected by the periodically observed mycoplasma in some of the cell lines. The definitive experiments reported below

were done on cells which were demonstrated to be free of mycoplasma. Also, there was no difference in the results between infected and uninfected cell cultures (Brady, unpublished results).

Medium and growth conditions: The AL/N cell lines were cultivated in 2-liter glass roller bottles in fortified Eagle's medium in the presence of 10% fetal bovine serum as described.[1,2] The Swiss lines were cultivated similarly in the Dulbecco-Vogt modification of Eagle's medium.[1,2] Cells were harvested with saline as described, when in similar rapid growth, just before reaching a confluent monolayer. The physiological stage of growth in the various cell lines was therefore similar and the ganglioside pattern was reproducible whether the cell lines were or were not eventually subject to inhibition of growth in a monolayer.[1,2]

Assay of ganglioside catabolism: Gangliosides were labeled in the *N*-acetylneuraminyl portion of the molecule by the intracerebral administration of *N*-[^{3}H]-acetyl-D-mannosamine to weanling rats.[8] Mixed radioactive gangliosides were separated into individual components by column chromatography on silicic acid to provide a homologous series of radioactive gangliosides. The catabolism of labeled gangliosides was measured in homogenates of freshly harvested cells which had been washed twice with an isotonic solution of NaCl. The cell pellets were suspended in 10 volumes (w/v) of 0.25 M sucrose solution containing 0.001 M disodium EDTA. The cells were disrupted with 12 up-and-down strokes of the pestle in an all-glass TenBroeck homogenizer. The suspensions were centrifuged at 600 $\times$ *g* for 15 min and the enzymatic hydrolysis of the labeled gangliosides was determined in both the supernatant fraction and in the pellet, which was resuspended with homogenization as above in an equivalent of the original volume of sucrose EDTA solution. The incubation mixtures contained the disrupted cell preparations (0.15–0.70 mg of protein), 10 μmol of potassium acetate buffer (pH 5.0), 10–47 nmol of labeled ganglioside (745 cpm/nmol of sialic acid), and water in a final volume of 0.1 ml. The tubes were stoppered and incubated with shaking for 2 hr at 37°C. The reaction was terminated by adding 0.75 ml of cold water, 0.05 ml of a solution of human serum albumin (100 mg/ml), and 0.1 ml of a 100% solution of trichloroacetic acid. The suspensions were clarified by centrifugation, the supernatant solutions were decanted, and the precipitated protein was washed with 1 ml of cold 10% trichloroacetic acid solution. The suspensions were centrifuged and the supernatant solutions were combined with those obtained from the preceding step. The unhydrolyzed gangliosides coprecipitated with the denatured serum albumin. The enzymatically released radioactivity was determined in 1-ml aliquots of the combined supernatant solutions by liquid scintillation spectrometry. Boiled enzyme controls were run with each incubation.

Assay of uridine diphosphate *N*-acetylgalactosamine: hematoside *N*-acetylgalactosaminyltransferase. Preparation of substrates: *N*-acetylneuraminylgalactosylglucosylceramide ($G_{M3}NAc$) and *N*-glycolylneuraminylgalactosylglucosylceramide ($G_{M3}NGlyc$) were obtained from dog and horse erythrocytes respectively. The procedures employed for the extraction and purification of these substances were essentially those of Yamakawa *et al.*[9] and Handa *et al.*[10] for the respective compounds. These substrates were further purified by preparative thin-layer chromatography followed by passage of the recovered lipids over a column of Sephadex G-25 in order to eliminate the last traces of nonlipid contaminants.[11] Chemical analysis of the two hematosides gave theoretical values for the respective sialic acids and lactose. Both sialic acid moieties were completely hydrolyzed by *Vibrio cholera* neuraminidase. The identity of the liberated sialic residue was established by analytical thin-layer chromatography with authentic *N*-acetylneuraminic acid and *N*-glycolylneuraminic acid as standards.[12] The other product of the neuraminidase reaction cochromatographed with lactosylceramide on thin-layer chromatography.

Enzyme preparation: The harvested cells were washed three times with cold saline and were suspended in 10 volumes of ice-cold 0.25 M sucrose in 0.1 M Tris·HCl-buffered saline (pH 7.4) containing 0.001 M Mg^{++}. In the experiments in Table 3 in which total cell homogenates were used, the sucrose concentration was 0.06 M. The

cells were ruptured by sudden decompression from 800 psi to 1 atmosphere.[13] A "crude" nuclear fraction was sedimented by centrifugation at 250 × *g* for 5 min. The supernatant was made 0.002 M in EDTA to avoid the aggregation of microsomes which occurs in the presence of Mg^{++} and a mitochondria-rich fraction was pelleted by centrifugation at 20,000 × *g* for 10 min. The supernatant was diluted four-fold with Tris-buffered saline and then made 0.004 M in Mg^{++}; the microsomal fraction was obtained after centrifugation for 90 min at 105,000 × *g*. The pellets were resuspended in various quantities of 0.2 M sodium cacodylate–HCl buffer solution (pH 7.3) so that the protein concentration ranged from 2.5 to 6.25 mg/ml.

Enzyme assay: Portions of the various hematoside acceptors (0.1 μmol) in chloroform-methanol, 2:1 (v/v) were taken to dryness under a stream of nitrogen. To the dried residue were added 40 μl of a solution of Triton X-100 (12.5 mg/ml), 5 μmol of sodium cacodylate–HCl buffer (pH 7.3), 2.5 μmol of $MnCl_2$, 0.6 nmol of UDP-*N*-acetyl-[1-^{14}C]galactosamine (43 mCi/mmol, New England Nuclear), 40 μl of enzyme preparation (0.10–0.25 mg of protein), and water in a final volume of 0.12 ml. The mixtures were incubated for 3 hr at 37°C and the reaction was stopped by the addition of 2.4 ml of chloroform–methanol 2:1. The suspensions were shaken and allowed to stand overnight. The precipitated protein was removed by filtration through Whatman #50 filter paper and the retentate and filter paper were washed with 2 ml of chloroform–methanol–water 60:30:4.5 (CMW). The filtrate was passed over a 1 × 7 cm column of superfine Sephadex G-25 previously equilibrated with the chloroform–methanol–water solution to remove ions and unreacted UDP-[^{14}C]GalNAc. The column was washed with 4 ml of the same solvent after all of the filtrate had penetrated the column. The effluent solution was taken to dryness with mild warming and the radioactivity was determined on a suitable aliquot by liquid scintillation spectrometry.

Results. The initial step in the biological degradation of the major gangliosides (G_{D1a} and G_{M3}) of the cultured mouse cell lines is the enzymatic cleavage of the terminal molecules of sialic acid from these glycolipids. The catabolism of G_{M1}, the third major ganglioside, occurs primarily via the enzymatic hydrolysis of the terminal molecule of galactose, although cleavage of the *N*-acetylneuraminyl moiety does occur to a small extent.[14] A systematic investigation of the enzymatic hydrolysis of the *N*-acetylneuraminic acid residues of these gangliosides was undertaken in parent cell lines and in SV40-transformed cell lines. There was no indication of excessively rapid ganglioside catabolism in the DNA virus-transformed cells (Table 1).

The observation that increased catabolism of gangliosides probably could not account for the change in the ganglioside pattern of the virally transformed

TABLE 1. *Enzymatic hydrolysis of sialic acid-labeled gangliosides.*

Expt.	Substrate concentration (M × 10^{-4})		Cell line: N AL/N (nanomol of sialic acid cleaved per mg of protein per hr)*	Cell line: SVS AL/N (nanomol of sialic acid cleaved per mg of protein per hr)*
1	G_{D1a}	1.1	0.68	0.89
2	"	0.93	0.56	0.99
3	"	2.2	0.78	0.50
1	G_{M1}	0.76	0.08	0.10
2	"	1.0	0.12	0.11
3	"	2.3	0.08	0.11
1	G_{M3}	1.3	1.1	1.3
2	"	2.1	1.7	1.6
3	"	4.7	4.8	7.6

* Combined activity in pellet and supernatant fractions.

cell lines, coupled with the evidence in previous experiments that only hematosides became labeled when radioactive precursors of gangliosides were added to the tissue culture medium, suggested that the enzyme which catalyzed the next step in the synthesis of higher ganglioside homologs might be impaired in the virus-transformed cells. This enzyme catalyzes the transfer of a molecule of *N*-acetylgalactosamine from uridine diphosphate *N*-acetylgalactosamine (UDP-GalNAc) to hematoside (G_{M3}) to form G_{M2} (Reaction 1):[15]

1. Ceramide-Glc-Gal-NANA + UDP-GalNAc $\xrightarrow{\text{transferase}}$ ceramide-Glc-Gal (NANA)-GalNAc + UDP.

Accordingly, an investigation of the activity of this *N*-acetylgalactosaminyl transferase was undertaken in whole homogenates and in subcellular fractions obtained from control, spontaneously transformed, and SV40- and polyoma-transformed mouse cell lines. Under the assay conditions employed in the present experiments, the activity of this transferase is indicated by the Δ increase in the amount of radioactivity incorporated from UDP-[1-^{14}C]GalNAc when glycolipid acceptor was added to the incubation mixture. The activity of this enzyme was drastically reduced (Tables 2 and 3) in preparations obtained from the DNA virus-transformed AL/N and Swiss mouse cell lines compared with that in the parental cell lines and cell lines which had transformed spontaneously in culture. Nearly all (>90%) of the enzyme was found associated with the various particulate subcellular fractions. No increase in enzymatic activity

TABLE 2. *Uridine diphosphate N-acetylgalactosamine:hematoside N-acetylgalactosaminyl-transferase activity in AL/N mouse cell lines.*

Expt.	Cell line	Subcellular fraction	Glycolipid acceptor: None	G_{M3}NAc	Δ	G_{M3}NGlyc	Δ
			Radioactivity incorporated (cpm/mg Protein)				
1.	N AL/N	Nuclear	1,370	7,220	5,850	...	...
	"	Mitochondrial	67	7,320	7,260	...	...
	"	Microsomal	0	13,200	13,200	...	...
	T AL/N	Nuclear	6,770	29,980	23,200	...	...
	"	Mitochondrial	6,060	25,200	19,100	...	...
	"	Microsomal	5,330	30,200	24,900	46,800	41,400
	SVS AL/N	Nuclear	6,400	7,500	1,100	...	...
	" "	Mitochondrial	6,130	6,170	40	...	...
	" "	Microsomal	15,500	15,300	...	...	...
	Py AL/N	Nuclear	3,270	3,900	630	...	...
	" "	Mitochondrial	10,600	12,000	1,370	...	...
	" "	Microsomal	13,300	12,800	...	...	...
2.	T AL/N	Mitochondrial	14,400	...	...	78,900	64,500
	"	Microsomal	7,590	...	...	25,200	17,600
	SVS AL/N	Mitochondrial	8,920	...	...	13,700	4,800
	" "	Microsomal	6,940	...	...	8,640	1,700
	Py AL/N	Mitochondrial	8,090	...	...	10,200	2,100
	" "	Microsomal	7,320	...	...	6,080	...
3.	T AL/N	Microsomal	15,100	...	...	49,200	34,100
	SVS AL/N	"	1,750	...	...	3,220	1,470
4.	T AL/N	"	14,800	...	...	40,300	25,500
	SVS AL/N	"	59	...	...	1,190	1,130

The figures are corrected for radioactivity in controls without enzyme (100–200 cpm).

TABLE 3. *Uridine diphosphate N-acetylgalactosamine:hematoside N-acetylgalactosaminyltransferase activity in Swiss mouse cell lines.*

Cell line	Glycolipid acceptor: None	$G_{M3}NAc$	Δ	$G_{M3}NGlyc$	Δ
	Radioactivity incorporated (cpm/mg of protein)				
Swiss 3T3	1,280	11,100	9,820	15,600	14,300
" SV 101	642	3,860	3,220	4,930	4,290
" Py 11	2,950	5,510	2,560	3,260	310

Whole homogenates of the various cells were used as the source of enzyme in these experiments. The values are corrected for radioactivity in controls without enzyme.

was found in the supernatant solutions obtained from the virally transformed cells. Previous experiments indicated that the mouse cell lines in question contained both *N*-acetylneuraminyl and *N*-glycolylneuraminyl gangliosides.[1,2] Both of these types of G_{M3} were found effective as acceptors in the transferase reaction (Tables 2 and 3 and Fig. 1). When $G_{M3}NAc$ was included in the reaction mixture, the radioactive product cochromatographed with authentic G_{M2} obtained from human brain tissue of patients with Tay-Sachs disease. When $G_{M3}NGlyc$ was employed, the R_f of the radioactive product was slightly less than that of the authentic $G_{M2}NAc$ standard, a finding which is consistent with the migration of a slightly more polar derivative (in this case the presence of one additional hydroxyl group). Completely characterized, authentic *N*-

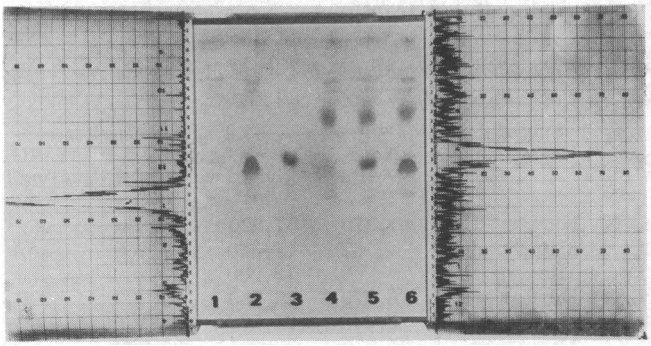

FIG. 1. Identification of the products of the uridine diphosphate *N*-acetylgalactosamine: hematoside *N*-acetylgalactosaminyltransferase reaction. Aliquots (0.2) of the effluent solutions from the Sephadex G-25 columns obtained from the T AL/N microsomal enzyme preparation in Expt. 1, Table 2 were dried under N_2 and the residues were redissolved in 30-μl portions of chloroform–methanol 2:1. The radioactive products were applied to a Silica Gel G thin-layer chromatogram and the plate was developed with chloroform–methanol–10% NH_4OH 60:35:8. After the plate was dried, the migration of the labeled compounds was determined with a Berthold chromatogram scanner (Varian Aerograph, Palo Alto, California) and the plate was then sprayed with resorcinol reagent.[16] *Lane 1*, reaction mixture without added glycolipid acceptor; *Lane 2*, same, but with $G_{M3}NGlyc$ as acceptor; *Lane 3*, authentic G_{M2} standard; *Lane 4* is similar to Lane 2 except that $G_{M3}NAc$ was the acceptor; *Lane 5* is the same as Lane 4, but authentic unlabeled G_{M2} was added as internal standard prior to the application of the product of the reaction to the thin-layer chromatogram; *Lane 6* is a mixture of equal aliquots of the solutions applied to *Lanes 2 and 4*. The radioactivity scan of *Lane 2* is shown at the left of the thin-layer plate and the scan of *Lane 4* is shown at the right. The predominantly staining spots in *Lanes 2 and 4* are the added glycolipid acceptors $G_{M3}NGlyc$ and $G_{M3}NAc$ respectively.

glycolylneuraminyl G_{M2} was not available for exact comparison. The nature of the radioactive product(s) which is formed in the absence of added glycolipid acceptor is unknown at present. This radioactive material does not migrate from the origin with the solvent system employed and in this area no bluish-purple color characteristic of gangliosides was detected with the resorcinol reagent.

An investigation was undertaken to determine whether the decrease in the UDP-*N*-acetylgalactosaminyl:hematoside *N*-acetylgalactosaminyltransferase activity was due to the presence of an inhibitory substance in extracts of the virus-transformed cells. When equal portions of microsomal preparations from T AL/N and SVS AL/N cells were incubated with UDP-*N*-acetyl-[1-^{14}C]galactosamine and G_{M3}NGlye as the glycolipid acceptor, 3890 cpm was incorporated into the radioactive reaction product. The predicted value based on the counts incorporated in aliquots of the respective microsomal preparations incubated separately was 4110 cpm. Therefore the decrease in *N*-acetylgalactosaminyl transferase activity is probably not due to the presence of an inhibitor of the enzyme in the subcellular preparation of the SV40-transformed cells.

Discussion. The present experiments clearly demonstrate in both SV40- and polyoma virus-transformed mouse cell lines a profound diminution of the activity of the enzyme that is required for the synthesis of gangliosides having an oligosaccharide moiety larger than sialyllactose. This observation is consistent with the marked decrease in the quantity of tri- and tetrahexosyl ganglioside homologs in the DNA virus-transformed cells and with the confinement of radioactivity to *N*-acetylneuraminyl and *N*-glycolylneuraminyl G_{M3} when labeled precursors of gangliosides are added to the tissue culture medium in which the virus-transformed cells are grown.[2] Attenuation of the UDP-*N*-acetylgalactosamine:hematoside *N*-acetylgalactosaminyltransferase was seen when either the naturally occurring *N*-acetylneuraminyl or *N*-glycolylneuraminyl G_{M3} was used as acceptor.

A number of possibilities must be considered when attempting to account for the diminution of the glycolipid *N*-acetylgalactosaminyltransferase in the virally transformed cell line. The data appear to rule out the presence of an inhibitor of this enzyme in the transformed cell preparation; the biochemical lesion is therefore likely to be an alteration of the biosynthesis of the enzyme or steps which precede its formation. Among the feasible alternatives are the following: the synthesis of the enzyme may be repressed, a catalytically inactive protein may be synthesized, or the enzyme may be converted to an inactive form after its synthesis. The various possibilities are currently under investigation. We shall also examine whether the subsequent steps for the formation of the higher ganglioside homologs are similarly attenuated in the virus-transformed cell lines.

In very recent experiments we have observed a tendency for the pattern of gangliosides to return towards normal in a flat (contact-inhibited) "revertant" cell line, derived from SV40-transformed cells. The variant cells still carry the virus genome, but phenotypically exhibit normal growth properties in culture.[7] Together with the recovery of a normal ganglioside pattern there appears to be

a concomitant return of UDP-*N*-acetylgalactosamine:hematoside *N*-acetylgalactosaminyltransferase activity in extracts obtained from the flat variant cell line.[17]

We conclude that the alteration of ganglioside synthesis in mouse cell lines transformed in tissue culture by tumorigenic SV40 and polyoma virus is an unambiguous biochemical effect shared by these viruses. One biochemical lesion was localized as a specific block in the enzymatic pathway in the synthesis of higher gangliosides. Highly purified plasma membrane preparations were obtained in too low yields for quantitation of gangliosides in the cell lines investigated.[18] In the present work we report on the biochemical changes in the gangliosides of both inner and peripheral membranes. However, it has recently been demonstrated that these complex lipids occur in especially high concentration in the plasma membrane of cells.[19,20] Thus, these observations provide an opportunity for clarifying the exact biochemical change, which, while distal to the viral DNA, is common in both DNA virus-induced tumorigenic transformations. This effect occurs on the protein (enzyme) biosynthesis level that causes a change on the surface of the transformed cells, and is apparently pertinent to the regulation of the growth of the cells when in contact with each other.

Abbreviations: $G_{M3}NAc$ and $G_{M3}NGlyc$, *N*-acetyl and *N*-glycolylneuraminylgalactosylglucosylceramides.

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[1] Mora, P. T., R. O. Brady, R. M. Bradley, and V. W. McFarland, *Proc. Nat. Acad. Sci. USA*, **63**, 1290 (1969).
[2] Brady, R. O., and P. T. Mora, *Biochim. Biophys. Acta*, in press.
[3] Takemoto, K. K., R. C. Y. Ting, H. L. Ozer, and P. Fabish, *J. Nat. Cancer Inst.*, **41**, 1401 (1968).
[4] Smith, R. W., J. Morganroth, and P. T. Mora, *Nature*, in press.
[5] Takemoto, K. K., G. J. Todaro, and K. Habel, *Virology*, **35**, 1 (1968).
[6] Todaro, G. J., and H. Green, *J. Cell Biol.*, **17**, 299 (1963).
[7] Pollack, R. E., and M. M. Burger, *Proc. Nat. Acad. Sci. USA*, **62**, 1074 (1969).
[8] Kolodny, E. H., R. O. Brady, J. M. Quirk, and J. N. Kanfer, *J. Lipid Res.*, **11**, 144 (1970).
[9] Yamakawa, T., R. Irie, and M. Iwanaga, *J. Biochem. (Tokyo)*, **48**, 490 (1960).
[10] Handa, S., and T. Yamakawa, *Japan J. Exp. Med.*, **34**, 293 (1964).
[11] Wells, M. A., and J. C. Dittmer, *Biochemistry*, **2**, 1259 (1963).
[12] Schoop, H. J., R. Schauer, and H. Faillard, *Z. Physiol. Chem.*, **350**, 155 (1969).
[13] Wallach, D. F. H., and V. B. Kamat, in *Methods in Enzymology*, eds. S. P. Colowick and N. O. Kaplan (New York: Academic Press, 1966), vol. 8, p. 164.
[14] Kolodny, E. H., J. F. Tallman, and R. O. Brady, manuscript in preparation.
[15] Steigerwald, J. C., B. Kaufman, S. Basu, and S. Roseman, *Federation Proc.*, **25**, 587 (1966).
[16] Svennerholm, L., *Biochim. Biophys. Acta*, **24**, 604 (1957).
[17] Mora, P. T., R. O. Brady, and F. A. Cumar, unpublished observations.
[18] Mora, P. T., R. O. Brady and R. W. Smith, *Proceedings, Xth International Cancer Congress*, Houston, USA (1970).
[19] Klenk, H.-D., and P. W. Choppin, *Proc. Nat. Acad. Sci. USA*, **66**, 57 (1970).
[20] Renkonen, O., C. G. Gahmberg, K. Simons and L. Kääriäinen, *Acta Chem. Scand.*, **24**, 733 (1970).