effect. During historic times this has not occurred, and the deep-sea core record has not been adequately correlated on a global scale, nor adequately resolved on the vertical time scale, to determine whether such a high frequency of explosive volcanism occurred during the Cenozoic. It is true, however, that some explosive volcanism in the geologic past greatly exceeded in magnitude that in the historic past. Such events, when occurring at critical times of climate evolution, might have strongly modulated the intensity of climate change. We believe that a careful global chronology of explosive volcanism should be developed from available piston and DSDP core data, together with a related chronology of climate evolution. Only a study of this kind can settle the question of the possible influence of volcanism on climate.

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Biosynthesis and Function of Gangliosides

Gangliosides appear to participate in the transmission of membrane-mediated information.

Peter H. Fishman and Roscoe O. Brady

Gangliosides comprise a family of acidic glycolipids that are characterized by the presence of sialic acid. They are unusual compounds in that they contain both hydrophilic and hydrophobic regions, and they bear a strong negative charge. Gangliosides are primarily membrane components, and plasma cell membranes are highly enriched in these materials. The carbohydrate portion of gangliosides is made up of molecules of sialic acid, hexoses, and *N*-acetylated hexosamines. The hydrophobic moiety is called ceramide, and it consists of a long-chain fatty acid linked through an amide bond to the nitrogen atom on carbon 2 (C-2) of the amino alcohol sphingosine. Oligosaccharides are linked through a glycosidic bond to C-1 of the sphingosine portion of ceramide. The structure of one of the more common gangliosides called G_{M1} is illustrated in Fig. 1.

Gangliosides were first identified in brain more than 30 years ago by Klenk (*1*). In the ensuing years, much effort has been devoted to establishing the structures and physical properties of gangliosides. Within the past few years, the individual steps involved in the biosynthesis of gangliosides have been elucidated (*2–4*), and the reactions involved in the impaired catabolism of these compounds in heritable metabolic disorders such as Tay-Sachs disease have been established (*5*). Until recently, however, knowledge of the function of these components was extremely limited. Good evidence that these substances play significant roles in membrane-related phenomena is now available. Since the ceramide portion of the ganglioside molecule is embedded in the fluid phase of membranes and the negatively charged oligosaccharide chain is exposed to the external environment (*6*), these substances are well suited to participate in external signals and other events that occur on the surfaces of cells. In this article we briefly review current knowledge regard-

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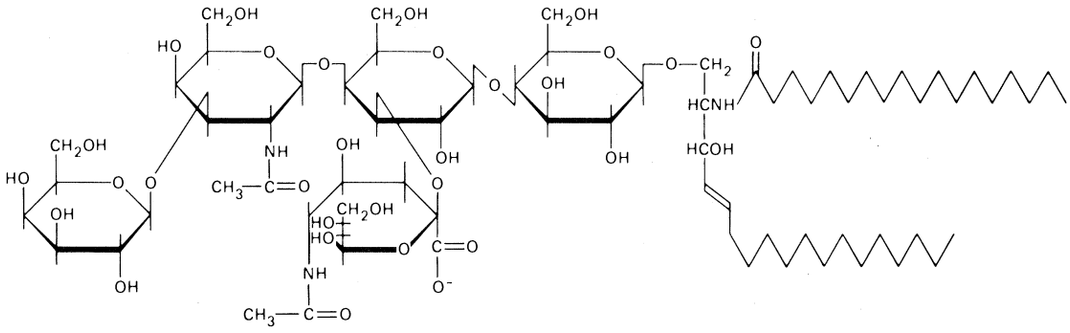

Fig. 1. Structure of the monosialoganglioside G_{M1}. Abbreviations of gangliosides are those of Svennerholm (*109*).

ing the biosynthesis of gangliosides, describe several instances of abnormalities of ganglioside formation, and indicate the detrimental consequences of such impairment. We then summarize recent observations on the physiological roles of gangliosides and offer some predictions concerning other potential functions of these important membrane components.

Biosynthesis of Gangliosides

We discuss here only the reactions involved in the biosynthesis of the oligosaccharide chains of gangliosides which appear to be the functional components of these complex lipids. The sequential addition of monosaccharide residues is catalyzed by enzymes known as glycosyltransferases. Each reaction involves the transfer of a sugar residue from a sugar nucleotide donor (XDP-monosaccharide) to an acceptor

XDP-glycose + acceptor →

XDP + glycose-acceptor

The pathway for the biosynthesis of the major gangliosides has been worked out in detail by Roseman (*2*) and Basu *et al.* (*3*) and in our laboratory (*4*) (Fig. 2).

The addition of the sugar residues appears to be closely ordered, and each glycosyltransferase is highly specific. Thus, although G_{M2} is a branched molecule (Fig. 2), the sialic acid residue is added before the addition of *N*-acetylgalactosamine. This pathway has been demonstrated in vitro; lactosylceramide is a poor substrate for *N*-acetylgalactosaminyltransferase activity compared to G_{M3} (*7–9*). Furthermore, G_{A2} (*N*-acetylgalactosaminylgalactosylglucosylceramide) is a poor acceptor for sialyltransferase activity compared to lactosylceramide (*4*). However, the specificity of these enzymes may not be absolute. The appearance of minor gangliosides may be a consequence of this incomplete specificity. *N*-Acetylneuraminylgalactosylceramide has been isolated in small quantities from human brain (*10*). The in vitro biosynthesis of this ganglioside from galactosylceramide and cytidine monophosphate (CMP)–sialic acid has been demonstrated (*11*). Mouse brain microsomes were used as the enzyme source; the sialyltransferase activity appeared to be identical to the enzyme that catalyzes reaction 3a in Fig. 2. The apparent Michaelis constant (K_m) for lactosylceramide was tenfold less than for galactosylceramide. However, the sequence of reactions in the pathway establishes some additional constraints on enzyme specificity. In vitro synthesis of an isomer of G_{M1} with the structure *N*-acetylneuraminylgalactosyl-*N*-acetylgalactosaminylgalactosylglucosylceramide has been observed (*12*), but this ganglioside has not been detected in vivo. Presumably, the same sialyltransferase that converts G_{M1} to G_{D1a} (Fig. 2, reaction 6a) also catalyzes this reaction; but nonavailability of asialo-G_{M1} accounts for absence of the G_{M1} isomer in vivo. As is indicated in Fig. 2, the biosynthesis of disialogangliosides proceeds by analogous reactions (*7, 13*), commencing with formation of G_{D3} (*14*). Recently, a sialyltransferase activity has been identified in vitro that converts G_{D1b} to trisialoganglioside G_{T1} (Fig. 2, reaction 6b) (*15*). These reactions were examined with exogenous glycolipids as acceptors. Experiments with endogenous acceptors (*16*) as well as studies in vivo (*17*) suggest that interactions between enzymes and substrates may be more complex than indicated by the pathway in Fig. 2.

Cellular Localization of Ganglioside Glycosyltransferases

In the early studies of these biosynthetic reactions, developing chick (*2, 3*) and rat brains (*4*) were the sources of the enzymes. Similar glycosyltransferase activities have been detected in liver (*8*), mammary gland (*18*), thyroid gland (*19*), and a variety of cultured cells (*20*).

The localization of glycosyltransferases within cells is somewhat controversial. Keenan *et al.* demonstrated that in liver these enzymes are associated with the Golgi apparatus (*8*). The situation seems complicated in brain. Some studies indicated that these biosynthetic enzymes are associated with synaptosomes (*21*), while others suggested that the enzymes are localized in the microsomes and myelin [for a recent review, see (*4*)]. In cultured cells, there is some evidence that a portion of the glycosyltransferase activities is part of the plasma membrane. The status of cell surface glycosyltransferases has been reviewed (*22*). In most studies, transfer of sugars from exogenous donors to endogenous acceptors on the cell surface has been detected; however, the reaction products have not been identified. Patt and Grimes observed the synthesis of G_{M3}, G_{M2}, and G_{D1a} when intact mouse cells were incubated with the appropriate sugar nucleotide donors (*23*). Yogeeswaran *et al.* were able to demonstrate surface sialyltransferase activity (*24*). The acceptor was lactosylceramide immobilized on glass, and, in the presence of CMP–sialic acid, the transfer of sialic acid could be demonstrated with intact hamster cells.

Regulation of Ganglioside Structure and Synthesis

The ganglioside composition of various tissues and cultured cells is dictated by and is a reflection of the glycosyltransferases found within them. Brain has the highest quantity of lipid-bound sialic acid and a complex ganglioside pattern. All of the enzyme activities indicated in Fig. 2 are readily detected in brain. Most extraneural tissues contain mainly G_{M3} and G_{D3} and have very low (or lack) uridine diphosphate (UDP)-*N*-acetylgalactosamine : G_{M3} *N*-acetylgalactosaminyltransferase activity (*4*). This enzyme catalyzes a key reaction in the synthesis of the more complex ganglio-

sides and is thus a principal regulator of the ganglioside composition in tissues and cells. Support for this concept is provided by experiments with normal human skin fibroblasts in culture. The major ganglioside in these cells is G_{M3} (*25, 26*) and only trace amounts of more complex gangliosides are demonstrated (*26*). The cells lack detectable *N*-acetylgalactosaminyltransferase activity but contained substantial galactosyltransferase (Fig. 2, reaction 5a) and sialyltransferase (Fig. 2, reaction 6a) activities. Thus, except for this reaction, all of the enzymes in ganglioside biosynthesis are operative in these cells (*26*). Studies with bovine thyroid tissue provide further support for this concept. In contrast to most other extraneural tissues, bovine thyroid contains higher order gangliosides that appear to be G_{M1}, G_{D1a}, G_{D1b}, and G_{T1} (*27*). All of the glycosyltransferase activities involved in the synthesis of G_{D1a} can be detected in thyroid extracts, including the elusive *N*-acetylgalactosaminyltransferase (*19*).

Ganglioside Biosynthesis via a Multienzyme Complex

Roseman originally proposed that the biosynthesis of the oligosaccharide chains of complex carbohydrates was catalyzed by multiglycosyltransferase systems (*2*). The various glycosyltransferases involved in the synthesis of a particular glycolipid (or glycoprotein) were associated within the cell as an enzyme complex. Such a system would provide for efficiency and regulation of synthesis and reduce the probability of synthetic errors. It is important to realize that the synthesis of these compounds is not directed by a template. The localization of ganglioside glycosyltransferases in the same subcellular organelles of various cells has provided support for this concept (*2, 8, 19, 21*).

If such an enzyme complex existed, one would predict that efficient sequential transfer of sugars to an acceptor should occur in vitro. We were able to demonstrate the sequential synthesis of G_{M1} from G_{M3} using UDP-*N*-[^{14}C]acetylgalactosamine and UDP-[^{3}H]galactose as sugar donors with rat brain membrane preparations as the source of the enzymes (*4, 7*). As indicated in Fig. 3, *N*-[^{14}C]acetylgalactosamine is transferred to G_{M3} to form [^{14}C]G_{M2}, which in turn receives [^{3}H]galactose to form the doubly labeled G_{M1}. In addition, the galactosyltransferase had a tenfold higher affinity for the G_{M2} synthesized de novo than for exogenous G_{M2} (*4*).

These observations support the concept of a glycosyltransferase complex that catalyzes the synthesis of gangliosides. Such a complex is bound to membranes either as part of the Golgi apparatus or other subcellular organelles. It is important to note that in the proposed pathway there are several reactions involving the transfer of galactose and sialic acid. Based on kinetic studies, each of these reactions is catalyzed by a different enzyme. However, in analogy with the lactose synthetase complex (*28*), there may be a common galactosyltransferase (or sialyltransferase) protein and several modifier proteins which confer specificity on the catalytic protein. We would predict that such modifier proteins would also be membrane-bound as part of the multienzyme complex. Existence of such modifier proteins would provide insight into the altered ganglioside biosynthesis observed in transformed cells and genetic disorders that we will discuss subsequently. Therefore, attempts to solubilize and purify these glycosyltransferases have considerable importance and may yet have wide application (*7*).

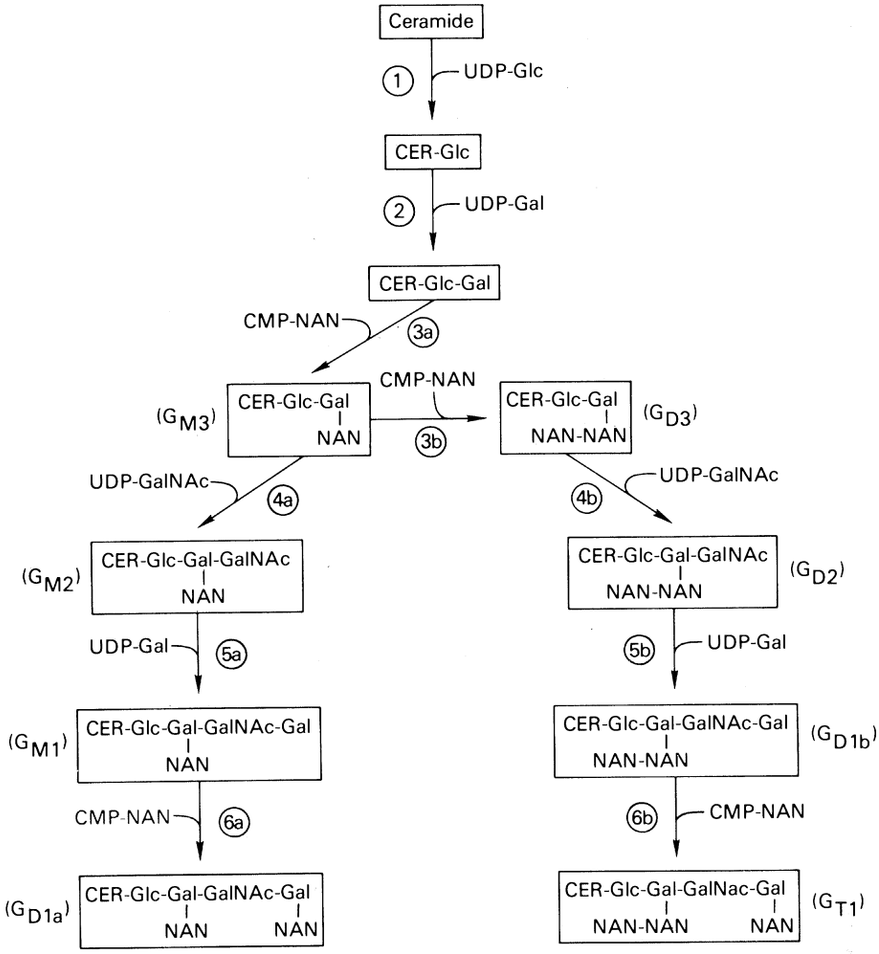

Fig. 2. Pathway for the biosynthesis of gangliosides. Each reaction is catalyzed by a specific glycosyltransferase. Abbreviations are CER, ceramide; Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; and NAN, *N*-acetylneuraminic acid.

Ganglioside Biosynthesis During Development

There are striking changes in the pattern and quantities of brain gangliosides during development (*29*). There are corresponding changes in the activities of the requisite glycosyltransferases (*9, 12, 30*). Their levels rise rapidly preceding the onset of myelination and then decrease as the brain matures. Taken together with the reported localization of gangliosides (*31*) as well as these enzymes (*3, 21*) in the synaptosomes, the data suggest an important role for gangliosides during brain development (*32, 33*). Inability to synthesize gangliosides during this critical period should result in severe neurological dysfunction.

We have observed the consequences of this prediction. The propositus was a male infant who began to have respira-

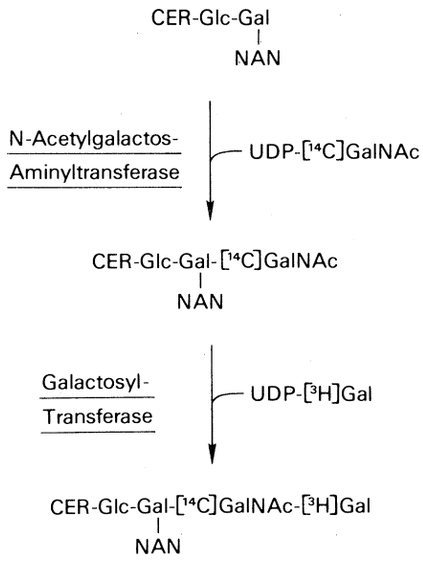

Fig. 3. Sequential synthesis of gangliosides in vitro. In the presence of exogenous G_{M3}, a rat brain particulate preparation contains the two glycosyltransferase activities that catalyze the synthesis of G_{M2} and G_{M1} by the sequential transfer of *N*-[^{14}C]acetylgalactosamine and [^{3}H]galactose from the respective sugar nucleotides.

tory difficulties and convulsions a few days after birth. Physical and motor development were poor, the skin was thickened and coarse, the liver and spleen were enlarged, and there were bilateral inguinal hernias (*34*). The propositus died at 3½ months. At autopsy the central nervous system showed spongy degeneration, and there was a severe lack of myelin in large areas of the brain (*35*). The major biochemical abnormality was a dramatic alteration in the pattern of gangliosides in the brain. Most of the gangliosides in normal human brain consist of G_{M1} and corresponding di-, tri-, and tetrasialylated homologs. The only gangliosides in the patient's brain were G_{M3} and G_{D3} (Fig. 4) (*36*). The absence of the higher gangliosides was shown to be due to a dramatic reduction in the activity of the amino-sugar transferase that catalyzes reaction 4a in Fig. 2 (*37*). This metabolic defect is exactly the opposite of that in Tay-Sachs disease where patients cannot cleave the *N*-acetylgalactosamine residue from G_{M2} (*38*).

There are several important aspects to this discovery. It seems likely that this metabolic defect was inherited as an X-chromosomal recessive characteristic since a maternal uncle of the child had many similar clinical features and died at the age of 2½ months 30 years previously; and subsequently, a brother of the propositus was born with identical physical and clinical characteristics.

Some remarks concerning the pathogenesis of the organomegaly and lack of myelin in these patients seem appropriate. In most metabolic disorders that are associated with organomegaly, there is an accumulation of a substance such as a glycolipid or mucopolysaccharide in the organs and tissues of the patients. A peculiarity of this observation lies in the fact that there is really quite a small amount of stored material in a number of these individuals compared with the extensive enlargement of organs such as the liver and spleen (*39*). Thus, one can speculate that the organomegaly may be due, at least in part, to a compensatory hypertrophy of the organ so that it supplies additional quantities of the deficient enzymes rather than simply satisfying a spatial requirement for the accumulating substance (*40*). An increase in galactosyltransferase activity (Fig. 2, reaction 5a), observed in the brain of the propositus, supports this concept (*37*).

The cause of the hypomyelination in these patients is not understood at this time. It had been postulated earlier that the formation of the myelin sheath occurs because of recognition of some similarity of surface components between oligodendroglial cells and neurons which become myelinated, and that gangliosides play a role in this phenomenon (*33*). The absence of the higher ganglioside homologs in this patient seems superficially to offer substantiation of this hypothesis. However, such an explanation may be an oversimplification of the situation in the developing nervous system. Gangliosides may be involved in trophic reactions on neural cell surfaces, as will be shown for other systems. Thus, a particular ganglioside may be required on the surface of the neuron or glial cell to receive the signal that initiates metabolic events operative in myelination. Further experimentation is required to determine whether either of these phenomena are involved in myelination.

Altered Ganglioside Biosynthesis in Transformed Cells

We have been actively investigating the changes in ganglioside pattern and synthesis that occur in transformed cells. Because our findings as well as those of other investigators have been reviewed (*20, 41, 42*), we summarize these observations only briefly. In contrast to normal cells, transformed cells lack the growth restraints in culture known as contact or density-dependent inhibition of growth. In addition, transformed cells have reduced requirements for nutrients and serum factors, they can grow in suspension, and they are morphologically distinct from normal cells. Finally, transformed cells may produce tumors in appropriate animals. Many of these novel properties of transformed cells suggested that changes in the surfaces of the cells accompany transformation. Since gangliosides are well-characterized components of the plasma cell membrane, an investigation of these compounds and their metabolism appeared to offer a reasonable approach to the biochemistry of cell transformation.

Table 1. Altered ganglioside biosynthesis in oncogenically transformed mouse cells.

Cell line	Oncogenic agent	Enzyme block
	Decrease in gangliosides more complex than G_{M3}	
Swiss 3T3	SV40	$G_{M3} \rightarrow G_{M2}$
	Polyoma	
	Moloney sarcoma virus	
AL/N	SV40	
	Polyoma	
BALB 3T3	SV40	
	Moloney sarcoma virus	
	Decrease in gangliosides more complex than G_{M2}	
	Kirsten sarcoma virus	$G_{M2} \rightarrow G_{M1}$
	Benzo[*a*]pyrene	
	Methylcholanthrene	
	Dimethylbenzanthrene	
	X-irradiation	

The availability of established cloned mouse cell lines and derivative lines that had been transformed by a number of oncogenic agents provided a well-controlled system for investigating the relationship of gangliosides to transformation. Normal mouse cells contain a homologous series of gangliosides consisting of G_{M3}, G_{M2}, G_{M1}, and G_{D1a}. When these cells are transformed by oncogenic viruses, chemical carcinogens, or x-irradiation, the transformed cells lack the more complex gangliosides (Table 1). In each instance, the loss of these gangliosides can be attributed to a decrease in the activity of a specific glycosyltransferase. Cells transformed by the DNA tumor viruses SV40 and polyoma (*43, 44*) or the RNA Moloney sarcoma virus (*45*) are missing gangliosides more complex than G_{M3}, and *N*-acetylgalactosaminyltransferase activity is diminished in these cells. A simplification of ganglioside pattern in plasma membranes (*46*)

and a reduction in surface amino-sugar transferase (*23*) was also observed in SV40- and polyoma-transformed 3T3 cells. BALB 3T3 cells transformed by Kirsten sarcoma virus, chemicals, or x-irradiation have reduced levels of G_{M1} and G_{D1a}, and galactosyltransferase activity is virtually undetectable in these cells (*47, 48*).

Relation of Ganglioside Alterations to Oncogenic Transformation

We undertook a series of experiments to determine whether the changes in ganglioside synthesis were related to oncogenic transformation. The small tumorigenic DNA viruses can cause lytic infection in appropriate cells (*49*). When mouse or monkey kidney cells were infected with polyoma or with SV40, respectively, there were no significant changes in *N*-acetylgalactosaminyltransferase activity during the course of the virus infection (*41, 50*). The RNA sarcoma viruses require a helper leukemia virus in order to replicate. When mouse cells were infected with murine leukemia viruses, the ganglioside pattern and the activities of *N*-acetylgalactosaminyl- and galactosyltransferase remained unchanged (*45, 48*). We conclude that the altered ganglioside synthesis observed in virus-transformed mouse cells is related to the transforming properties of the viruses.

Since transformed cells are metabolically more active, are less dependent on serum factors, and attain higher population densities than their untransformed counterparts, we considered the possibility that changes in ganglioside synthesis might be a secondary consequence of transformation. However, varying the *p*H of the cell cultures, the composition of the medium, or the amount of fetal calf serum had little or no influence on ganglioside composition and synthesis in either normal or transformed cells (*51*). In addition, cell density had no significant effect on either the pattern of gangliosides or the activities of glycosyltransferases (*44, 46*).

Finally, we examined the composition and biosynthesis of gangliosides in revertants derived from DNA virus-transformed mouse 3T3 cells. These revertants had regained many of the phentotypic properties of normal cells, such as low saturation density in culture. Although these flat revertants still possessed the viral genome, they exhibited a complex ganglioside pattern and a high amino-sugar transferase activity similar to that of normal 3T3 cells (*50, 52*). The coordinate restoration of normal growth properties and ganglioside synthesis may be a consequence of the hyperploidy of the revertant cells. Current theories suggest that the expression of malignant transformation may depend on either the balance between normal and malignant chromosomes or the suppression of a recessive malignant trait by specific normal chromosomes (*53*).

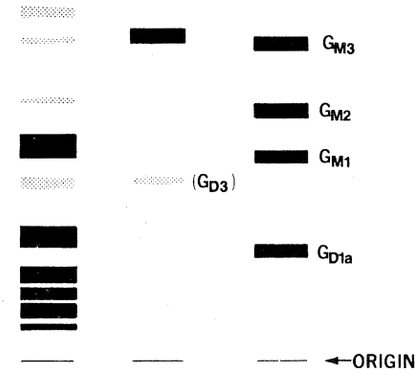

Fig. 4. Ganglioside patterns in a normal human brain and in a patient with deficiency in ganglioside biosynthesis (*108*). [Courtesy of *Archives of Neurology*]

Altered Ganglioside Biosynthesis, a General Phenomenon?

The changes in ganglioside synthesis that we have observed in oncogenically transformed mouse cells have also been observed in transformed cells of other species (*54, 55*). It is interesting that certain hamster and rat cell lines that contain complex gangliosides show a reduction in gangliosides more complex than G_{M3} and in amino-sugar transferase activity after transformation (*55*). Similar alterations in ganglioside pattern and biosynthesis have been observed in tumor cells isolated in vivo and in vitro (*56*) as well as in the plasma membranes derived from tumor cells (*57*). Of additional interest is the report of Skipski *et al.* (*58*) that the ganglioside pattern of serum from rats bearing the Morris hepatoma reflected the altered ganglioside patterns observed in the tumors. Skipski *et al.* observed a reduction in more complex gangliosides and an increase in the less complex ones compared to gangliosides extracted from normal serums.

We, as well as others, have observed several exceptions to the phenomenon of altered ganglioside synthesis in malignant transformation. Spontaneously transformed cells such as TAL/N and BALB/3T12, which are highly tumorigenic, appeared to have a normal ganglioside pattern and normal levels of glycosyltransferase activities (*43, 50, 51*). However, continuous cultivation of the spontaneously transformed cells did result in changes in ganglioside composition and reduced glycosyltransferase activities (*51, 52*). This situation did not occur when nontumorigenic cells were maintained in culture over similar periods of time (*51*). More complex gangliosides such as G_{D1a} have been observed in several clones of SV40-transformed mouse cells [(*46, 51, 59*); see also (*20, 42*)].

Finally, we observed a normal ganglioside composition and glycosyltransferase activities in BALB 3T3 cells transformed with a primate virus, woolly monkey sarcoma virus (*48*). The tissue culture properties of these cells were very similar to cells transformed by Kirsten murine sarcoma virus (*48*). Although the tumorigenicity of these cells could not be determined because of the presence of helper leukemia virus, it was apparent that viral transformation properties in culture could not be correlated with quantitative changes in ganglioside composition. It is possible that changes in the organization of membrane glycolipids or in the appearance of unusual glycolipids, as suggested by Hakomori (*42*), may still be related to cell transformation. However, we decided that it was first essential to establish the function of these complex membrane components.

Role of Gangliosides in Cell Morphology

Abnormalities of ganglioside metabolism—such as in Tay-Sachs disease, general gangliosidosis, or the newly discovered anabolic gangliosidosis—result in severe neurological dysfunction in the afflicted patients. Plasma membranes of nonneuronal cells also are enriched in gangliosides, the synthesis of which often is simplified after malignant transformation. In order to explore the relation of these changes in ganglioside synthesis to the role or roles of gangliosides in cell growth and behavior, several model systems have been developed.

The growth-inhibiting properties of lipophilic acids are well known. These agents inhibit the growth of bacteria (*60*) and mammalian cells (*61*). Butyrate in millimolar concentrations, in addition to inhibiting growth, causes a number of epithelioid cell lines to change shape. These striking morphological changes are readily observed in HeLa cells, which extend long processes in the presence of butyrate (Fig. 5) (*61, 62*). Buty-

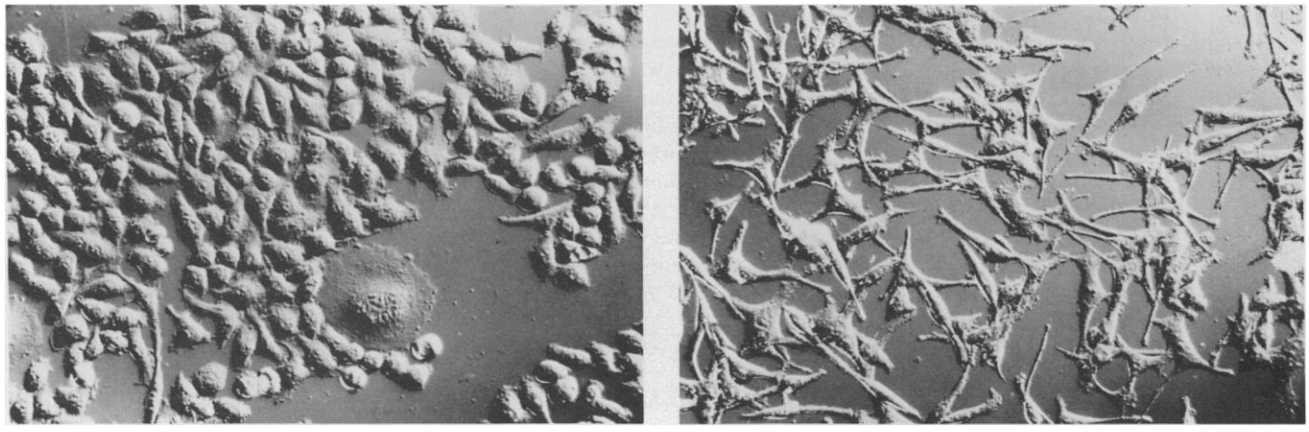

Fig. 5. Photomicrographs of normal and butyrate-treated HeLa cells. HeLa cells were grown for 12 hours in normal medium (left) or in medium supplemented with 5 m*M* sodium butyrate (right). Photomicrographs (× 265) were taken with Nomarski interference optics and are reproduced from (*4*). [Courtesy of *Chemistry and Physics of Lipids*]

rate-treated HeLa cells contain 3.5 to 5 times more G_{M3} than do untreated cells (*62, 63*). Although the ganglioside pattern of the HeLa cell is simple and consists of G_{M3} and G_{D3}, the effect of butyrate on G_{M3} is specific in that the content of G_{D3} and other glycosphingolipids does not change substantially (*63*).

We were able to demonstrate that the increase in G_{M3} content was due to elevated CMP–sialic acid: lactosylceramide sialyltransferase activity (Fig. 2, reaction 3a) in the butyrate-treated cells (*62, 63*). The increase in sialyltransferase activity depended on the concentration of butyrate in the culture medium and the time of exposure to this fatty acid. The activity of this enzyme may increase as much as 20-fold in cells exposed to 5 m*M* butyrate for 20 hours (*64*). Under these conditions, G_{M3}-sialidase activity remained unchanged (*65*). Of the many analogs and homologs of butyrate tested, only pentanoate and propionate caused a similar increase in sialyltransferase activity (*63*). Induction of enzyme activity was blocked by actinomycin D or cycloheximide, agents that inhibit messenger RNA and protein synthesis, respectively (*62, 63*). Neither thymidine nor Colcemid, which block the cells in specific phases of the cell cycle, inhibited enzyme induction (*63*).

There is a close association between the induction of G_{M3} synthesis and changes in HeLa cell morphology. Increase in sialyltransferase activity precedes the formation of cell processes; and on removal of butyrate, sialyltransferase activity declines prior to process retraction and a return to the usual HeLa cell morphology (*63*). Only propionic, butyric, and pentanoic acids induce these characteristic changes in shape and enzyme activity (*63*). The formation of neurite-like processes is inhibited by actinomycin D and cycloheximide (*61*). Colcemid, which blocks microtubule assembly, and the calcium ionophore A23187, which alters intracellular calcium levels, prevent or reverse the butyrate-induced shape changes (*63, 66*). Under these conditions, the induction of sialyltransferase activity is not inhibited or reversed. Thus, process formation requires microtubule assembly which, in turn, may be regulated by calcium. At higher concentrations of the calcium ionophore, induction of sialyltransferase activity is blocked (*66*). Finally, when butyrate-treated cells are transferred to fresh medium containing low concentrations of cycloheximide (0.5 μg/ml), the cells maintain their neurite-like processes and elevated G_{M3} content for up to 72 hours in the absence of butyrate (*65*). Surprisingly, under these conditions sialyltransferase activity returns to basal levels (*65*).

At present we can only speculate about the relationship between induction of G_{M3} biosynthesis and morphological differentiation in HeLa cells. The newly synthesized G_{M3} may be incorporated into the plasma membrane where it functions in a physical-chemical manner to promote extension of cell processes. In support of this idea is the observation that using a procedure for labeling surface gangliosides, about fivefold more G_{M3} was labeled in butyrate-treated cells than in control cells (*67*). Regardless of the mechanism whereby gangliosides function in this morphological differentiation, these results have important implications for the morphological and biochemical differentiation that occurs as the brain develops. Although there is controversy concerning the distribution and localization of gangliosides among different types of nerve cells (*68*), studies with cultured glial and neuroblastoma cells suggest that glial cells contain mainly G_{M3} and G_{D3} whereas neuroblastoma cells contain the more complex gangliosides (*69*). Treatment of neuroblastoma cells with butyrate resulted in a substantial change in ganglioside composition and an increase in two sialyltransferase activities (Fig. 2, reactions 3a and 6a) (*70*). Moskal *et al.* also reported that sialyltransferase activity increased when neuroblastoma cells were treated with dibutyryl cyclic adenosine monophosphate (AMP), an agent that causes morphological, biochemical, and physiological changes in these cells (*71*).

Role of Gangliosides as Surface Membrane Receptors

Potentially the most exciting research on ganglioside function is related to recent observations that gangliosides serve as surface membrane receptors. The best-characterized receptor is the monosialoganglioside G_{M1}, which binds cholera toxin, the enterotoxin from *Vibrio cholera*. Van Heyningen *et al.* originally showed that brain gangliosides bound cholera toxin and blocked its physiological effect (*72*). Subsequently, in 1973, three independent groups of investigators reported that the ganglioside G_{M1} was the most effective inhibitor (*73, 74*). At a toxin concentration of $10^{-9}M$, 50 percent inhibition was achieved at $10^{-8}M$ G_{M1} (*73*). In addition, prior incubation of liver membranes, fat cells, and erythrocytes with mixed brain gangliosides (*73*) or prior incubation of erythrocytes (*75*) and intestinal cells (*76*) with G_{M1} substantially increased the amount of cholera toxin bound after excess ganglioside was washed away. These and other experiments suggested that G_{M1} was the receptor for cholera toxin.

Cholera toxin causes a biological response in a wide variety of cells; these include fluid accumulation in intestinal loops (*77*), lipolysis in fat cells (*78*), inhibition of DNA synthesis in fibroblasts (*79, 80*), and steroidogenesis in mouse adrenal cells (*81*). These diverse biological effects of cholera toxin are believed to be mediated through cyclic AMP after activation of adenylate cyclase bound to the plasma membrane (*82*). If G_{M1} is the receptor for cholera toxin, then the ability of cells to bind and respond to the toxin should depend on their content of this ganglioside. In order to test this hypothesis, transformed mouse cells with different ganglioside compositions were treated with cholera toxin (*80*). For example, SV40-transformed cells in which G_{M3} was the only detectable ganglioside bound less ^{125}I-labeled cholera toxin than cells containing more complex gangliosides, including G_{M1}. As measured by activation of adenylate cyclase and inhibition of DNA synthesis, SV40-transformed cells were less responsive to cholera toxin than were G_{M1}-containing cells. This correlation between G_{M1} content and toxin binding and action was supported by the work of Holmgren *et al.* (*76*), who examined the G_{M1} content of intestinal mucosal cells from different species. They found a close correlation between G_{M1} content and the amount of ^{125}I-labeled cholera toxin bound to the different cells.

Interaction of Cholera Toxin with Ganglioside-Deficient Cells

It was still not clear why the SV40-transformed mouse cells bound some cholera toxin; these cells lacked demonstrable sugar transferase activities required for G_{M1} synthesis as well as any chemically detectable G_{M1} (*80*). Possibly cholera toxin was binding to some other membrane component. However, on the basis of the amount of toxin known to be bound by these cells (0.2 pmole per 10^6 cells), the quantity of G_{M1} required would be below the level of analytic detection. Thus, cholera toxin might be an ultrasensitive indicator of G_{M1}. Even if the cells were incapable of synthesizing these minute amounts of G_{M1}, the calf serum, which was a component of the culture medium, contains gangliosides (*83*) that might be taken up by the cells.

In order to avoid these complications, we investigated the effects of cholera toxin on transformed mouse fibroblasts that had been adapted to grow in serum-free medium. These cells were unresponsive to cholera toxin and were deficient in G_{M1} as well as other gangliosides (*84*). The cells lack two sugar transferase activities (Fig. 2, reactions 3a and 5a) (*85*). G_{M1} of high specific radioactivity (4.37 c/mmole) was prepared by oxidation of the terminal galactose with galactose oxidase and reduction with sodium borotritide (NaB $^3H_4$). When [^{3}H]G_{M1} was added to the culture medium, it was taken up by the cells which then responded to the toxin (*84*). A significant response to cholera toxin was observed with as few as 17,000 molecules of G_{M1} per cell. Maximal response was obtained with 100,000 molecules per cell. When the [^{3}H]G_{M1}-containing cells were extracted with a mixture of chloroform and methanol, 90 percent of the [^{3}H]G_{M1} was recovered intact.

Although other exogenous labeled gangliosides could be taken up by the cells, they did not confer cholera toxin sensitivity to the cells (*85*). When cells containing equal amounts of G_{M1}, G_{M2}, and G_{M3} were treated with ^{125}I-labeled cholera toxin, only G_{M1}-containing cells bound the radioactive ligand, and binding was proportional to the amount of G_{M1} in the cells (*86*). Thus, the interaction of cholera toxin with G_{M1} is specific, and the inability of other gangliosides to confer toxin sensitivity on these cells appears to be due to their failure to bind the toxin.

These results indicate that G_{M1} can be taken up by cells and become functionally integrated into the plasma membrane. Further evidence for a direct interaction between cholera toxin and membrane-bound G_{M1} was provided by the following experiment. Mouse fibroblasts were first incubated with G_{M1} and then half of the cells were exposed to an excess of cholera toxin. The cells were then treated with galactose oxidase and sodium borotritide and G_{M1} was isolated from both sets of cells. It was labeled only in the cells that were not treated

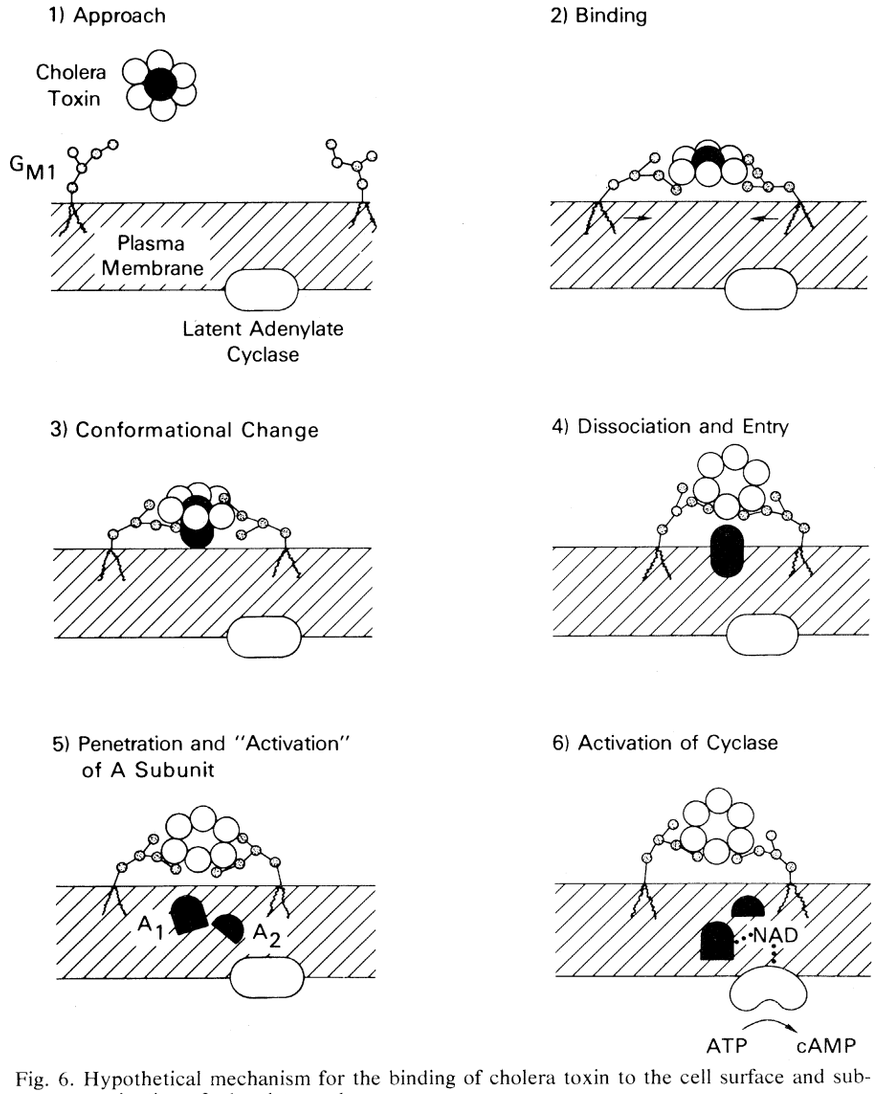

Fig. 6. Hypothetical mechanism for the binding of cholera toxin to the cell surface and subsequent activation of adenylate cyclase.

with cholera toxin (*86*). The toxin completely protected the integrated G_{M1} from the action of galactose oxidase. Similar results were obtained with cells that contain endogenous G_{M1} (*86*).

Role of G_{M1} in Mediating the Action of Cholera Toxin

Besides serving as the surface receptor for cholera toxin, G_{M1} may have another function. We have observed that G_{M1} induces a conformational change in cholera toxin based on changes in the fluorescence spectrum (*87*). Other gangliosides do not cause a shift in the fluorescence spectrum. Cholera toxin is composed of two subunits, A and B, and each toxin molecule appears to consist of six B subunits and one A subunit (*88*). The B subunits are involved in the binding of the molecule to the G_{M1} receptor (*73, 74, 89*) and the A subunit, or part of it, is involved in the activation of adenylate cyclase (*75, 90*). Although the various steps between toxin binding and cyclase activation are unknown, there are several lines of evidence suggesting that the cholera toxin complex undergoes dissociation. There is a characteristic lag between binding of cholera toxin to intact cells and activation of adenylate cyclase; there is no lag when intact cells are treated with "active" A subunit (*90*) or in cell homogenates exposed to cholera toxin (*84*) or the "active" A subunit (*75*). The lag period presumably represents the time required for the cholera toxin to dissociate and for the A subunit to penetrate the cell membrane (*75, 90*). There may be some additional cellular factors required for the formation of "active" A subunit and the subsequent activation of adenylate cyclase (*75, 84, 91*). Figure 5 schematically depicts our concept for the binding of cholera toxin and its dissociation and stimulation of adenylate cyclase. The important aspect of our scheme is that, following binding of the B subunits to G_{M1}, the toxin complex undergoes a conformational change that promotes dissociation of the complex and entry of the A subunit into the plasma membrane. This process may involve multivalent binding of one toxin molecule to several ganglioside receptors and redistribution of surface components by lateral movement of the toxin-ganglioside complex in the fluid phase of the membrane (*90*). Evidence for such redistribution (patching and capping) has been observed in lymphocytes directly with fluorescein-labeled cholera toxin (*92*) or indirectly with immunofluorescence (*93*). After the A subunit, which consists of two peptides linked by disulfide bridges (*75, 89*) is internalized, it becomes converted to an active form. This process apparently involves cleavage of the disulfide bonds and formation of an A_1 fragment which can, in turn, stimulate adenylate cyclase by an unknown mechanism involving NAD (nicotinamide adenine dinucleotide) and other cellular factors (*75, 84, 91*).

Interaction of Gangliosides with Glycoprotein Hormones

Studies with cholera toxin are potentially important for understanding the mechanism of the binding and the subsequent biological effects of glycoprotein hormones. Many of these hormones bind to specific receptors on the cell surface and activate adenylate cyclase (*94*). It seems likely that cholera toxin may be subverting a normal mechanism for the transfer of information across cell membranes. Recent work on the interaction of thyroid-stimulating hormone (TSH) with gangliosides supports this idea (*27*). Gangliosides inhibit the binding of [^{125}I]-TSH to thyroid membranes whereas comparable amounts of sialic acid or fetuin, a sialoglycoprotein, do not. The most potent inhibitor of TSH was G_{D1b} followed by $G_{T1} > G_{M1} > G_{M2} = G_{M3} > G_{D1a}$. Thus the inhibition is highly specific and depends on the carbohydrate structure of the ganglioside. G_{D1a}, the structural isomer of G_{D1b}, inhibits TSH binding only 4 percent at a concentration of G_{D1b} that is 100 percent inhibitory. Gangliosides inhibit binding by interacting with the hormone and alter the conformation of TSH as measured by fluorescence changes. The degree of fluorescence change produced by a particular ganglioside directly correlates with its ability to inhibit binding. Furthermore, in contrast with other extraneural tissues, bovine thyroid membranes contain substantial amounts of gangliosides more complex than G_{M3}, including G_{M1}, G_{D1a}, G_{D1b}, and G_{T1}.

Thyroid-stimulating hormone as well as related glycoprotein hormones are composed of two subunits, α and β. The α subunit is common to these hormones whereas the β subunit confers target organ specificity (*95*) and appears to contain the primary determinants for binding to membranes (*96*). These phenomena have certain obvious analogies with the mechanism of action of cholera toxin. We recently observed that there are regions of amino acid sequence homology between the cholera toxin B fragment and the β-chains of TSH, luteinizing hormone, human chorionic gonadotropin, and follicle-stimulating hormone (*27, 97*). In addition, there is sequence homology between the A_1 subunit of cholera toxin and the α-chain of these hormones (*97*). Cholera toxin can partially inhibit the binding of [^{125}I]TSH to thyroid membranes, suggesting that there are at least two classes of TSH receptors on the membrane (*87*). At low concentrations of cholera toxin, binding of [^{125}I]TSH is actually enhanced, indicating "cooperativity" among the receptors (*87*). In this regard, these concentrations of cholera toxin will protect G_{M1} on thyroid membranes from labeling by the galactose oxidase-borotritide method but actually increase the labeling of other membrane glycolipids (*87*). As is expected, gangliosides inhibit the stimulation of thyroid membrane adenylate cyclase by cholera toxin or TSH. However, in contrast with mechanisms of action of cholera toxin, NAD was not required for the stimulation of adenylate cyclase by TSH (*87*).

Role of Gangliosides in Glycoprotein Hormone Action

These results strongly implicate a role for gangliosides in the biological effects of glycoprotein hormones of the TSH superfamily. In addition, rat thyroid tumor membranes which bind ^{125}I-labeled TSH poorly contain only G_{M3}, whereas rat thyroid membranes which bind TSH avidly contain more complex gangliosides (*98*). The binding of human chorionic gonadotropin to rat testicular membranes is also inhibited by gangliosides (*99*). However, the function of gangliosides in these hormone-responsive systems is not clear. There is evidence that the membrane receptor for TSH is a glycopeptide, which can be released from the membranes by trypsin or solubilized with detergents (*100*). Similarly, the complex formed between human chorionic gonadotropin and its surface receptor has been solubilized and found to have a molecular weight of more than 200,000 (*101*). This raises the possibility that our observations on the interactions of glycoprotein hormones with gangliosides is happenstance. The carbohydrate structures on the glycoprotein receptor may be similar to the oligosaccharide chains of certain gangliosides. However, we prefer an alternate explanation, which may be visualized as a modification of the scheme outlined in Fig. 5 in the following fashion. After the hormone becomes bound to the glycopeptide receptor, it interacts with a specific ganglioside in the membrane and undergoes a conformational change. The change in

the structure of the hormone promotes the stimulation of adenylate cyclase by the α subunit. This slight difference would be consistent with differences between the action of cholera toxin and the action of these glycopeptide hormones. Cholera toxin has six binding subunits per molecule, whereas the hormones have only one. The cholera toxin becomes dissociated, and part of the molecule must penetrate the membrane in order for adenylate cyclase to become activated. There is no evidence that hormone molecule must separate and the α subunit penetrate the membrane.

Gangliosides as Receptors for Other Biologically Active Agents

There is considerable evidence that specific gangliosides can inhibit the action of tetanus toxin (*102*), botulinum toxin (*103*), serotonin (*104*), and interferon (*105*). Prior incubation of gangliosides with interferon (*105*) will inhibit the antiviral activity of the interferon, and interferon will bind to gangliosides coupled to agarose (*105, 106*). In addition, prior treatment of some transformed mouse cells that are deficient in certain gangliosides with gangliosides will increase the sensitivity of the cells to interferon (*106*). These results indicate that gangliosides and interferon can interact at the cell surface and that gangliosides may have a function in the antiviral activity of interferon.

Undoubtedly, other biologically active agents may interact with gangliosides, and additional functions for these membrane-bound complex carbohydrates will be discovered. It is well known that stimulation of adenylate cyclase or addition of derivatives of cyclic AMP to the culture medium will alter the growth properties of many cultured cells (*107*). In addition, a number of different transformed cell lines have decreased levels of adenylate cyclase. It has been suggested that the loss of growth control in certain transformed cells may be related to their altered ganglioside composition since the adenylate cyclase in these cells would be less sensitive to undetermined "physiologic" stimuli which interact with ganglioside receptors (*4, 80*). These stimuli may be serum factors or substances secreted by cells that are involved in the regulation of growth of normal cells. In addition, membrane components on the surface of one cell could interact specifically with surface gangliosides on another cell. This association would lead in turn to a stimulation of adenylate cyclase and to subsequent changes in cell growth (*80*). In this regard, it has been shown that the addition of cholera toxin to transformed mouse cells inhibited DNA synthesis and cell growth and led to more "contact-inhibited" cells (*80*). Thus gangliosides may be essential components of a system that transfers information across cell membranes and the absence or structural defect of any one component could result in an inability of the cells to respond to the stimulus. Spontaneously transformed cells that contain no apparent alterations in membrane gangliosides could be defective in another component of this system such as adenylate cyclase or a protein kinase (*4, 80*).

Summary

Gangliosides are unique acidic glycolipids that are selectively concentrated in the plasma membrane of cells. Surface labeling studies have demonstrated that at least a portion of the oligosaccharide chain of gangliosides extends beyond the hydrophobic region of the cell membrane, whereas the lipid moiety of gangliosides (ceramide) is imbedded in the membrane bilayer. It is becoming increasingly apparent that gangliosides participate in the internalization of environmental signals elicited by cholera toxin and glycoprotein hormones such as thyrotropic hormone and chorionic gonadotropin as well as other substances such as interferon and possibly serotonin. The mechanism by which cholera toxin binds to a specific ganglioside receptor on the cell surface and subsequently activates adenylate cyclase provides a model for the interaction of trophic agents with gangliosides. We would predict that analogous phenomena involving gangliosides will be discovered in brain.

The biosynthesis of gangliosides proceeds by the ordered sequential addition of sugars to the lipid moiety. These reactions are catalyzed by a cluster of membrane-bound glycosyltransferases. Any alteration in the activity or specificity of one of these enzymes will result in a dramatic change in the ganglioside pattern of an afflicted cell or organ. The drastic consequences that accompany abnormalities of ganglioside synthesis have been documented in a heritable metabolic disorder in vivo and in tumorigenic transformation of cells in vitro. In this article, we have attempted to unify these observations and to provide a reasonable interpretation of the role of gangliosides in mediating cell surface phenomena.

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