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Cell Contact-Dependent Ganglioside Changes in Mouse 3T3 Fibroblasts and a Suppressed Sialidase Activity on Cell Contact†

Ganesa Yogeeswaran and Sen-itiroh Hakomori*

ABSTRACT: Certain enzyme activities for synthesis and degradation of gangliosides and the chemical quantity and incorporation of radioactivity from [^{14}C]galactose into gangliosides have been studied in 3T3 cells and their transformed counterparts at various cell population densities. The chemical quantity of and the incorporation of radioactivity into GD1a ganglioside increased at the early stage of cell contact ("contact response" of ganglioside), whereas this response was not detectable in transformed 3T3 cells at any stage of cell contact. These phenomena were reproduced in five separate qualitative analyses and two quantitative determinations of gangliosides. As the basis of these phenomena, a membrane-bound sialidase activity which acted on gangliosides was suppressed in 3T3 cells at the "touching" stage of cell-to-cell contact. Transformed cells did not display the change of sialidase activity at any stage of cell contact.

Nontransformed cells in culture reduce their growth rate significantly when their cell population density increases ("contact inhibition", "topoinhibition", or "density-dependent inhibition") (Abercrombie and Ambrose, 1962; Todaro and Green, 1963; Stoker and Rubin, 1967; Dulbecco, 1970). Because transformed cells do not significantly reduce their growth rate at higher cell population densities, the phenomenon is regarded as the basis for loss of growth control in transformed cells, although the essential biological difference between normal and transformed cells could be a difference in membrane susceptibility to "serum factor" and other nutrients (Holley, 1972).

The biochemical basis of cell surface changes associated with cell contact is important not only for understanding

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the growth control of normal cell proliferation, but also for elucidating the mechanism of cell recognition and sorting and organization of cells in tissues and organs during the process of histogenesis and morphogenesis (Lilien and Moscona, 1967; Gottlieb et al., 1974).

An intriguing phenomenon is the significant increase in chemical concentration and synthesis of certain glycolipids, particularly neutral glycolipids, when cell population density increases (Hakomori, 1970; Robbins and Macpherson, 1971; Sakiyama et al., 1972; Critchley and Macpherson, 1973; Kijimoto and Hakomori, 1971). Such a response of glycolipids on cell contact was considered to be related to "contact inhibition" of cell growth, since contact inhibitability and contact-dependent glycolipid synthesis showed parallelisms in various cells, and the loss of contact inhibition was associated with the loss of contact-dependent enhancement of glycolipid synthesis (Hakomori, 1970; Sakiyama et al., 1972; Sakiyama and Robbins, 1973). However, it is an enigma that highly "contact inhibitable" mouse fibroblasts, such as 3T3 cells, did not show contact-dependent change of glycolipid concentration or rate of synthesis, although an increase in cell surface galactosyl residues at the early stage of cell contact was well documented by Nicolson and Lacorbiere (1973) in their study with *Ricinus communis* lectin. Fishmann et al. (1972) reported that the activities of four glycosyltransferases involved in ganglioside synthesis in various mouse cell lines (N-AL/N, Swiss 3T3) did not alter at different cell population densities. Yogeeswaran et al. also observed that the ganglioside pattern of 3T3 cells under sparse growing conditions was nearly identical with that of confluent cells (1972, and unpublished observation). If ganglioside synthesis has no response to cell contact in contact inhibitable 3T3 cells, it is improbable that contact-dependent glycolipid synthesis is correlated with "contact inhibition" in general. Consequently, we initiated this study in order to settle whether contact-dependent enhancement of ganglioside concentration occurred in contact sensitive mouse fibroblasts 3T3 cells. A few experiments were carried out to find the enzymatic mechanism existing behind this phenomenon.

An increase of ganglioside concentration, particularly GD1a ganglioside, was found to occur at the very early stage of cell contact or at slight cell contact ("touching"), but the response was not obvious in cells at crowded populations. Such a response of ganglioside on cell contact was not observed at all in transformed 3T3 cells during different degrees of cell contact.

Materials and Methods

Cell Culture. Cloned Swiss 3T3 lines were obtained from Dr. Keller, Department of Biochemistry, University of Washington, who obtained the cells originally from the American Type Culture Association. The cells were grown in Dulbecco's modified Eagle's medium and 10% fetal calf serum in 6% carbon dioxide–air incubator. The 3T3 cells were well contact inhibited and showed a cobblestone appearance at confluency (saturation density: about $6-7 \times 10^4$ cells/cm^2). Spontaneously transformed cells (3T6), SV40 transformed, 3T3sv101, and double transformed 3T3 cells with SV40 and polyoma viruses were also obtained from the same source and propagated in the same manner. Cultures of different cell population densities were obtained by the following procedure. To obtain a sparse culture (Figure 1A and D), $1-2 \times 10^3$ cells were seeded per cm^2 and harvested after 14–20 hours. To obtain a "touching culture" (Figure 1B and E), $1-2 \times 10^4$ cells were seeded per cm^2 and harvested after 30–48 hr. The same number of cells were seeded and harvested after 50–58 hr in order to obtain a "crowded" culture (Figure 1C and F). Cells were grown in either roller bottles (surface area 1260 or 625 cm^2) or Falcon Petri dishes (surface area 145 or 72 cm^2), depending on the experimental design. When the cells reached the appropriate density, the cells were labeled with 0.2 μCi of uniformly labeled [^{14}C]galactose (60.5 Ci/mol, New England Nuclear) per ml of culture medium for 6 hr for radiochemical analysis. For chemical and radiochemical analysis of the gangliosides, approximately 1.5×10^7 cells was used. For enzyme assay 2×10^5 cells was used per analysis.

Ganglioside Analysis. Gangliosides were extracted from cell pellets by the procedure of Suzuki (1969) and were separated and detected by thin-layer chromatography (TLC) on glass plates coated with silica gel G, as described previously (Yogeeswaran et al., 1970). The standard solvent system was chloroform–methanol–concentrated ammonia–water (60:35:1:7, v/v). The sialic acid content of the individual gangliosides was quantitated by the method of MacMillan and Wherrett (1969). Autoradiography of the TLC-separated gangliosides (approximately 16,000 cpm per single TLC channel) was performed by covering the TLC plates with "Kodak No-Screen X-ray film" and kept in the dark for 8 days. The plates were then sprayed with resorcinol reagent so that the resorcinol-positive ganglioside zones could be compared with the corresponding radioactive zones.

Sialidase assay was performed both on crude membrane preparations according to the procedure of Schengrund et al. (1973) and on intact cells grown as monolayer culture with exogenously added ganglioside as a substrate. The latter method was used, as exogenously added ganglioside may be a suitable substrate for surface-localized sialidase. The crude membrane preparations were suspended in 1 ml of aqueous solution of human brain ganglioside mixture in 0.02 *M* acetate buffer, or in 1 ml of aqueous solution of labeled ganglioside with a radioactivity of 34,000 cpm. The radioactive ganglioside was prepared from 3T3 cells cultured in the presence of uniformly labeled [^{14}C]galactose. The suspension was incubated for 2 hr. The liberated sialic acid was estimated by extracting the incubation mixture with 20 volumes of chloroform–methanol (2:1) and recovering the ganglioside and free sialic acid in the Folch upper phase and performing thin-layer chromatography in chloroform–methanol–ammonia–water (60:35:1:7, v/v). The free sialic acid migrated on the silica gel plate close to the origin, which was scraped and transferred to a Teflon-lined screw-capped test tube; 1 ml of Svennerholm's resorcinol reagent (1957) was added, and the tube was heated in a boiling water bath for 15 min. After cooling in ice-water, the color which developed was extracted with 1 ml of butanol–butyl acetate (15:85, v/v), and the optical density was determined at 580 mμ. Alternatively, the radioactivity of the sialic acid zone on the thin-layer chromatogram was scraped, and its radioactivity was counted.

Sialidase activity of intact cells was performed by incubating the monolayer cells with exogenously added ganglioside in culture medium. The cells grown on Falcon Petri dishes were washed with phosphate buffered saline (pH 6.0), and the buffer was aspirated thoroughly. The monolayer cultures having approximately the same number of cells were grown in different sizes of Petri dishes, with dif-

fering growing area (145 cm^2 for sparse culture; 72 cm^2 for "touching", and 19 cm^2 for "crowded"), and were incubated with 4 ml of 0.02 *M* acetate buffer (pH 4.2) containing 1 μmol of brain ganglioside. The cells were incubated at 37° for 45 min, and the cells were separated from the supernatant by centrifugation. Cells were intact during this incubation period and able to exclude Trypan Blue. The supernatant was lyophilized and extracted with chloroform–methanol–water (2:1:0.1). The extracts containing ganglioside and free sialic acid were analyzed on TLC, as described above.

Biosynthesis of Ganglioside with Membrane-Bound Endogenous Substrate at Physiological Condition. The activities for sialyl transferase and galactosyl transferase of crude membrane preparations were determined according to the method of Maccioni et al. (1972). The membrane was prepared from hypoosmotically ruptured cells by the same method as was used for the determination of sialidase activity, according to Schengrund et al. (1973). A typical assay system is as follows: 200 μl of a suspension of membrane pellet in 0.1 *M* phosphate buffer (pH 6.8), 20 μl of magnesium chloride (5 μmol), 20 μl of manganese chloride (5 μmol), 0.75 μCi of cytidine monophosphate [^{14}C]-*N*-acetylneuraminic acid (labeled at C-4,-5,-6,-7,-8,-9; 150–250 Ci/mol) in 10 μl of saline, and 6 μCi of uridine diphosphate [^{3}H]galactose (labeled at C-1; 1.23 Ci/mmol) in 10 μl of saline. Total volume was made up to 0.3 ml. The mixture was incubated for 20 min at 37° and frozen at −70° pending extraction. The gangliosides were extracted from the incubation mixture as described before, and the individual ganglioside was isolated by TLC using carrier gangliosides for chemical detection by resorcinol reagent. The ^{14}C and ^{3}H radioactivity in each zone was measured and expressed as a ratio of these two activities.

Characterization of Gangliosides. Characterization of the biosynthesized gangliosides was performed from a large scale incubation of membranes derived from 1×10^7 cells with sugar nucleotide, and individual ganglioside was separated by preparative TLC. The ratio of the activities of ^{14}C and ^{3}H was first measured for the individual ganglioside. Then each ganglioside extracted was subjected to methanolysis and GLC, as described by Laine et al. (1971). The GLC pattern of the sugars, identified by the retention times, was first recorded; subsequently, each peak of sugars was collected by quenching the flame of the GLC detector and using capillary pipets cooled with Dry Ice. The condensed sugar derivatives in the capillary pipets were eluted with chloroform–methanol, and the eluate was dried in scintillation vials. The ^{14}C or ^{3}H radioactivity was measured in toluene-based scintillation fluid and expressed as the ratio of these two activities.

For chemical characterization of the gangliosides, 3T3svpy cells were grown in 40-cm long roller bottles to give approximately 15 ml of packed cells. The gangliosides were prepared and subjected to chromatography on an Anasil-S column, according to the procedure of Penick et al. (1966). Preparative TLC was also used to separate GM1 and GD1a gangliosides which were eluted as a mixture. The stoichiometric analysis of the carbohydrate moieties of the individual gangliosides was performed as trimethylsilyl derivatives of the *O*-methyl glycosides by gas chromatography (Laine et al., 1971). The carbohydrate sequence was determined by sequential degradation of gangliosides by 0.5 *N* HCl at 80° for 1 hr and identification of the degradation product on TLC using standard reference glycolipids. TLC

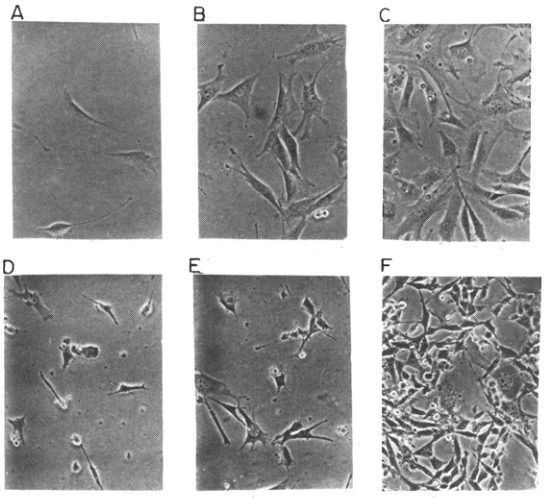

FIGURE 1: Microphotograph of mouse fibroblasts. (A) 3T3 cells sparse growing; (B) growing phase quoted as "touching"; (C) growing phase quoted as "crowded"; (D) 3T3sv cells sparse growing; (E) 3T3sv cells "touching"; (F) 3T3sv cells "crowded".

was run in solvent systems, chloroform–methanol–water (60:35:8) and chloroform–methanol–concentrated ammonia–water (60:35:1:7, by volume). The susceptibility of the sialyl residues to neuraminidase was determined by incubating gangliosides with *Vibrio cholerae* neuraminidase at pH 4.2 in 0.02 *M* acetate buffer. Subsequently the hydrolysis products were analyzed by TLC with solvent chloroform–methanol–concentrated ammonia–water (55:40:2:10, v/v). Sialic acid was not liberated from ganglioside at pH 4.2 in acetate buffer without neuraminidase.

Inhibition of sialidase activity was tried in order to find any possibility to exclude the effect of sialidase upon sialyltransferase. *N*-Phenyloxamic acid was synthesized according to Edmond et al. (1966) from ethoxylyl chloride and aniline. The product was crystallized from benzene, and the melting point agreed with that recorded (153°). Other inhibitors, such as copper acetate, sialyllactose, and "sialostatin B" were used. "Sialostatin B" (2-acetamido-3,4-dihydroxy-5-carboxypiperidine) was donated by Dr. Takaaki Aoyagi, Institute for Microbial Chemistry, Sinagawa-ku, Tokyo (Umezawa et al., 1974).

Results

Change of Ganglioside Patterns on Cell Contact as Determined by Chemical Quantity and by Isotope Incorporation. Qualitative analysis of ganglioside pattern of 3T3 cells at different cell population densities by thin-layer chromatography indicated that "band 4" (corresponding to GD1a ganglioside) increased significantly at the "touching" stage (Figure 1B and E). The increase of "band 4" was indicated not only by isotope incorporation from [^{14}C]galactose (Figure 2A), but also by an increase in the resorcinol positive spot on TLC (Figure 2B and C). However, the ganglioside patterns of "crowded" 3T3 cells were almost identical with those of "sparse" 3T3 cells in isotope incorporation pattern and in chemical quantity (lanes 2–4, Figure 2C). These qualitative changes of GD1b ganglioside on cell contact, as seen in Figure 2, have been reproduced in five separate experiments, whereas quantitative analyses of gangliosides, sialidase activities, and ganglioside synthesis were carried out twice, as seen in Tables I–III.

The results of duplicate quantitative analyses of lipid-

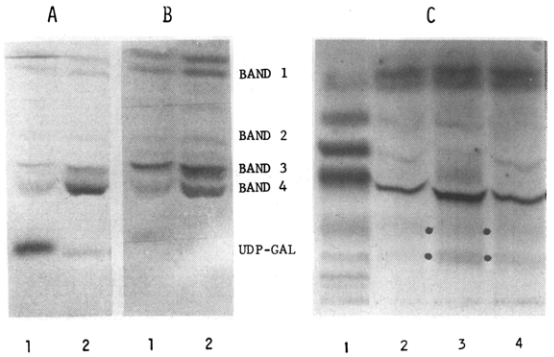

FIGURE 2: Autoradiogram of radioactive ganglioside incorporated from [^{14}C]galactose (A) and chemical pattern of ganglioside as revealed by resorcinol reaction (B and C). (A) Lane 1, sparse growing cells and lane 2, touching cells: both autoradiograms from 16,000 to 24,000 cpm of ganglioside derived from 3 mg of cell residue. (B) Lane 1, sparse growing cells and lane 2, touching cells: both revealed by resorcinol reagent, ganglioside derived from 3 mg of cell residue. (C) Lane 1, reference gangliosides: bands from top to bottom, double band for GM3, GM2, GM1, GD1a, GD1b (faint), GT (faint). Lane 2, sparse growing and lane 3, touching, and lane 4, crowded: bands shown by dots indicate nonresorcinol positive yellow-colored substance which is not gangliosides. Details of the conditions are described in the text. These qualitative data were reproduced in five separate experiments.

Table I: Comparison of Chemical Quantities and Radioactive Incorporation of [^{14}C] Galactose into the Gangliosides in Different Cell Population Densities of 3T3 Cells.[b]

	nmoles of LBSA/10 mg of Cell Residue wt.			Cpm/10 mg of Cell Residue wt.		
Samples	Sparse	Touching	Crowded	Sparse	Touching	Crowded
Band 1	18.1	19.4	16.6	7200	7500	7025
Band 2	6.6	3.3	5.4	1150	1200	1050
Band 3	6.2	5.0	5.7	1700	2000	1800
Band 4	19.0	28.4	19.8	6590	14800	7200

[a] Lipid-bound sialic acid. [b] Results are the average of duplicate analysis. Each experiment was repeated, and the values from one experiment are represented above.

Table II: Activity of Membrane Sialidase of Mouse 3T3 Fibroblasts at Different Cell Population Densities.[a]

Population Density	nmoles of Sialic Acid Released per mg of Cell Residue per 2 hr			
	3T3	3T3svpy	3T3sv101	3T6
I. Determined Using Membrane Preparation				
Sparse	12.0 (12.0)[c]	11.0 (11.0)[c]	7.3 (7.3)[c]	11.6 (11.5)[c]
Touching	6.5 (6.4)[c]	10.5 (10.2)[c]	6.5 (6.5)[c]	11.6 (11.2)[c]
Crowded	12.3 (12.2)[c]	14.3 (14.3)[c]	9.2 (9.3)[c]	13.4 (13.1)[c]
II. Determined Using Intact Cells Grown in Petri Dishes[b]				
Sparse	7.5	6.6	5.0	6.2
Touching	3.7	6.3	5.4	6.3
Crowded	6.6	6.2	7.5	7.5

[a] Results are an average of duplicate analysis. Each experiment was repeated twice with separate batches of cells, and data from one experiment are shown above. [b] Calculated value for 2 hr from the values obtained for 45 min of incubation time. [c] These values were determined from the release of radioactivity of sialic acid from radioactive gangliosides that were used as the substrate (values in parentheses).

Table III: Biosynthesis of Ganglioside by Membrane Preparation of 3T3 Cells with Different Degree of Cell Contact as Measured According to the Method of Maccioni et al. (1972).[a]

	Isotope Ratio cpm ^{14}C/^{3}H[b]					
Ganglioside Zones	3T3 Sparse	3T3 Touching	3T3 Crowded	Svpy Sparse	Svpy Touching	Svpy Crowded
1. GM3	1.25	1.25	1.52	1.44	1.28	1.34
2. GM2	1.52	0.89	1.00	1.25	1.24	1.24
3. GM1	1.32	1.80	1.12	1.22	1.30	1.35
4. GD1a	2.20	3.92	2.10	2.60	1.35	2.10

[a] Membranes derived from 2×10^5 cells were incubated with UDP-[^{3}H] galactose, and CMP-[^{14}C] sialic acid and the radioactive gangliosides were extracted with chloroform–methanol and were separated by TLC. Numbers are the ratio of ^{14}C/^{3}H, representing degree of sialylation and galactosylation. [b] Results are an average of duplicate analysis from separate batches of cells.

bound sialic acid of each ganglioside and the radioactive incorporation from [^{14}C]galactose into each ganglioside are shown in Table I. Band 4 ganglioside increased to a small degree in chemical quantity, whereas radioactive incorporation increased over twofold at the early stage of cell contact; "crowded" cells showed the same gangliosides as sparse-growing cells.

3T3 cells transformed by Simian virus 40, those double transformed by polyoma virus and SV40, and spontaneously transformed cells (3T6) did not show any difference in ganglioside pattern at different cell population densities.

Sialidase Activity of Sparse, "Touching", and "Crowded" Normal and Transformed 3T3 Cells. Sialidase activities of crude membrane preparations of 3T3 cells grown at different population densities are reported in Table II, which indicates that sialidase activity of normal 3T3 cells, either the membrane preparation or intact cells as monolayer, showed about 50% reduction at the "touching" stage, as compared to sparse growing and "crowded" states. In striking contrast, spontaneously transformed 3T3 cells or those transformed with SV40 virus or double transformed with SV40 and polyoma viruses did not show any detectable difference of sialidase activities at different cell densities.

Biosynthesis of Ganglioside. Incorporation of the radioactivity from UDP-[^{3}H]galactose and CMP-[^{14}C]-*N*-acetylneuraminate into "band 4" ganglioside of 3T3 cells at various cell population densities has been studied using crude membrane fractions under physiological conditions according to the method described by Maccioni et al. (1972), and the results are reported in Table III. Under these assay conditions, the incorporation of ^{3}H into ganglioside indicates the activity of galactosyltransferase and that of ^{14}C indicates the activity of sialyltransferase. As shown in Table III, the ratio ^{14}C/^{3}H of "band 4" (GD1a ganglioside) increases at the "touching" phase of 3T3 cells. This change was not observed in transformed 3T3 cells.

Characterization of the Biosynthesis Product. The isotope ratio ^{14}C/^{3}H (^{3}H present in the galactose moiety and ^{14}C present in the sialic acid moiety) of the newly synthesized ganglioside before methanolysis was determined and compared with the ratio of ^{14}C/^{3}H after collecting the individual sugar peaks from the gas chromatograph after methanolysis. As shown in Table IV, the isotope ratio in the intact molecule closely resembled the ratio of the activities of collected sugar peaks.

Carbohydrate compositions of the gangliosides and their degradation studies by neuraminidase and by weak acid

Table IV: Comparison of the Radioactivities of Intact Biosynthesized Gangliosides and that of Isolated Galactose and Neuraminate found in the Methanolysate of Biosynthesized Gangliosides.

Initial	cpm $^3H/^{14}C$	Ratios
1. (GM_3)	26610/6900	3.86
2. (GM_1)	3270/690	4.74
3. (GD_{1a})	38610/16200	2.38
By GLC[a]	Gal/Neuraminate	
1	2260/610	3.80
2	1200/254	4.70
3	5500/2380	2.30

[a] = 2–5 μl injected/total 20 μl.

Table V: Effect of Sialidase Inhibitors on Sialyltransferase.[a]

Inhibitor Solution in 500 μl	Percent Inhibition of: Vibrio Cholerae Sialidase	3T3 Cells Crude Membrane Sialidase	Sialyltransferase of 3T3 Cells Crude Membrane
Copper acetate (5 m*M*)	70.0[b] (N.D.)	71.7[b]	78.1
N-Phenyloxamic acid (10 μmol)	80.0[b] (67.0[c])	40.0[b]	81.8
Sialyl lactose (20 μg)	60.0[b] (N.D.)	89.5[b]	63.5
Sialostatin-β[d] (40 μg)	1.5[b] (34.0[c])	10.0[b]	45.3

[a] Enzyme activity of control without inhibitor was taken as 100%, and percent inhibition was calculated from this value. [b] Values determined with brain ganglioside as substrate. [c] Values in parentheses represent corresponding percent inhibition determined with submaxillary mucin as substrate. N.D. not determined. [d] 2-Acetamido-3,4-dihydroxy-5-carboxypiperidine.

were carried out as previously described. The results confirmed the structures of bands 1–4 to be GM3, GM2, GM1, and GD1a ganglioside, respectively, as previously reported (Yogeeswaran et al., 1972).

Discussion

Cell contact-dependent or density-dependent increase of glycolipid synthesis was found in hamster fibroblasts (BHK and NIL) and in human fibroblasts (8166), but ironically, the phenomenon was not easily observed in highly contact inhibitable mouse 3T3 cells. The enzymatic basis of an enhanced glycolipid synthesis on cell contact has been well studied in hamster fibroblasts. Kijimoto and Hakomori (1971) and Critchley et al. (1974) both observed a several-fold increase of uridine diphosphate galactose:lactosylceramide-α-galactosyltransferase activity in NIL and BHK cells at high cell population densities, as compared to sparse cell populations, in striking contrast to the enzyme activity not being enhanced in BHKpy and NILpy cells. Fishmann et al. (1972) found that a few glycosyltransferases for synthesis of ganglioside in mouse fibroblasts did not vary significantly at different cell population densities. In contrast, the results of the present paper clearly indicate that "band 4" ganglioside significantly increases on cell contact. This response occurred at the early stage of cell-to-cell contact, rather than during extensive cell conact at "crowded" cell populations. A similar early response of glycolipid synthesis was observed previously in hematoside and Forssman antigen (Kijimoto and Hakomori, 1971). It is likely that the earlier the glycolipid synthesis response occurred, the greater was the sensitivity of contact inhibition demonstrated by the cells. In all these studies, the response on cell contact was not observed in various transformed cells. The loss of contact inhibition associated with transformation may indeed be due to loss of the response in glycolipid concentration on membranes at cell contact.

Holley (1972) postulated that the phenomenon of "contact inhibition" in 3T3 cells may not be ascribed to cell contact, as any specific signal between cells cannot be assessed. The postulation is also based on a well-established fact that increased serum concentration evokes release of "contact inhibition", and the cell saturation density of 3T3 cells is exactly proportional to the amount of serum added to the medium (Todaro and Green, 1963; Holley, 1972). The loss of "contact inhibition", often described for transformed phenotype, could be ascribed to a membrane change for transport of nutrients and susceptibility to a "serum factor". On the other hand, cells may secrete a factor called a "chalone" to control mitosis (Weiss and Kavanu, 1957; Bullough and Laurence, 1960). Recently, "chalone" was purified and its kinetics have been studied (Houck et al., 1972, 1973). Cell contact may alter membrane conformation making it susceptible to "chalone". It is of crucial importance therefore to find a qualitative molecular change in membrane, which is dependent on cell contact and which varies for normal and transformed 3T3 cells. The cell contact-dependent increase of galactosyl residue, indicated by a higher level of absorption of *Ricinus communis* lectin observed in normal but not in transformed 3T3 cells, is one indication of a biochemical surface membrane difference (Nicolson and Lacorbiere, 1973). It is possible that a specific chemical response of membrane indeed exists on contact between 3T3 cells, as enhanced glycolipid concentration occurs at the early stage of cell contact. Another important fact revealed by this study is that the glycolipid accumulation occurring on cell contact results not from inhibited cell growth but rather from actual cell contact, as the enhanced GD1a concentration is clearly observable at the early stage of cell contact when cell growth is still not inhibited, but the change of GD1a concentration is no longer obvious after cell growth is inhibited. Cell contact may induce an enhanced ganglioside concentration in the membrane, which in turn might lead to cessation of DNA synthesis.

If cell contact-dependent enhancement of glycolipid synthesis is a general phenomenon for nontransformed cells and its absence is a common denominator for transformed phenotypes, this mechanism is extremely important and could be a key factor in understanding malignancy.

In this study the changes of both glycosyltransferase and sialidase activities on cell contact were demonstrated. The sialidase changes observed at the early stage of cell contact should be significant, as the change of sialidase activity is not affected by and is independent from sialyltransferase activity. However, it is highly probable that an apparent increase of sialyltransferase detected at the early stage of cell-to-cell contact could be due to a suppressed sialidase activity. We therefore focussed our study on the possible elimination of sialidase activity by "sialidase inhibitors". As shown in Table V, many sialidase inhibitors so far tested also inhibit sialyltransferase activity. Ideally we should determine sialidase activity completely separate from sialyltransferase

activity, although this is impossible by presently available methods.

Determination of various glycosyltransferases and comparison of the activities as related to the function of cells have become increasingly popular, and a number of valuable data have been reported. The approach is, however, difficult if one considers a number of factors involved for the quantitative determination of membrane-bound glycosyltransferase. Complete solubilization of the enzyme without partial destruction of the enzyme in situ, a reconstruction of enzyme activity, and the exclusion of sugar nucleotide phosphatase and glycosylhydrolases are particularly important. The conditions usually employed for demonstration of sialyl- or glycosyltransferases in general are unphysiological, with inclusion of detergents, unusually high concentrations of magnesium and of manganese, unphysiological buffer systems, etc.

In this investigation we therefore employed a "physiological condition" for biosynthesis as described by Maccioni et al. (1972). However, elimination of coexisting sialidase activity was practically impossible. As the conditions solely depended on endogenous acceptors, the relative activities of sialyl- and galactosyltransferases could also reflect acceptor pool changes.

Schengrund et al. (1970, 1973) and Visser and Emmelot (1973) recently described the presence of sialidase in plasma membranes, distinguishable from lysosomal sialidase. We have used in this study two methods for determination of surface sialidase activity: (1) crude membrane fraction obtained by hypotonic lysis of cells according to Schengrund et al. (1973) and (2) direct application of ganglioside substrate to monolayer culture of cells and determination of the liberated sialic acid. Interestingly, by either method, decreased sialidase was demonstrated at the early stage of cell-to-cell contact. It is highly probable that the surface sialidase plays an important role in determining cell social activity and also in determining surface-mediated regulation of ganglioside metabolism during cell growth.

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