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Cell Surface Glycosyltransferases—Do They Exist?

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The presence of glycosyltransferases on surfaces of mammalian cells has been reported by many investigators and a biological role for these enzymes in cell adhesion and cell recognition has been postulated. Critical analysis, however, showed 2 major complications regarding the assay for cell surface glycosyltransferases: 1) hydrolysis of the nucleotide sugar by cell surface enzymes and subsequent intracellular use of the free sugar and 2) loss of cell integrity if trypsinized or EDTA-treated cells were used in suspension asays. We have assayed intact, viable cells in monolayer for cell surface glycosyltransferases using conditions under which intracellular utilization of free sugars generated by hydrolysis of the nucleotide sugar was prevented. Our data demonstrate that the presence of galactosyltransferases on the surface of a variety of cells, including established (normal and virally transformed) as well as non-established cells, is unlikely. No evidence for the existence of cell surface fucosyl- and sialyltransferases could be obtained, but our data do not exclude the possibility that low levels of these enzymes are present.

Key words: cell viability, nucleotide sugar hydrolysis, intracellular glycosylation

A large number of publications have reported glycosyltransferase activities on the surface of whole cells (cf. Ref. 1 for a review). These studies were initated by a hypothesis put forward by Roseman (2) suggesting that glycosyltransferases on the cell surface are

Abbreviations: EDTA – (ethylenedinitrilo) tetraacetic acid; UDP-galactose – uridine diphosphate galactose; DME – Dulbecco and Vogt's modified Eagle's medium; BHK – baby hamster kidney cells; Py BHK – polyoma virus-transformed BHK cells; ME – mouse embryo cells; SV 3T3 – SV40-transformed BALB/c 3T3 cells; CHE – Chinese hamster embryo cells; AGMK – African green monkey kidney cells; Hepes – N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; SDS – sodium dodecyl sulfate; AFP – antifreeze proteins; Fetuin-desial. – desialized fetuin; AFP-degal. – degalactosized antifreeze proteins; Fetuin-desial.-degal. – desialized and degalactosized fetuin; Gal-1-P – galactose-1-phosphate; GDP-fucose – guanosine diphosphate fucose; CMP sialic acid – cytidine monophosphate sialic acid; 5′-AMP – 5′-adenosine monophosphate; ND – experiment not done; BSA – bovine serum albumin.

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involved in intercellular adhesion and cell recognition. According to this hypothesis, glycosyltransferases on the cell surface interact with their substrate carbohydrate chains of glycoproteins or glycolipids on the surfaces of neighboring cells.

Evidence consistent with this idea has come from experiments in which single cell suspensions, prepared by trypsin or EDTA treatment of cells grown in monolayer cultures, were incubated with nucleotide sugars and the transfer of sugar residues to either endogenous or exogenous glycoproteins or glycolipids was observed. The conclusion drawn from these experiments that the transfer of the sugar residue from the added nucleotide sugar was mediated by cell surface glycosyltransferases is ambiguous for the following reasons: 1) The cell surface of many cells (see Results) contain 2 enzymatic activities, a nucleotide pyrophosphatase and a monophosphoesterhydrolase, which together catalyze the hydrolysis of nucleotide sugars to free sugar. The generation of free sugar, its transport into the cells, and its incorporation into carbohydrate-containing macromolecules would simulate the activity of cell surface glycosyltransferases. 2) The choice of assay systems using cells in suspension after trypsin or EDTA treatment further complicates the evaluation of the data reported in the literature. Cells which are normally cultured in monolayers are viable in suspension only for a limited period of time (see Results). Therefore, many of the experiments reported may have been performed with dead or dying cells. A small fraction of dead cells and the accompanying availability of intracellular membranes could account for the incorporation data described in many studies. 3) The possibility that trypsin or EDTA treatment might alter the permeability of cells for small molecules, and macromolecules as well, has to be considered.

In a previous report (3), we have shown that the incubation of BHK cells in monolayer with exogenous UDP-galactose does not result in a measurable transfer of galactose residues from UDP-galactose to cellular acceptor molecules if the utilization of free galactose as a hydrolysis product of UDP-galactose was blocked. Since we also could not find any detectable transfer to exogenous acceptor molecules, we concluded that the cell surface of BHK cells does not contain detectable cell surface galactosyltransferase activity. In this report, we demonstrate that the ability to hydrolyze exogenous nucleotide sugars is not confined to BHK cells and that cell surface glycosyltransferase assays using trypsinized cells in suspension may lead to artefacts due to the altered permeability and decreased viability of these cell preparations. Furthermore, various cells grown and assayed in monolayer do not show conclusive evidence for nucleotide sugar-dependent cell surface glycosylation if criteria for the differentiation between intracellular and cell surface glycosyltransferases (3) are applied.

MATERIALS AND METHODS

Cell Culture

BHK 21/13 were cultured in Dulbecco and Vogt's modified Eagle's medium (DME) (4) supplemented with 10% calf serum and 0.3% tryptose phosphate.

Polyoma virus-transformed BHK cells (Py BHK) were obtained from Dr. W. Eckhart (The Salk Institute), as were primary mouse embryo cells (ME). Mouse BALB/c 3T3 and Swiss 3T3 4A cells, SV40-transformed BALB/c 3T3 cells (SV 3T3), and Chinese hamster embryo cells (CHE) at passage 16 after establishing the primary culture were obtained from Dr. M. Vogt (The Salk Institute). Primary African green monkey kidney cells (AGMK) were obtained from Microbiological Associates, Inc., Los Angeles, California.

Py BHK, 3T3 (BALB/c and Swiss 4A), CHE, and AGMK cells were cultured in DME supplemented with 10% calf serum. BHK, Py BHK, 3T3 (BALB/c and Swiss 4A), SV 3T3, and CHE cells were free of mycoplasma as tested by labeling with [^{3}H] thymidine followed by autoradiography.

Incubation Assay

If not stated otherwise, subconfluent to confluent cells in monolayer were used for the experiments. Cells were seeded 2 days before the start of the experiments. After removal of the medium, the cells were washed 3 times with prewarmed Mg^{2+} and Ca^{2+} free isotonic salt solution, buffered at pH 7.4 with 20 mM Hepes (isotonic Hepes buffer). Cells were incubated in the same buffer, containing 1–5 mM $MnCl_2$ as stated. Uridine diphosphate D-[1-^{3}H] galactose (1.23 Ci/mmole, New England Nuclear Corporation, Boston, Massachusetts), D-[6-^{3}H] galactose (168 mCi/mmole, New England Nuclear Corporation), guanosine diphospho-2-[U-^{14}C] fucose (170 mCi/mmole, Amersham/Searle), 2-[U-^{14}C]-fucose (133 mCi/mmole, Amersham/Searle), and cytidine-5′-monophospho-[G-^{3}H] sialic acid (2.33 Ci/mmole, New England Nuclear Corporation) were added in the concentrations indicated. The plates were incubated at 37°C in a moist chamber mounted on a rocking shaker.

Paper Chromatography

The hydrolysis products of the nucleotide sugars were determined by analyzing aliquots of the incubation mixture by descending paper chromatography on Whatman No. 3 MM paper. The following solvent systems were used: ethanol:1 M ammonium acetate, pH 3.8, (5:2) for analysis of UDP-galactose; ethanol: 1 M ammonium acetate (7:3) for analysis of CMP-sialic acid; and ethanol:1 M ammonium acetate:acetic acid (50:20:33) for analysis of GDP-fucose. The running time was 17 h. The paper was then dried, cut into 1-cm strips, eluted with 1 ml of distilled water, and counted in Aquasol (New England Nuclear Corporation) using a Beckman liquid scintillation counter.

Determination of Incorporated Label

After the incubation times indicated, the medium was removed, the cells washed twice with isotonic Hepes buffer, dissolved in 0.5 ml of 1% SDS solution per 3-cm dish, and homogenized by sonication (10 sec at full power in a Branson sonifier equipped with a mictrotip). To 200-μl aliquots, diluted with 2 ml of ice cold water, 3 ml of 1% phosphotungstic acid in 0.5 N HCl were added. After 45 min at 0°C, the precipitates were collected on Whatman GF/C filters and washed with 10% trichloroacetic acid. The filters were then dried and their radioactivity was measured.

High-Molecular-Weight Acceptors

The freezing point depressing proteins AFP (antifreeze proteins), a mixture of 3 components with molecular weights of 10,500, 17,000, and 21,000 isolated from the serum of the Antarctic fish Trematomus borchgrevinki (5), and bovine fetuin were used as high-molecular-weight acceptors. Terminal galactose residues of AFP were removed by Smith degradation as described (6); terminal sialic acid and penultimate galactose residues were removed from fetuin by the method of Kim et al. (7). AFP and desialized fetuin (fetuin-desial.) were used as acceptors in sialyltransferase assays, degalactosized AFP (AFP-degal.), and desialized and degalactosized fetuin (fetuin-desial.-degal.) as acceptors for galactosyltransferase assays. AFP was a gift from Dr. T. Shier, The Salk Institute.

TABLE I. Hydrolysis of UDP[^{3}H]galactose by Various Cells*

Cells	Percent of radioactivity				Protein (μg)/incubation
	UDP-galactose	Gal-1-P	X	Galactose	
A. Established cell lines					
BHK 21/13	0	16.6	8.2	75.1	630
BALB/c 3T3	0	90.8	2.1	7.1	430
Swiss 3T3 4A	0	94.5	1.1	4.4	440
Py BHK	0	5.7	7.4	86.9	830
SV3T3	26.2	61.3	11.9	0.6	800
B. Nonestablished cells					
AGMK primary	28.4	32.4	3.0	36.2	560
ME secondary	0.6	14.9	2.6	81.9	720
CHE passage 18	2.1	24.7	4.2	69.0	480
C. BHK cells in suspension					
trypsin treated	4.7	73.0	0	22.3	430
EDTA treated	43.0	50.9	2.1	4.0	430
control (on plate)	0	55.0	3.8	41.2	430
No cells (control)	99.6	< 1.0	< 1.0	< 1.0	

*2×10^5 cells were seeded on 3-cm dishes and tested for hydrolysis of UDP[^{3}H]galactose 2 days after seeding. Cells were incubated for 3 h with 0.5 ml of isotonic Hepes buffer containing 1.0 μCi UDP[^{3}H]-galactose and 1 mM $MnCl_2$, and hydrolysis products were determined as described in Materials and Methods. "X" is an unidentified compound, whose chemical nature was not further characterized. For testing BHK cells in suspension, the cells were removed from plates either by treatment with 0.1% trypsin or with 10 mM EDTA in isotonic Hepes buffer. The cell suspension was incubated for 30 min in 5 ml of growth medium and then for 3 h in 0.5 ml of isotonic Hepes buffer, containing 1.0 μCi UDP[^{3}H]galactose and 1 mM $MnCl_2$. Protein was determined according to Lowry et al. (29).

RESULTS

Hydrolysis of Nucleotide Sugars by Intact Cells

The cell surface of BHK 21/13 cells contains 2 enzymatic activities, a nucleotide pyrophosphatase and a monophosphoester hydrolase, which together hydrolyze exogenous UDP-galactose to free galactose in a 2-step mechanism with galactose-1-phosphate (gal-1-P) being the intermediate product (3). The ability of intact cells to hydrolyze UDP-galactose is not unique to BHK cells. Table I, A and B, shows that all cells exhibited considerable hydrolytic activity. Differences, however, were observed between the different types of cells in their ability hydrolyze gal-1-P to free galactose. Whereas with BHK, Py BHK, AGMK, CHE, and ME cells, the major hydrolysis product after a 3 h incubation period was galactose, with BALB/c 3T3, Swiss 3T3 4A, and SV 3T3 cells, the major hydrolysis product was gal-1-P. Single cell suspensions of BHK cells, obtained either by trypsin or EDTA treatment, also hydrolyzed UDP-galactose to free galactose, although to a lesser extent (Table IC).

We have also tested the hydrolysis of GDP-fucose, the substrate for fucosyltransferases, and CMP-sialic acid, the substrate for sialyltransferases by BHK cells in monolayer (Table II). GDP-fucose was hydrolyzed at about the same rate as UDP-galactose with fucose-1-phosphate being the intermediate, and free fucose being the end product of

TABLE II. Hydrolysis of UDP[^{3}H]Galactose, GDP[^{14}C]Fucose and CMP[^{3}H]Sialic Acid by BHK Cells*

Nucleotide sugar	Percent of radioactivity		
	Nucleotide sugar	Sugar-1-phosphate	Free sugar
UDP-galactose	0	51.1	46.8 (galactose)
GDP- fucose	0	48.4	50.7 (fucose)
CMP-sialic-acid	65.1	nonexistent	34.9 (sialic acid)

*Monolayers of BHK cells (1×10^6 per 3-cm dish) were incubated for 3 h with 0.5 ml of isotonic Hepes buffer, containing 1.0 μCi UDP[^{3}H]galactose, GDP[^{14}C]fucose, or CMP[^{3}H]sialic acid, respectively, and 1 mM $MnCl_2$. Hydrolysis products were determined as described in Materials and Methods.

hydrolysis. The hydrolysis of CMP-sialic acid, on the other hand, occurs at a much slower rate, but still yields a considerable amount of free sialic acid during a 3 h incubation period. Most of this hydrolysis reaction is not mediated by cell surface enzymes but is due to the chemical instability of CMP-sialic acid (3, 8).

The hydrolysis data shown in Table I and Table II were obtained by analysis of an aliquot of the incubation medium after 3 h of incubation. During that time period, a considerable amount of the free sugar, generated by hydrolysis, was taken up by the cells, whereas neither the nucleotide sugar nor the sugar-1-phosphate were able to permeate (3). The total amount of free galactose generated by hydrolysis, therefore, is higher than the amount of free galactose found in the incubation medium at the end of the assay. This to be considered in cases where the hydrolysis of UDP-galactose to free galactose is not very effective.

Comparison of the Incorporation of Galactose by BHK Cells in Monolayer and by Trypsinized BHK Cells in Suspension

In any assay for cell surface glycosyltransferases, the hydrolysis of nucleotide sugars to the free sugar by cell surface enzymes will simulate or obscure cell surface glycosyltransferase activity if the intracellular utilization of the free sugar is not prevented. Inhibition of the intracellular utilization of the radioactive free sugar can be achieved 1) by the addition of transport inhibitors, such as phloridzin (9), 2) by competition with high concentrations, e.g., at or above the K_m for the sugar transport system of the nonradioactive free sugar, or 3) by poisoning the cells with azide. We have shown in a previous study (3) that neither phloridzin, nor high concentrations of galactose, nor azide have any effect on the galactosyltransferase reaction in cell homogenate.

The effect of these inhibitors on the galactose incorporation of BHK cells in monolayer and in suspension assays with trypsinized cells was compared. Table III shows that high concentrations of nonradioactive galactose as well as the addition of 15 mM sodium azide inhibited galactose incorporation into BHK cells in monolayer and in suspension to a similar degree. In contrast, phloridzin inhibited galactose incorporation during a 3 h incubation period only by about 60% with BHK cells in a suspension assay, but inhibited incorporation by 95% with BHK cells assayed in monolayer. Phloridzin acts as an inhibitor of galactose incorporation by blocking the transport sites for galactose (9). The failure of phloridzin to inhibit galactose incorporation with trypsinized cells to the same extent as with BHK cells in monolayer, therefore, may indicate either altered binding properties of

TABLE III. Inhibition of [^{3}H]Galactose Incorporation With BHK Cells in Monolayer and With Trypsinized BHK Cells in Suspension*

Addition	BHK cells in monolayer		BHK cells in suspension	
	cpm/10^6 cells	% Incorporation	cpm/10^6 cells	% Incorporation
None	2,535	100	1,487	100
1.5 mM nonradioactive galactose	335	13	223	15
15 mM NaN_3	483	19	252	17
1.5 mM nonradioactive galactose, 10 mM NaN_3	228	9	45	3
5 mM phloridzin	127	5	565	38

*BHK cells (3×10^6 per assay) either in monolayer or in suspension after trypsin treatment (see Legend to Table I) were incubated for 1 h with 0.5 ml of isotonic Hepes buffer, containing 1.0 μCi [^{3}H]-galactose. Additions to the incubation medium were as indicated. The reaction was stopped by the addition of 50 μl of 10% SDS and incorporation of radioactive galactose was determined in 200-μl aliquots as described in Materials and Methods.

this inhibitor at the galactose transport sites or altered permeability properties of trypsinized cells. That the latter may indeed be the case is suggested by the different kinetics of galactose incorporation with BHK cells in monolayer and BHK cells in suspension. As shown in Fig. 1, the incorporation of [^{3}H]galactose by BHK cells in monolayer into phosphotungstic acid precipitable material showed a lag phase of 10–15 min which can be explained by the time required for the equilibration of the radioactive galactose with the intracellular sugar pool. With trypsinized cells, on the other hand, the incorporation occurred without an apparent lag. A possible explanation for this result is that the intracellular sugar pool had decreased due to leakiness of the trypsinized cells and, therefore, equilibrium with the transported radioactive sugar was reached much earlier. If sugar molecules can leak out of trypsinized cells as shown by Hirschberg et al (8), one can expect also that sugar molecules may permeate into the cells unspecifically, even if the specific transport sites are blocked.

Viability of BHK Cells in Monolayer and in Suspension Assays

An absolute requirement for an assay of cell surface glycosyltransferases is that the cells remain viable during the incubation period. Dead cells could either leak out intracellular glycosyltransferases or exogenous nucleotide sugar might be made available to glycosyltransferases inside the cell. We have previously found that trypan blue exclusion is not a very sensitive measure for cell viability (3) and therefore we decided to investigate the viability of BHK cells by their ability to grow after addition of growth medium at the end of the assay.

Two types of cell preparations were tested: BHK cells grown and assayed in monolayer cultures and BHK cells which were removed from the plate with trypsin, resuspended in complete growth medium for a "recovery period" of 30 min, and then assayed in suspension. BHK cells incubated in monolayer with isotonic Hepes buffer (see Materials and Methods) did not significantly increase in number during a 3 h incubation period, but doubled in number within 16 h following the readdition of growth medium. Trypsinized BHK cells which were reseeded immediately after the recovery period (control cells)

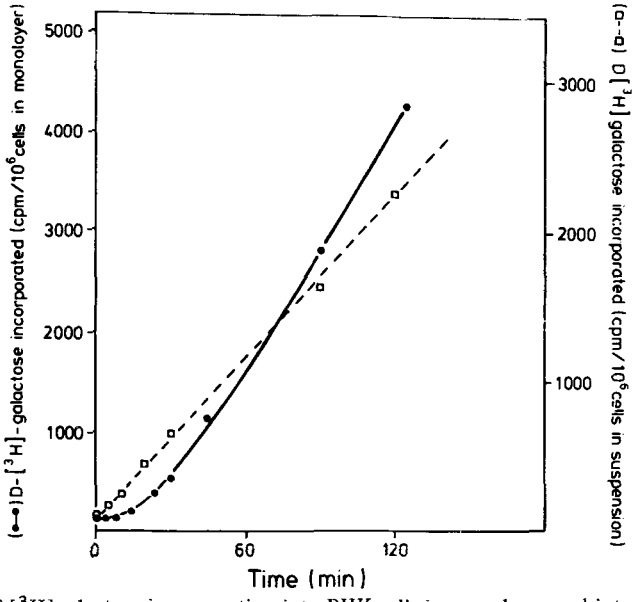

Fig. 1. Kinetics of [^{3}H]galactose incorporation into BHK cells in monolaver and into BHK cells in suspensions. BHK cells (1 X 10^6) in monolayer on 3-cm dishes (•) or in suspension after trypsin treatment (see legend to Table I) were incubated with 1.0 μCi of [^{3}H]galactose in isotonic Hepes buffer. At the times indicated, galactose incorporation was determined as described in Materials and Methods.

attached almost completely to the plate within 4 h and doubled within 20 h. However, these same cells if incubated for 3 h with isotonic Hepes buffer following the recovery period attached much more poorly (about 65%), and only the cells attached to the plate were able to resume normal growth. This indicated that after suspension of trypsinized cells in buffer for a 3-h incubation period about one third of the cells were no longer viable according to our standards.

The decreased viability of trypsinized cells maintained in suspension during an assay is a particular problem when highly unphysiological conditions, such as the presence of high concentrations of $MnCl_2$ and sodium azide, are used. When trypsinized BHK cells were incubated with isotonic Hepes buffer containing 10 mM $MnCl_2$ and 10 mM sodium azide (conditions routinely used by Roth and White, Ref. 10), all cells were killed during a 3 h incubation period as judged by their inability to reattach to the plate within 24 h after reseeding (Fig. 2B). BHK cells in monolayer, on the other hand, survived under the same assay conditions. Although these monolayer cells started to round up during the 3 h incubation period, they recovered completely after readdition of growth medium (Fig. 3). The altered permeability and decreased viability of trypsinized cells in suspension assays, in our opinion, renders such a cell preparation unsuitable for the assay of cell surface glycosyltransferases.

Assay for Cell Surface Glycosyltransferase Activity Towards Endogenous Acceptors

In a previous paper we have provided evidence that the incorporation of galactose from exogenous UDP-galactose with BHK cells can be inhibited if the intracellular utilization of free galactose is blocked by various inhibitors (3). We, therefore, concluded that

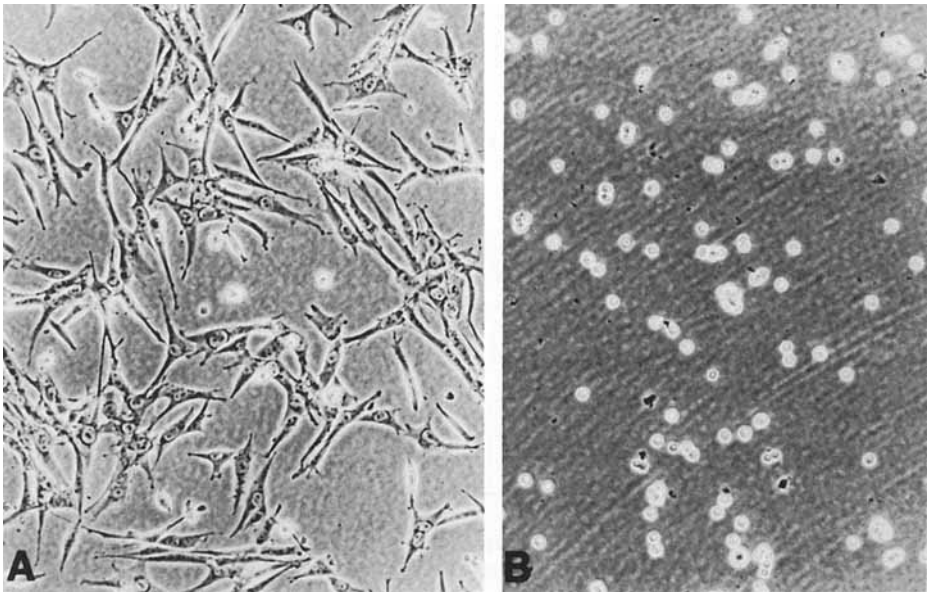

Fig. 2. Survival of BHK cells in suspension following incubation with isotonic Hepes buffer containing manganese chloride and sodium azide. BHK cells (1.3×10^6 per 3-cm dish) were removed from petri dishes by treatment with 0.1% trypsin in isotonic Hepes buffer. The cell preparations were preincubated with 1 ml of normal growth medium for 30 min, washed twice with isotonic Hepes buffer and then incubated for 3 h with isotonic Hepes buffer containing 10 mM $MnCl_2$ and 10 mM sodium azide. A) Control cells were incubated for approximately 5 min, washed twice with growth medium, and then reseeded onto 10-cm dishes. B) Cells were incubated for 3 h with occasional shaking, washed twice with growth medium, and then reseeded onto 10-cm dishes. Phase contrast microscopy pictures (magnification 165 ×) were taken 24 h after reseeding the cells.

the incorporation observed in the absence of inhibitors is due to hydrolysis of the nucleotide sugar and to the subsequent intracellular use of free galactose. This conclsuion is further substantiated by the experiment shown in Table IV. By cloning BHK cells, it is possible to obtain BHK subclones, whose ability to hydrolyze UDP-galactose is greatly reduced (11). If these BHK subclones are assayed for cell surface galactosyltransferase activity in the absence of inhibitors of galactose incorporation, a close relationship between the hydrolytic activity of these cells and the amount of galactose incorporated is found. BHK cells which exhibit strong hydrolytic activity, show high incorporation values. This incorporation can be inhibited by the addition of phloridzin. On the other hand, BHK cells which do not measurably hydrolyze UDP-galactose, also do not incorporate radioactive galactose to a significant amount, even in the absence of phloridzin.

The experiment shown in Tables IV further demonstrates that upon incubation of BHK cells with UDP-galactose a basal level of incorporation of radioactive galactose cannot be inhibited by the addition of phloridzin. Since this basal level of incorporation also is observed upon incubation of cells with radioactive galactose (Table III) it most likely does not represent specific incorporation by cell surface glycosyltransferases.

An argument against the validity of our finding that BHK cells do not exhibit endogenous cell surface galactosyltransferase activity (3) was put forward by Shur and Roth (1).

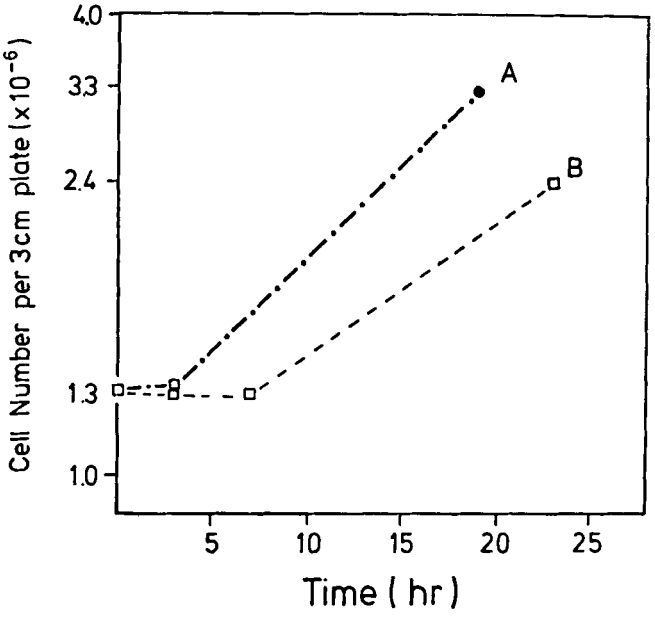

Fig. 3. Growth of BHK cells in monolayer following incubation with isotonic Hepes buffer containing manganese chloride and sodium azide. BHK cells in monolayer (1.3×10^6 per 3-cm dish) were incubated for 3 h with isotonic Hepes buffer (A) or with isotonic Hepes buffer containing 10 mM sodium azide and 10 mM $MnCl_2$ (B). The cells were washed twice with growth medium and 3 ml of growth medium was added. Cell numbers were determined from parallel plates at the times indicated.

They feel that monolayer cultures are not suitable for any assay of endogenous cell surface glycosyltransferase activity, because "cells from subconfluent cultures may have a low endogenous activity, because they were separate, while cells from confluent cultures may have already glycosylated their acceptors" (cited from Ref. 1). For the reasons given earlier in this paper, we believe that any assay system using trypsinized cells will possibly lead to artefacts due to the altered permeability and decreased viability of these cells. In order to test whether contact dependent cell surface galactosyltransferase activity could be detected with BHK cells in monolayer, we have seeded BHK cells at different concentrations, ranging from sparse (2.9×10^4 cells/cm^2) to dense (2.9×10^5 cells/cm^2). Approximately 6–8 h after seeding, when the cells were attached to the plate and no longer rounded up, e.g., at a time when cell contact was most likely to be (re)established in the more dense cultures, these cells were assayed for endogenous cell surface galactosyltransferase activity in monolayer. Table V shows that the incorporation of radioactive galactose from UDP-galactose by the BHK cell monolayers was independent of cell density and could be inhibited with phloridzin, suggesting the absence of contact-dependent cell surface galactosyltransferase activity on BHK cells.

We then examined whether the absence of cell surface galactosyltransferase activity on BHK cells in monolayer might be due to the fact that all endogenous acceptors were already glycosylated. BHK cells in confluent monolayers were pretreated with a) 0.5 μg/ml trypsin, b) 0.5 μg/ml pronase, or c) a glycosidase mixture containing 50 μg/ml β-galactosidase, 50 μg/ml β-glucosidase, 50 μg/ml hyaluronidase, and 10 μg/ml sialidase, for 45 min. This treatment did not affect the viability of these cells, although a few of the

TABLE IV. Incorporation of [^{3}H] Galactose From UDP[^{3}H]Galactose by BHK-Subclones With Different Hydrolytic Activities*

BHK subclone	Percent of free galactose generated by hydrolysis	[^{3}H] Galactose incorporated (cpm/10^6 cells)		Percent residual incorporation in the presence of phloridzin
		No inhibitor	8 mM phloridzin	
scl 3	58.8	10,671	127	1.2
scl 16	7.1	1,410	50	3.5
scl 15	2.6	227	46	20.3
scl 23	0.8	107	69	64.5

*Monolayers of BHK cells (1×10^6 per 3-cm dish) were incubated for 3 h with 0.3 ml of isotonic Hepes buffer, containing 1.0 μCi UDP[^{3}H]galactose and 5 mM of $MnCl_2$. Phloridzin was added as indicated. Hydrolysis of UDP[^{3}H]galactose and incorporation of [^{3}H]galactose was determined as described in Materials and Methods. Designation of BHK subclones is according to (11).

TABLE V. [^{3}H] Galactose Incorporation From UDP[^{3}H] Galactose by BHK Cells at Different Cell Densities*

Cell density (cells/cm^2)	[^{3}H] Galactose incorporated (total cpm/assay)		Percent residual activity in the presence of phloridzin
	No inhibitor	5 mM Phloridzin	
2.9×10^4	1,407	84	6.0
7.1×10^4	3,545	137	3.9
1.0×10^5	6,113	122	2.0
1.4×10^5	7,697	159	2.1
2.9×10^5	16,349	346	2.2

*BHK cells were seeded on 3-cm dishes at the cell densities indicated approximately 6–8 h before the experiment. Monolayers were incubated for 3 h with 0.3 ml of isotonic Hepes buffer, containing 2.0 μCi UDP[^{3}H]galactose and 5 mM $MnCl_2$. Phloridzin was added as indicated. Incorporation of [^{3}H]-galactose was determined as described in Materials and Methods.

cells treated with trypsin rounded up while remaining on the plate. The cells were then assayed for cell surface galactosyltransferase activity in the absence or presence of 5 mM phloridzin either directly after the pretreatment or after a recovery period of 45 min in normal growth medium. The result of this experiment is summarized in Table VI. None of the pretreatments caused an enhancement of galactose incorporation. Furthermore, in all samples the incorporation observed could be inhibited with phloridzin.

Cells of Various Origins

The absence of detectable surface galactosyltransferase activity of BHK cells is not an unique property of these cells. We have tested Py BHK, BALB/c 3T3, and SV 3T3 cells as representatives of established cell lines and primary AGMK cells, secondary ME cells, and CHE cells in passage 18 as representatives of nonestablished cells. Table VII shows that with all cells tested the incorporation of radioactive galactose from exogenous UDP-

TABLE VI. Assay for Endogenous Cell Surface Galactosyltransferase Activity With Protease (or Glycosidase) Pretreated BHK Cells on Plate*

Pretreatment	Recovery period	Additions to the incubation medium	[^{3}H] Galactose incorporation	
			cpm/10^6 cells	% incorporation
A. Trypsin	none	none	1,168	100
	none	5 mM Phloridzin	164	14.0
	45 min	none	1,365	100
	45 min	5 mM Phloridzin	130	9.5
B. Pronase	none	none	1,300	100
	none	5 mM Phloridzin	111	8.5
	45 min	none	1,289	100
	45 min	5 mM Phloridzin	97	7.5
C. Glycosidase mixture	none	none	1,205	100
	none	5 mM Phloridzin	94	7.8
	45 min	none	1,292	100
	45 min	5 mM Phloridzin	69	5.3
D. None (Control)	none	none	1,292	100
	none	5 mM Phloridzin	98	7.6

*Monolayers of BHK cells (3×10^6 per 3-cm dish) were pretreated for 45 min with A) 0.5 μg/ml trypsin in isotonic Hepes buffer, B) 0.5 μg/ml pronase in isotonic Hepes buffer, or C) a glycosidase mixture containing 50 μg/ml β-galactosidase, 50 μg/ml hyaluronidase, and 10 μg/ml sialidase in isotonic Hepes buffer. Cells incubated with isotonic Hepes buffer for 45 min were used as control (D). Following the pretreatment, the monolayers were washed twice with isotonic Hepes buffer and the cells assayed for cell surface glycosyltransferase activity either directly or after a recovery period of 45 min in normal growth medium. The incubation medium was 0.3 ml of isotonic Hepes buffer, containing 1.0 μCi UDP[^{3}H]galactose and 5 mM $MnCl_2$. Phloridzin was added as indicated. Incubation was for 3 h. Incorporation of [^{3}H]galactose was determined as described in Materials and Methods.

galactose could be inhibited by either phloridzin or azide to a similar extent as the incorporation resulting from the incubation with radioactive-free galactose. This result strongly suggests that none of the cells tested exhibits cell surface galactosyltransferase activity.

The criteria developed in our earlier study (3) to differentiate between intracellular and cell surface galactosyltransferase activity can also be applied for testing whether glycosyltransferases other than galactosyltransferases may be located on the cell surface.

Assay for Fucosyl- and Sialyltransferases

We decided to test for cell surface fucosyl- and sialyltransferases because both fucose and sialic acid are terminal sugars on carbohydrate chains of glycoproteins and glycolipids (2) and neither sugar is rapidly metabolized in cells.

Table VIII shows that the incorporation of radioactive L-fucose into BHK cells is not inhibited by either high concentrations (1.5 mM) of glucose or by the addition of 8 mM phloridzin. This indicates that L-fucose is transported into the cells by a different transport system than glucose or galactose. However, inhibition of radioactive fucose incorporation can be obtained by competing with nonradioactive fucose, by inhibition of the cellular metabolism with sodium azide (10), and most effectively by a combination of both (Table VIII). Similarly, the incorporation of radioactive fucose upon incubating BHK cells with GDP-fucose can be inhibited by the addition of either cold fucose or azide, or

TABLE VII. Assay for Endogenous Cell Surface Galactosyltransferase Activity With Various Cells*

Cells	Additions to the incubation medium	Incubation with [^{3}H]galactose		Incubation with UDP[^{3}H]galactose		μg Cell protein per assay
		cpm/assay	% Incorporation	cpm/assay	% Incorporation	
A. Established cells						
BALB/c3T3	none	1,781	100	203	100	170
	4 mM Phloridzin	358	20	32	16	170
	10 mM Sodium azide	610	34	58	28	170
	20 mM Sodium azide	432	24	38	19	170
SV 3T3	none	ND		696	100	270
	4 mM Phloridzin	ND		108	15	270
	8 mM Phloridzin	ND		78	11	270
Py BHK	none	21,347	100	9,204	100	350
	4 mM Phloridzin	2,989	14	1,473	16	350
	8 mM Phloridzin	1,281	6	828	9	350
B. Nonestablished Cells						
AGMK, primary	none	ND		2,132	100	220
	8 mM Phloridzin	ND		175	8	220
	20 mM Azide	ND		327	15	220
	1.6 mM nonradioactive galactose	ND		220	10	220
ME, secondary	none	4,163	100	3,202	100	290
	8 mM Phloridzin	281	7	95	3	290
CHE, passage 18	none	ND		8,497	100	190
	4 mM Phloridzin	ND		202	2.4	190
	8 mM Phloridzin	ND		119	1.4	190

*2×10^5 cells were seeded on 3-cm dishes and assayed for cell surface glycosyltransferases 2 days after seeding. The incubation medium consisted of 0.3 ml of isotonic Hepes buffer containing 5 mM $MnCl_2$ and 1.0 μCi UDP[^{3}H]galactose or 1.0 μCi [^{3}H]galactose, respectively. Additions to the incubation medium were as indicated. Incubation was for 3 h and incorporation of [^{3}H]galactose was determined as described in Materials and Methods. Protein was determined according to Lowry et al. (29).

TABLE VIII. Assay for Endogenous Cell Surface Fucosyltransferase Activity With BHK Cells*

Additions to the incubation medium	[^{14}C] Fucose (1.0 μCi)		GDP[^{14}C] Fucose (1.0 μCi)	
	cpm/10^6 cells	% Incorporation	cpm/10^6 cells	% Incorporation
None	375	100	153	100
8 mM Phloridzin	382			
1.5 mM D-glucose	376			
15 μM L-fucose	352	94		
75 μM L-fucose	217	58		
150 μM L-fucose	152	40		
750 μM L-fucose	95	25		
1,500 μM L-fucose	24	6	27	17
5 mM Sodium azide	86	22	23	15
10 mM Sodium azide	36	10	16	10
15 mM Sodium azide	34	9	10	6
5 mM Sodium azide +1,500 μM L-fucose	20	5	3	2

*BHK cells in monolayer (1×10^6 per 3-cm dish) were incubated for 3 h with 0.3 ml of isotonic Hepes-s buffer, containing 1 mM Mn Cl_2 and μCi 2-[^{14}C] fucose or 1.0 μCi GDP-2-[^{14}C] fucose, respectively. Additions to the incubation medium were as indicated. Incorporation of 2-[^{14}C] fucose was determined as described in Materials and Methods.

a combination of both, suggesting the absence of surface fucosyltransferases on BHK cells (Table VIII).

Datta (12) has reported that BHK cells in monolayer catalyze the transfer of sialic acid from exogenous CMP-sialic acid onto acceptor molecules on the surface of these cells. He found that neither a 1,000-fold molar excess of nonradioactive sialic acid (1 mM) in the labeling medium nor the addition of transport inhibitors such as phloridzin or a mixture of sodium cyanide and iodoacetate (13) reduced the incorporation of radioactivity into the acid-insoluble fraction. The addition of 20 mM sodium azide reduced the incorporation only by about 20%. Datta, therefore, concluded that the incorporation of sialic acid from CMP-sialic acid was mediated by cell surface sialytransferase.

Hirschberg et al. (8) recently have demonstrated that the uptake of sialic acid by BHK cells and other fibroblasts is a saturable process with an apparent K_m of 10 mM. The incorporation of radioactive sialic acid as a breakdown product of CMP-sialic acid, therefore, should not be affected by the addition of unlabeled sialic acid in concentrations lower than the K_m for sialic acid uptake. Table IX shows that radioactive sialic acid incorporation from radioactive CMP-sialic acid and from radioactive free sialic acid can be effectively inhibited by the addition of 10 mM or 20 mM unlabeled sialic acid. This result may confirm the conclusion of Hirschberg et al. (8) that the incorporation of sialic acid observed after incubation of monolayer cells with CMP-sialic acid is more likely due to the uptake and intracellular utilization of free sialic acid as a breakdown product of CMP-sialic acid than to cell surface transferase activity. On the other hand, our finding that the addition of 5 mM unlabeled sialic acid to the incubation medium hardly inhibited radioactive sialic acid incorporation, whereas the addition of 10 mM unlabeled sialic acid inhibited almost completely is difficult to understand. A possible explanation is that high concentrations of unlabeled sialic acid in the incubation medium may inhibit the incor-

TABLE IX. Assay for Endogenous Cell Surface Sialyltransferase Activity With BHK Cells*

Additions to the incubation medium	Incubation with [^{3}H] sialic acid (1.0 μCi) cmp/10^6 cells	% Incorporation	CMP[^{3}H] sialic acid (1.0 μCi) cmp/10^6 cells	% Incorporation
None	256	100	224	100
5 mM sialic acid	213	83	205	92
10 mM sialic acid	36	14	16	7
20 mM sialic acid	8	3	3	1

*BHK cells in monolayer (1×10^6 per 3-cm dish) were incubated for 3 h with 0.3 ml of isotonic Hepes buffer containing 1.0 μCi [^{3}H] sialic acid or 1.0 μCi CMP[^{3}H] sialic acid, respectively. Nonradioactive sialic acid was added to the incubation medium as indicated. Incorporation of [^{3}H] sialic acid was determined as described in Materials and Methods.

poration of sialic acid unspecifically either because of the presence of a nonspecific inhibitor in the sialic acid preparation or because of toxic effects of the sialic acid itself.

Assay for Cell Surface Glycosyltransferase Activity Towards High-Molecular-Weight Exogenous Acceptors

A demonstration that viable, intact cells were able to catalyze the transfer of the sugar moiety from a nucleotide sugar directly onto an exogenous acceptor molecule present in the incubation medium would be strong support for the existence of cell surface glycosyltransferases. Experimental data suggesting this transfer reaction have been reported (see review by Shur and Roth, Ref. 1) but, unfortunately, in most of these studies trypsinized or EDTA-treated cells in suspension assays were used. In these studies, therefore, the transfer reaction may have been catalyzed by dead cells, where the acceptor molecules as well as the nucleotide sugars were available to intracellular glycosyltransferases. We have tested various cells in monolayer for cell surface galactosyltransferase and cell surface sialyltransferase activity using exogenous high-molecular-weight acceptors (see Materials and Methods). Table X shows that the degalactosized antifreeze protein as well as the desialized and degalactosized fetuin were active as acceptors in homogenate of BHK cells. Intact cells, however, were not able to transfer galactose onto these acceptor proteins (Table XI). Similar results were obtained, when acceptor proteins for the sialic acid transfer were tested in BHK cell homogenate and on intact cells. Whereas the antifreeze protein (AFP) and the desialized fetuin acted as acceptor proteins for sialic acid transfer in BHK cell homogenate (Table XII), no transfer reaction could be detected when these acceptor proteins were added to intact cells (Table XIII).

DISCUSSION

Prevention of intracellular utilization of the radioactive sugar as a breakdown product of nucleotide sugars and the use of intact viable cells are prerequisites for studies on the existence of cell surface glycosyltransferases. We have shown in this and in previous studies (3, 11) that the cell surface of various cells contains hydrolytic enzymes which catalyze the conversion of nucleotide sugars to the free sugar. Intracellular utilization of the free radioactive sugar generated by hydrolysis, however, can be prevented by com-

TABLE X. Galactosyltransferase Activity Towards Exogenous Acceptors in BHK Cell Homogenate*

Addition	[^{3}H] Galactose incorporation (cpm/100 μg cell homogenate)
None	3,870
100 μg AFP	3,756
100 μg AFP-degal.	18,212
100 μg fetuin	3,945
100 μg fetuin-desial.-degal.	23,109

*Cells were removed from plates with 0.1% trypsin in isotonic Hepes buffer, suspended in isotonic Hepes buffer, and homogenized by sonication (60 sec in 5-sec intervals at full power in a Branson sonifier equipped with a microtip). Cell homogenate at a concentration of 100 μg in a final volume of 50 μl was incubated with 1.0 μCi UDP[^{3}H] galactose in isotonic Hepes buffer containing 5 mM $MnCl_2$ and 0.1% Triton X-100. Acceptor protein (see Materials and Methods) was added as indicated. After 3 h incubation at 37°C the reaction was stopped by adding 10 μl of 10% SDS. Samples were precipitated and their radioactivities measured as described in Materials and Methods. Background incorporation with 1.0 μCi of [^{3}H] galactose was 200 cpm. Protein was determined according to Lowry et al. (29).

petition with the nonradioactive free sugar in concentrations higher than the K_m for the transport system of the free sugar or by inhibiting the transport of the radioactive sugar with appropriate inhibitors or by inhibiting cellular metabolism with sodium azide.

We have compared the permeability and the viability of trypsinized BHK cells in suspension and of BHK cells in monolayer. Our finding that phloridzin inhibits galactose incorporation to 95% with BHK cells in monolayer but only to about 60% with trypsinized BHK cells, as well as the different kinetics of galactose incorporation with these 2 cell preparations strongly suggests that trypsinization alters the permeability of BHK cells for galactose. In a more extensive study, Hirschberg et al. (8) have shown that trypsin or EDTA treatment of fibroblasts grown on plates leads to leakage of small molecules, as measured by the release of 2-[^{3}H]-deoxyglucose, as well as to leakage of macromolecules, as measured by the release of ^{51}Cr. In agreement with similar studies by the same authors, we found that trypsin treatment of BHK cells grown on plates significantly reduced the viability of these cells if they were kept suspended in buffer during the assay period. BHK cells on plates, on the other hand, stayed viable when incubated with buffer for the same period of time. The finding that trypsinized or EDTA-treated cells are leaky and lose their viability during the assay, severely questions the validity of all studies in which the assay for cell surface glycosyltransferases has been performed with such cell preparations.

In a previous paper (3), we have provided evidence that BHK cells in monolayer do not exhibit detectable cell surface galactosyltransferase activity. This conclusion is further substantiated in the present study. Under all experimental conditions applied, cell surface galactosyltransferase activity on BHK cells either endogenous or towards 2 different high-molecular-weight exogenous acceptor molecules could not be found. Similarly, all other cells tested, including transformed cells and primary cells, did not give any indication for the presence of galactosyltransferases on their surface. Our studies on the presence of cell surface fucosyl- and sialyltransferases were less extensive but point into the same direction. Inhibition of fucose incorporation from GDP-fucose into BHK cells could be obtained by adding high concentrations of unlabeled fucose, by metabolic inhibition with sodium azide, and by a combination of both, suggesting the absence of endogenous cell surface fucosyltransferases. On the other hand, inhibition with unlabeled fucose was not

TABLE XI. Galactosyltransferase Activity Towards Exogenous Acceptors With Cells in Monolayer*

Cells	Additions to the incubation medium	Precipitation I (cells + incubation medium) — cpm/10^6 cells	Precipitation II (cells + incubation medium separately) — cpm/10^6 cells	Precipitation II — cpm total incubation medium
BHK	10.0 μCi UDP[^{3}H]galactose 50 μg AFP 5 mM Phloridzin	188	–	–
	10.0 μCi UDP-gal 50 μg AFP-degal. 5 mM Phloridzin	212	–	–
ME, secondary	1.0 μCi UDP[^{3}H]galactose no acceptor	–	2,202	1,300
	1.0 μCi UDP[^{3}H]galactose 50 μg AFP	–	2,136	1,363
	1.0 μCi UDP[^{3}H]galactose 50 μg AFP-degal.	–	1,971	1,623
AGMK, primary	1.0 μCi UDP[^{3}H]galactose	2,132	–	–
	1.0 μCi UDP[^{3}H]galactose 200 μg AFP-degal.	2,037	–	–
	1.0 μCi UDP[^{3}H]galactose 200 μg fetuin-desial-degal.	2,209	–	–

*2×10^5 cells were seeded on 3-cm dishes and assayed for cell surface galactosyltransferase activity 2 days after seeding. The cells were incubated for 3 h with 0.3 ml of isotonic Hepes buffer, containing 5 mM $MnCl_2$. UDP[^{3}H]galactose, acceptor proteins (see Materials and Methods), and phloridzin were added as indicated. Incorporation of [^{3}H]galactose was determined by the following procedures: Precipitation I: The reaction was stopped by the addition of 50 μl of 10% SDS, the cell lysate diluted with 2 ml of ice cold water, precipitated with 3 ml of 1% phosphotungstic acid in 0.5 N HCl, and incorporation of radioactive sugar into cellular and acceptor proteins determined as described in Materials and Methods. Precipitation II: The incubation medium was collected with a Pasteur pipette, as were 2 subsequent washes of the cells with isotonic Hepes buffer. Incubation medium and the washes were pooled, 100 μg of BSA was added as carrier, and proteins precipitated with 1% phosphotungstic acid in 0.5 N HCl. Radioactivity of the samples was measured as described in Materials and Methods. Incorporation of radioactive sugars into cellular proteins was determined as described in Materials and Methods.

complete, and due to lack of an appropriate high-molecular-weight fucosyl acceptor, cell surface fucosyltransferase activity toward exogenous acceptors could not be tested. We therefore cannot exclude low levels of cell surface fucosyltransferase activity on BHK cells. In testing for cell surface sialyltransferase activity we were unable to obtain significant sialic acid transfer onto 2 different high-molecular-weight sialic acid acceptors using BHK and Balb/c 3T3 cells. We, therefore, conclude that these cells do not express cell surface sialytransferase activity towards exogenous acceptors. Analysis of BHK cells for cell surface sialyltransferase activity towards endogenous acceptors however, failed to give a conclusive result. Inhibition of radioactive sialic acid incorporation could only be achieved by using high concentrations of unlabeled sialic acid. Although this is in accordance with data obtained by Hirschberg et al. (8), and could mean that concentrations of unlabeled sialic acid above the apparent K_M of sialic acid transport are nec-

TABLE XII. Sialyltransferase Activity Towards Exogenous Acceptors in BHK Cell Homogenate*

Addition	[^{3}H] Sialic acid incorporation (cpm/100 μg cell homogenate)
None	1,142
100 μg AFP	38,737
100 μg AFP-degal.	1,203
100 μg fetuin	1,089
100 μg fetuin-desial.	21,045

*BHK cell homogenate was prepared as described in the legend to Table X and incubated at a concentration of 100 μg in a final volume of 50 μl with 1.0 μCi CMP[^{3}H] sialic acid in isotonic Hepes buffer containing 0.1% Triton X-100. Acceptor protein (see Materials and Methods) was added as indicated. After 3 h incubation at 37°C the reaction was stopped by adding 10 μl of 10% SDS. Samples were precipitated and their radioactivities determined as described in Materials and Methods. Protein was determined according to Lowry et al. (29).

TABLE XIII. Sialyltransferase Activity Towards Exogenous Acceptors With Cells in Monolayer*

		Precipitation I (cells + incubation medium)	Precipitation II (cells + incubation medium separately)	
Cells	Addition	cpm/10^6 cells	cpm/10^6 cells	cpm total incubation medium
BHK	1.0 μCi CMP[^{3}H] sialic acid	205		
	2.0 μCi CMP [^{3}H] sialic acid	389		
	1.0 μCi CMP 200 μg AFP [^{3}H] sialic acid	263		
	1.0 μCi CMP [^{3}H] sialic acid 200 μg AFP degal.	228		
	2.0 μCi CMP [^{3}H] sialic acid 200 μg AFP	441		
BALB c/3T3	1.0 μCi CMP[^{3}H] sialic acid		152	120
	1.0 μCi CMP [^{3}H] sialic acid 100 μg AFP		157	132
	1.0 μCi CMP [^{3}H] sialic acid 200 μg AFP		131	143

*BHK cells and BALB/c/3T3 cells in monolayers (1 × 10^6 per 3-cm dish) were incubated with 0.3 ml of isotonic Hepes buffer. CMP[^{3}H] sialic acid and acceptor proteins (see Materials and Methods) were added as indicated. Precipitation I and Precipitation II were described in the legend to Table XI.

essary to suppress the incorporation of radioactive sialic acid, another possible explanation for the suppression of radioactive sialic acid incorporation only with very high concentrations of unlabeled sialic acid is a toxic effect of this compound on the cells.

Obviously, different results are obtained when the assay for cell surface glycosyltransferases is performed with cells attached to plates and when performed with EDTA- or trypsin-treated cells. The finding that cells in monolayer do not contain detectable cell

surface glycosyltransferase activity (3, 14), whereas EDTA- or trypsin-treated cells appear to possess such an activity, is as easily explained by the increased permeability of EDTA- and trypsin-treated cells as by the speculation that the contact dependency of cell surface glycosylation might render it impossible to detect such activities with monolayer cells. Nevertheless, we have tried in this study to find conditions which might have allowed the detection of contact dependent endogenous cell surface galactosyltransferase activity on BHK cells but obtained a negative result.

Patt and Grimes (14) have tested BHK cells for cell surface glycosyltransferase activity in monolayer and in suspension after EDTA treatment. While no endogenous glycosyltransferase activity was found with BHK cell monolayers, if hydrolysis of the nucleotide sugars was inhibited by the addition of 5′-AMP, an inhibitor of nucleotide pyrophosphatase activity (15), the authors report a transfer reaction onto high-molecular-weight exogenous acceptors with BHK monolayer cells. Although Patt and Grimes do not offer direct proof for the transfer of the sugar specifically onto their acceptor molecules, they interpret their finding as evidence for the presence of cell surface glycosyltransferases on monolayer cells. In this study, we have assayed various cells for surface galactosyl-sialyltransferase activity using 2 different acceptors for each transferase reaction. In all cases we were unable to detect any significant transfer. In some experiments a slight stimulation of total incorporation was observed, but no correlation was found between the amount of acceptor or radioactivity added and the stimulation of incorporation.

Since the sialyltransferase reaction is very effective in cell homogenate (Table XII), less than 1% of broken cells would be sufficient to account for the incorporation data reported by Patt and Grimes (14) and would explain the small stimulation observed in some of our experiments.

Further support for our view that the surface of mammalian cells does not contain nucleotide sugar dependent glycosyltransferases comes from studies investigating the subcellular distribution of glycosyltransferases. If cell fractions of defined purity were analyzed, it was found that in rat liver cells the Golgi apparatus was the sole location of galactosyltransferase, N-acetylglucosaminetransferase, and transferases of ganglioside synthesis (16–23). A similar subcellular distribution of galactosyltransferase and cerebroside sulfotransferase was found in rat kidney cells (22, 23). In all of these studies, the plasma membrane fraction was either devoid of glycosyltransferases or the minute amounts detected could be assigned to contamination by Golgi membrane fragments. Glycosyltransferases were also not detected in bovine milk fat globule membrane (24, 25), a membrane known to be derived directly from apical plasma membrane of mammary secretory cells (26).

In sum, the experimental data available cause us to view with caution the concept of cell surface glycosyltransferases. We feel that the evidence presented in previous studies supporting the existence of cell surface glycosyltransferases is insufficient; recent studies (3, 27, 8, this paper) have provided evidence that either hydrolysis of the nucleotide sugars or altered permeability due to cell damage or a combination of both will account for the data presented in most reports of cell surface glycosyltransferases. On the other hand, failure to detect nucleotide sugar dependent cell surface glycosyltransferases does not necessarily rule out the possibility of other mechanisms of cell surface glycosylation. So, for instance, Yogeeswaran et al. (28) have suggested the existence of a hitherto unknown class of cell surface glycosyltransferases, which do not use nucleotide sugars as substrate but instead use another, yet unknown, sugar donor. Clearly, more experimental support for this kind of surface glycosylation would be desirable.

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