Labeling of the External Surface of Hamster and Mouse Fibroblasts with [^{14}C]Sialic Acid†

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ABSTRACT: A simple and direct method for specific labeling of cell surface components *in situ* by [^{14}C]sialic acid is described. At least 75–80% of cell-associated radioactivity could be accounted for by the labeling of external acceptors. The efficiency of labeling may be increased 2–3-fold by pretreating the cells with neuraminidase. The rates of incorporation of cell-bound radioactivity (measured at 37°) were higher in polyoma-transformed cells grown at 38.5° than in cells grown at 32°. In contrast, untransformed cells showed a lower incorporation rate when grown at 38.5° than when grown at 32°.

It is generally speculated that biochemical differences in the surface constituents of normal and virus-transformed cells may explain their altered responses to cellular regulatory mechanisms, and some evidence on the altered properties of the cell surface of transformed cells has been obtained. For these studies glycoproteins were labeled by growing cells in the presence of radioactive fucose or amino sugars (Sheinin and Onodera, 1970; Buck *et al.*, 1970; Sakiyama and Burge, 1972; Warren *et al.*, 1973) or the proteins of the outer cell layer were labeled by iodination with lactoperoxidase (Phillips, 1972; Poduslo *et al.*, 1972; Hynes, 1973). The surface components were then removed by mild trypsin digestion, or alternatively, labeled plasma membrane fractions were isolated by differential centrifugation (Sheinin and Onodera, 1970; Buck *et al.*, 1970; Sakiyama and Burge, 1972; Warren *et al.*, 1973; Phillips, 1972; Poduslo *et al.*, 1972). Using these procedures, certain differences in the glycoproteins and glycopeptides between transformed and untransformed cell lines were detected. Chemical analyses of various glycolipids also showed altered patterns (Hokomori, 1970; Brady *et al.*, 1973). However, these studies are often complicated due to lack of specific labeling procedures of the surface components. In this report, I describe a simple and direct procedure for labeling cell surface components *in situ* by [^{14}C]sialic acid. During these experiments, I have found that the rate of incorporation of sialic acid was higher in polyoma-transformed cells grown at 38.5° than in cells grown at 32°. In contrast, untransformed cells showed a lower incorporation rate when grown at 38.5° than when grown at 32°.

Materials and Methods

Cell Lines. The following cell lines were used: BHK-21 clone 13, an established line of hamster fibroblasts (Macpherson and Stoker, 1962); Py-BHK clone 1A, BHK-21 cells transformed by wild-type polyoma (Dulbecco and Eckhart, 1970); ts-3-BHK clone 7C, BHK-21 cells transformed by a temperature-sensitive mutant of polyoma which show agglutinability characteristic of transformed cells at 32° but have agglutinability behavior of normal cells at 38.5° (Eckhart *et al.*, 1971); ts-3-3T3 clone 1 was a recent isolate of Dr. W. Eckhart (unpublished data); Balb/C-3T3 was obtained from Dr. M. Vogt.

Chemicals. CMP-[^{14}C]Sialic acid (4,5,6,7,8,9-^{14}C, specific activity 229 Ci/mol) and [^{14}C]sialic acid (4,5,6,7,8,9,-^{14}C, specific activity 262 Ci/mol) were obtained from New England Nuclear and Amersham–Searle, respectively. Sialic acid was purchased from Sigma. *Clostridium perfringens* neuraminidase was obtained from the following sources. Partially purified enzyme preparation was purchased from P-L Biochemicals. Chromatographically purified enzyme (Type VI, specific activity 3 μmol of sialic acid liberated per min per mg of protein using sialyllactose as substrate) containing 0.002% protease activity was bought from Sigma. Highly purified neuraminidase (with a minimum specific activity of 153, see Cassidy *et al.*, 1965) completely free of protease, β-galactosidase, and *N*-acetylneuraminic acid aldolase was a gift from Dr. G. W. Jourdian of the Department of Biochemistry, The University of Michigan. Insoluble neuraminidase attached to beaded agarose (20 units/g) was supplied by Sigma. Receptor-destroying enzyme (a crude preparation of neuraminidase) was bought from Microbiological Associates.

Growth Conditions. Cells were grown in Dulbecco–Vogt modified Eagle's medium (1963) supplemented with 10% calf serum, and penicillin, streptomycin, and mycostatin. For some experiments, 0.3% tryptose phosphate was also included. Cultures were incubated in 50-mm plastic petri dishes (NUNC) in humidified incubators at 38.5 or at 32° in 10% CO_2. When specified, a 200-fold dilution of receptor-destroying enzyme (titer 1:1000) was added to the growth medium.

To compensate for different growth rates at low and high temperatures, 2.4 times more cells were plated at 32° and the plates were incubated for the same length of time to achieve similar cell density.

For all experiments reported here the cell number was determined by counting cell suspension in a Spencer hemacytometer. Cells were detached in trypsin–EDTA solution followed by centrifugation and resuspension of the cell pellet in complete medium. The average values given were from duplicate samples obtained from each of two separate plates and agreed within ±5%.

Labeling Procedure. Depending on the experiment, semiconfluent or confluent cultures were used. After removing the medium, cells were washed twice *in situ* with prewarmed phosphate-buffered saline (PBS),[1] and 2 ml of PBS was added to

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[1] Abbreviations used are: PBS, phosphate-buffered saline; TBS, Tris-buffered saline.

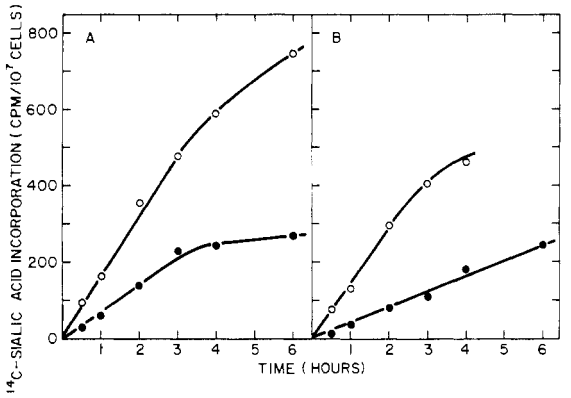

FIGURE 1: Incoporation of [^{14}C]sialic acid by ts-3-BHK cells. Cells were grown either at 38.5° (○) or at 32° (●). In (A) labeling with CMP-[^{14}C]sialic acid was carried out at 38.5°; in (B) the temperature during the labeling was 32°. In all cases the blank values (10–20 CPM) obtained at zero time have been subtracted. For other details, see Methods.

each plate. Following equilibration at 37° for 10 min, a solution of CMP-[^{14}C]sialic acid in PBS (1.9 nmol, 8.8 × 10^5 dpm) was added and the incubation continued at 37° if not otherwise stated. The reaction was stopped by adding 2 ml of cold 20% Cl_3CCOOH; cells were scraped off and deposited on a glass fiber filter (Whatman GF/C). The precipitate was washed thoroughly by suction with cold 5% Cl_3CCOOH, dried, and counted in a liquid scintillation mixture. Since some glycoproteins are known to be soluble in 10% Cl_3CCOOH, in selected experiments, the labeled cells were precipitated with phosphotungstic acid with or without added carrier bovine submaxillary mucin (Grimes, 1973). No significant difference was observed if the reaction was terminated with 1% phosphotungstic acid in 0.5 N HCl.

At the end of the incubation period, the cells in the monolayer showed a normal morphology and remained attached to the petri dish.

The viability of ts-3-BHK cells at the end of 2.5-hr incubation in PBS at 37° was determined by replacing the incubation fluid with fresh medium containing 10% calf serum. Upon incubation at 38.5° for 19 hr, the cell number increased from 3.0 × 10^6 to 6.1 × 10^6 indicating total cell viability. In a control culture without PBS treatment, the cell number increased from 3 × 10^6 to 7.4 × 10^6 in 22 hr.

In experiments where cells were pretreated with neuraminidase *in situ*, the plates were washed twice with prewarmed PBS and incubated with 0.2 unit/ml of neuraminidase in PBS for various time periods. The plates were again washed twice with PBS to remove the enzyme and incubation with CMP-[^{14}C]sialic acid was carried on as described.

Uptake of CMP-[^{14}C]sialic acid into acid-soluble form was measured according to the method described by Cunningham and Pardee (1969).

Results and Discussion

Incorporation of [^{14}C]*Sialic Acid.* Figure 1 shows the kinetics of [^{14}C]sialic acid incorporation by ts-3-BHK cells. The cells were grown either at 38.5° or at 32° and labeling in the presence of CMP-[^{14}C]sialic acid in PBS was carried out at 38.5 (Figure 1A) or at 32° (Figure 1B). The results indicate that (a) incorporation of radioactivity showed no lag and was linear at least up to 3 hr, (b) the rate of incorporation (CPM/10^7 cells/hr) was almost one-half for cells grown at 32° as compared to cells grown at 38.5°, and (c) the decreased incorporation rate by the cells grown at 32° was independent of the temperature during the labeling period. Additional experiments (not shown) in which the temperature during the labeling period was 25° or 37° gave similar results.

The results shown in Figure 1 were obtained with cells that have reached confluency. However, experiments with semiconfluent cultures (approximately 70% confluent) grown at high and low temperatures revealed the same relative labeling patterns as seen with cells harvested at confluency. These data suggest that the difference in the rates of sialic acid incorporation (measured at 37°) is dependent on the temperature during cell growth (also see Figure 6 and Table I) and not on saturation density.

With increasing concentrations of CMP-[^{14}C]sialic acid the rate of incorporation increased linearly up to 4.5 nmol/ml. Again as seen in Figure 1, the rates of incorporation of cell-bound radioactivity for ts-3-BHK cells grown at 32° were considerably reduced at all concentrations of CMP-sialic acid tested.

In the experiments described above, PBS containing 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ was used as the labeling medium. However, separate experiments revealed that the incorporation of [^{14}C]sialic acid by ts-3-BHK cells grown at 38.5° was unchanged (300 ± 20 cpm/10^7 cells/2 hr at 37°) if PBS was replaced with Tris-buffered saline (TBS), TBS minus the divalent cations, TBS minus the cations supplemented with 1 mM EDTA, or PBS minus the cations plus 0.5 mM EDTA. (In the last three incubation media, the cells became detached and rounded up within 10 min of the incubation period.)

Identification of Cell-Bound Radioactivity as [^{14}C]*Sialic Acid.* Two independent methods were used to demonstrate that the label incorporated by the cells was [^{14}C]sialic acid. In one experiment the cells prelabeled with CMP-[^{14}C]sialic acid (containing 3500 cpm as cell-associated radioactivity) were treated with 1 unit/ml of chromatographically purified neuraminidase (see Material and Methods) to hydrolyze sialic acid glycosides to free acid. In a second experiment free sialic acid was liberated by heating the labeled cells in 0.1 N H_2SO_4 at 80° for 60 min. Eighty per cent of cell-associated radioactivity was released by the neuraminidase treatment, and 96% of the label was liberated by acid hydrolysis. The presence of radiolabeled free sialic acid in the supernatant fluids was confirmed by passing the solutions through Dowex 2 columns, eluting with 0.3 N formic acid, followed by paper chromatography of the concentrated formic acid eluates in butanol–acetic acid–water (4:1:5) according to the method of Svennerholm (1963). Greater than 95% of the radioactivity present in the supernatant fluids eluted with formic acid and approximately 90% of the radioactivity recovered in the formic acid eluate cochromatographed with authentic unlabeled sialic acid in the same solvent system.

Evidence for the Labeling of the Cell Surface. Published reports (see Roseman, 1970) suggest that sialic acid is one of the terminal sugar residues of many glycoproteins and glycolipids thought to be localized on the cell surface of animal cells. Furthermore, the glycosyltransferase activities of animal cells have also been reported to be membrane bound (Den *et al.*, 1971; Bosmann, 1972; Grimes, 1973; Brady *et al.*, 1973; Warren *et al.*, 1973). The observed incorporation of [^{14}C]sialic acid by intact ts-3-BHK cells would then presumably be onto the cell surface. To examine this possibility, ts-3-BHK cells prelabeled with CMP-[^{14}C]sialic acid were treated *in situ* with soluble neuraminidase obtained from Sigma Chemical Co. The results given in Figure 2A show that between 60 and 75% of the cell-bound radioactivity was released by this enzyme. Identical results were obtained when cells in monolayer were treated with

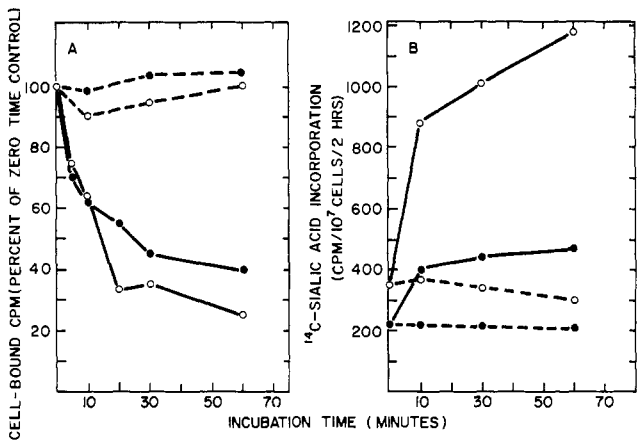

FIGURE 2: Effects of neuraminidase on [^{14}C]sialic acid incorporation. (A) Ts-3-BHK cells grown either at 38.5° (○) or at 32° (●) were incubated for 2 hr at 37° with CMP-[^{14}C]sialic acid *in situ*. The cells were washed twice with prewarmed PBS and neuraminidase (0.2 unit/ml in PBS) was added to each plate (—). After incubation at 37° for the indicated time periods, the reaction was terminated with Cl_3CCOOH and cell-bound radioactivity was determined as described in the Methods. In control plates (- - -), the cells were incubated with PBS without neuraminidase. (B) Ts-3-BHK cells grown either at 38.5° (○) or at 32° (●) were incubated with 0.2 unit/ml of neuraminidase in PBS at 37° for various time periods (—). Control plates were incubated with PBS (- - -). After treatments with or without neuraminidase, the cells were washed twice with prewarmed PBS and incubated with CMP-[^{14}C]sialic acid for 2 hr as described in Methods.

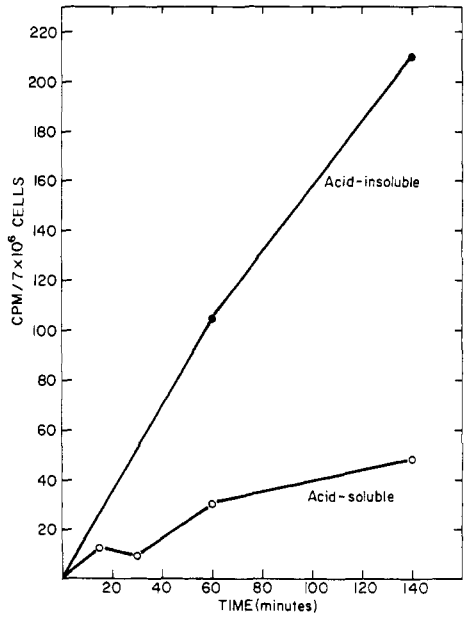

FIGURE 3: Incorporation of radioactivity into acid-soluble and acid-insoluble fractions from CMP-[^{14}C]sialic acid. Ts-3-BHK cells, grown at 38.5°, were washed and incubated in the presence of CMP-[^{14}C]sialic acid in PBS at 37° *in situ*. A 1000-fold molar excess of nonradioactive sialic acid was also included in the labeling fluid. Incorporation of radioactivity into acid-soluble fraction was measured by the method of Cunningham and Pardee (1969). Cell-bound radioactivity was measured as described in Methods.

highly purified (Cassidy *et al.*, 1965) neuraminidase that was completely free of protease, β-galactosidase, and *N*-acetylneuraminic acid aldolase. Seventy per cent of cell-bound radioactivity was released by 0.1 unit/ml of the enzyme in 60 min, and 87% of the radioactivity was released after incubation for 48 hr. In contrast, when prelabeled BHK cells or ts-3-BHK cells were incubated at 37° either in PBS or in complete medium containing 10% calf serum for various lengths of time up to 96 hr, less than 10% of the cell-bound radioactivity was released (*cf.* Figure 2) indicating no detectable neuraminidase activity in the serum or associated with the cell surface. Separate experiments in which cells grown at 38.5° or at 32° were exposed to purified neuraminidase *in situ* prior to labeling with CMP-[^{14}C]sialic acid showed a 2–3-fold increase in cell-bound radioactivity (Figure 2B) suggesting that acceptors that were already charged with sialic acid *in vivo* may be uncharged by neuraminidase and recharged with [^{14}C]sialic acid thus increasing the labeling efficiency. These results are consistent with the notion that a major fraction of [^{14}C]sialic acid is bound to sites on the surface that are accessible to neuraminidase. However, since it has been reported (Nordling and Mayhew, 1966) that 15–20% of added neuraminidase may be taken up in 30 min by human sarcoma cells and chicken erythrocytes in suspension, it is conceivable that neuraminidase could also penetrate ts-3-BHK cells and release sialic acid bound to intracellular acceptors. Several lines of evidence presented below argue against this possibility.

Since the sialyl donor in the sialyltransferase reaction is CMP-sialic acid and not free sialic acid (Roseman, 1970), the labeling of intracellular acceptors could occur by one of two following mechanisms: (a) transport of CMP-[^{14}C]sialic acid across the plasma membrane as intact nucleotide sugar, and (b) hydrolysis of CMP-[^{14}C]sialic acid to [^{14}C]sialic acid, transport of the sugar inside the cell which could then be "activated" to CMP-[^{14}C]sialic acid by an intracellular enzyme, and subsequently transferred to the acceptor moiety by sialyltransferases. The labeling of the intracellular acceptors resulting from hydrolysis of CMP-sialic acid and subsequent resynthesis of the nucleotide sugar within the cell (mechanism b) was ruled out by the fact that addition of a 1000-fold molar excess of nonradioactive sialic acid in the labeling fluid containing CMP-[^{14}C]sialic acid did not reduce the incorporation of radioactivity into the acid-insoluble fraction; two independent experiments gave an average of 270 ± 20 counts incorporated per 10^7 cells per 2 hr at 37° with and without nonradioactive sialic acid indicating no dilution of the specific activity of CMP-[^{14}C]sialic acid within the cell. This notion was further supported by the finding that incubation of ts-3-BHK cells with [^{14}C]sialic acid (specific activity, 262 Ci/mol) showed a rate of incorporation of 10–15% of that found when cells were incubated with CMP-[^{14}C]sialic acid with a lower specific activity of 229 Ci/mol.

To examine the possibility that CMP-[^{14}C]sialic acid might be transported across the plasma membrane as intact nucleotide sugar (mechanism a), ts-3-BHK cells were incubated with CMP-[^{14}C]sialic acid in PBS (in the presence of a 1000-fold molar excess of nonradioactive sialic acid) and the rate of incorporation of radioactivity into the *acid-soluble* fraction was measured according to the method described by Cunningham and Pardee (1969). In parallel cultures, incorporation of radioactivity into the *acid-insoluble* fraction was also determined. The data summarized in Figure 3 show that using an identical labeling procedure the incorporation of radioactivity into both fractions increased linearly with time and the initial rate of uptake of radioactivity into the acid-soluble fraction was about 25% of that found into the acid-insoluble fraction. Inhibitors such as phlorizin (Alvarado, 1967) and a mixture of sodium cyanide and iodoacetate (Cunningam and Pardee, 1969) that block transport of various sugars, amino acids, and nucleosides did not influence the rate of [^{14}C]sialic acid incorporation into the acid-insoluble fraction. Addition of 20 mM sodium azide, however, reduced the rate of incorporation by about 20% and may be due to a general toxic effect which resulted in the

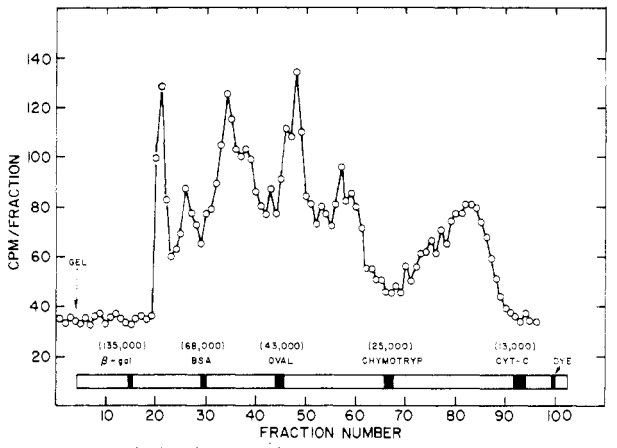

FIGURE 4: Analysis of cell surface acceptors labeled with [^{14}C]sialic acid on polyacrylamide gels. Ts-3-BHK cells grown at 38.5° were incubated *in situ* with CMP-[^{14}C]sialic acid in PBS as described in Methods. After labeling, cells were washed with cold PBS, dissolved directly in 0.01 M sodium phosphate buffer (pH 6.8) containing 2% sodium dodecyl sulfate and 1% β-mercaptoethanol, and immediately boiled for 3 min. Aliquots of the sample were subjected to electrophoresis on 20 × 0.6 cm 7.5% polyacrylamide gels for 20 hr at 50 V. The running buffer was 0.1 M sodium phosphate containing 0.1% sodium dodecyl sulfate. The gels were stained with Coomassie Blue according to the method of Fairbanks *et al.* (1971). For radioactivity determination gels were fractionated in a Maizel Autogeldivider (Savant Instrument Co.) and the fractions were counted in a toluene–Triton-X scintillation mixture. To determine the approximate size distribution of ^{14}C-labeled sialyl acceptors, identical gels were run using several standard proteins and stained with Coomassie Blue.

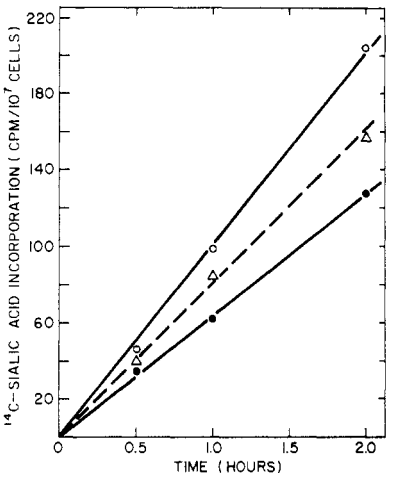

FIGURE 5: Effect of temperature during growth on [^{14}C]sialic acid incorporation by ts-3-BHK cells. (○) cells were grown for 66 hr at 38.5°; (●) cells were grown for 66 hr at 32°; (△) cells were grown for 48 hr at 32° and then shifted up to 38.5° for 18 hr. In all cases, the temperature during labeling was 37°. Blank values obtained at zero time have been subtracted. For other details, see Methods.

rounding up and subsequent detachment of the cells from the surface of the petri dish.

A direct experiment to uncharge sialic acid residues bound specifically to the cell surface acceptors was performed using insoluble neuraminidase attached to agarose beads of size larger than the cells. Cells growing in monolayer and prelabeled with [^{14}C]sialic acid were exposed to bead-bound neuraminidase (0.2 unit/ml) for various time periods and the amount of cell-associated radioactivity was determined as described in the Methods (*cf.* Figure 2A). In parallel experiments prelabeled cells were treated with the same concentration of soluble neuraminidase. The results showed that, in two separate trials, the enzyme attached to agarose beads released 65 and 70% of the radioactivity that was released by the soluble enzyme. The decreased efficiency of the insoluble enyzme to release cell-bound radioactivity may be attributable to the impaired accessibility of the enzyme to the surface acceptors due to steric hindrance.

The cumulative data described above clearly indicate that at least 75% of the cell-associated radioactivity is likely to be accounted for by labeling of external acceptors. The residual cell-bound radioactivity resistant to neuraminidase treatment (see Figure 2A) may be due to incomplete removal of terminal sialic acid residues from the cell surface acceptors by the enzyme. Using purified *C. perfringens* neuraminidase Cassidy *et al.* (1965) have shown that certain macromolecules containing terminal sialic acid residues (*e.g.*, bovine and porcine submaxillary mucins, and calf brain ganglioside) cannot be desialylated completely. On the other hand, since a low level of radioactivity was found in acid-soluble fraction (Figure 3) which may exist either in the form of intact nucleotide sugar or free sialic acid, it is conceivable that a small fraction if any of the cell-bound radioactivity not released by neuraminidase is bound to intracellular acceptors.

In order to determine the nature of the cell surface components that would serve as sialyl acceptors, ts-3-BHK cells labeled with [^{14}C]sialic acid *in situ* were dissolved directly in sodium dodecyl sulfate buffer and electrophoresed on sodium do-

TABLE I: A Comparison of [^{14}C]Sialic Acid Incorporation by Various Cell Lines Grown at 38.5 and at 32°.[a]

Cell Line	Growth Medium Supplementation	Phenotype	Rel Rate of Incorporation: Cells grown at 38.5°	Rel Rate of Incorporation: Cells grown at 32°
BHK 21 clone 13	None	Untransformed	1.00 (214)	2.15 (460)
BHK 21 clone 13	Tryptose phosphate	Untransformed	1.00 (210)	2.02 (425)
BALB/C-3T3	Receptor-destroying enzyme	Untransformed	1.00 (275)	1.60 (444)
Py-BHK clone 1A	None	Transformed	1.00 (335)	0.48 (161)
Py-BHK clone 1A	Tryptose phosphate	Transformed	1.00 (342)	0.76 (262)
Ts-3-BHK clone 7C	None	Temperature sensitive[b]	1.00 (265)	0.55 (146)
Ts-3-3T3 clone 1	Receptor-destroying enzyme	Temperature sensitive[b]	1.00 (1250)	0.68 (850)

[a] In all experiments labeling with CMP-[^{14}C]sialic acid in PBS was carried out at 37° as described in Methods and the rate of incorporation into acid-insoluble fraction calculated from the kinetics measured over 2 hr (*cf.* Figure 1). The number of counts per 10^7 cells in 2 hr are shown in parentheses. The data for ts-3-BHK clone 7C are averages obtained from ten independent experiments. [b] Untransformed phenotype when grown at 38.5°; transformed phenotype when grown at 32°.

decyl sulfate-polyacrylamide gels according to the general method of Maizel (1969). The results presented in Figure 4 show 6–8 ^{14}C-labeled glycoprotein peaks ranging in molecular weights from 100,000 to 30,000. A broad peak of labeled material amounting to about 25% of the total radioactivity recovered from the gel was also seen migrating behind the tracking dye. This material did not stain well with Coomassie Blue and from the relative migration rates on sodium dodecyl sulfate gels it appears to be a mixture of sialoglycolipids (*cf.* Fairbanks *et al.*, 1971). This notion was supported by the finding that approximately 20% of the cell-bound radioactivity was recovered in the organic phase by extracting Cl_3CCOOH precipitable material with a mixture of chloroform–methanol (2:1). Further characterization of these glycoproteins and glycolipids is in progress.

Effect of Temperature during Cell Growth on the Rate of [^{14}C]Sialic Acid Incorporation. The data shown in Figure 1 reveal that ts-3-BHK cells grown at 32° expressing transformed phenotype incorporated less radioactivity as compared to cells grown at 38.5° where the transformed phenotype is masked. (This labeling pattern was independent of the temperature used *during incubation* with CMP-[^{14}C]sialic acid in PBS.) Further, when cells grown at 32° were shifted up to 38.5° for 18 hr the rate of incorporation (measured at 37°) was intermediate to those observed for cells grown at 32 and at 38.5° (Figure 5). Eckhart *et al.* (1971) have shown that upon shift up from 32 to 39° for 20 hr the concentration of wheat germ agglutinin required for 50% agglutination of ts-3-BHK cells was also intermediate to that required for cells grown at 32° (transformed phenotype) and grown at 39° (untransformed phenotype). The data summarized in Table I reveal that two other polyoma-transformed cell lines, ts-3-3T3 and Py-BHK, also exhibited lower rates of sialic acid incorporation when grown at 32°.[2] It is reasonable to conclude therefore that the decreased incorporation rate for cells grown at lower temperature is a property related to the polyoma-transformed phenotypes.

In distinct contrast to the results obtained with polyoma-transformed clones, untransformed BHK and 3T3 cells showed a twofold *increase* in the rates of sialic acid incorporation when the cells were grown at 32° as compared to cells that were grown at 38.5° (see Table I). Several interpretations are plausible to explain the *growth temperature-dependent* alteration of sialylation reaction in both transformed and untransformed cells. They are (a) changes in the levels of all sialyl acceptor molecules on the cell surface, (b) changes in the synthesis of specific sialylacceptors, and (c) altered properties of sialyltransferases under specific growth conditions. Although the exact mechanism remains elusive at the moment the results may, in part, explain the apparent discrepancies in the observed levels of higher (Bosmann, 1972; Warren *et al.*, 1973) or lower (Den *et al.*, 1971; Grimes, 1973) sialyltransferase activities reported for various virus-transformed cell lines as compared to their untransformed counterparts.

[2] It is noteworthy that when ts-3-3T3 cells were grown in the presence of receptor-destroying enzyme (a crude preparation of neuraminidase) a significantly larger number of counts were incorporated, whereas, a smaller number of counts were incorporated by Balb/C-3T3 cells. These results may reflect the ability of neuraminidase to uncharge terminal sialic acid residues from the surface of ts3-3T3 cells and not from Balb/C-3T3 cells. Further experiments are necessary to clarify this point.

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