MORPHOLOGICAL ALTERATIONS AND GANGLIOSIDE SIALYLTRANSFERASE ACTIVITY INDUCED BY SMALL FATTY ACIDS IN HELA CELLS

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ABSTRACT

Incubation of HeLa cells in the presence of millimolar concentrations of propionate, butyrate, or pentanoate increases the specific activity of CMP-sialic acid:lactosylceramide sialyltransferase 7-20-fold within 24 h. Longer-chain saturated fatty acids or acetate are much less effective, decanoate showing no induction. Unsaturated fatty acid analogs of butyrate and other compounds are ineffective. Only the three most effective compounds also produce characteristic smooth extended cell processes in HeLa cells. Butyrate (5 mM) induces the sialyltransferase after a 4-h lag, producing maximum specific activity by 24 h. The amount of sialyl-lactosylceramide, the glycolipid product of the enzyme, increases during that time 3.5 times more than in control cultures. No other glycosphingolipid enzyme is significantly altered by butyrate exposure. The cellular shape changes occur 2-3 h later than the increase of sialyltransferase activity, and both processes require the continuous presence of inducer and the synthesis of RNA and protein but not the synthesis of DNA or the presence of serum.

Transformation of mammalian fibroblasts, which occurs spontaneously or after exposure to viruses or carcinogens, gives rise to cells that are more rounded and compact, have shorter processes, and grow without contact inhibition in tissue cultures (30, 27). Transformed cells are also unable to synthesize the more complex glycosphingolipids found in normal cells, because the activities of the corresponding glycosyltransferases are decreased (2, 5, 8, 21). Revertants of transformed cells have been isolated which exhibit a return of many normal phenotypic properties including cell growth and shape (24, 7). Exposure of transformed fibroblasts to dibutyryl cyclic adenosine-monophosphate (cAMP) or other N6-adenine derivatives produces a more normal phenotype, including more extended cell processes (16, 18, 19). Recently, it has been shown that short-chain fatty acids, such as butyrate, can alone produce a drastic extension of cell processes in epithelioid cells such as the (transformed) HeLa cell line (14). It was furthermore found that butyrate causes in HeLa cells the specific increase of one glycosphingolipid.

1 C. W. Sheu, D. Salomon, J. L. Simmons, T. Sreevalsan, and E. Freese, manuscript in preparation.
corresponding enzyme activity CMP-sialic acid: lactosylceramide sialyltransferase (13). This effect provides the unique opportunity to study the temporal relationship between enzyme increase and morphological alteration. This relationship has been examined, with inconclusive results, after reversion of transformed cells (22, 8, 6) or quantitative transformation of normal cells with RNA sarcoma viruses (23, 15, 28). In this paper we report on the specificity and kinetics of sialyltransferase induction and relate it to the change in morphology investigated in the following paper.

MATERIALS AND METHODS

Cell Culture

HeLa cells, strain R (Grand Island Biological Company, Grand Island, N. Y.), were cultured in minimal essential Eagle's medium supplemented with nonessential amino acids (MEM) (11) and 10% calf serum (Flow Laboratories, Inc., Rockville, Md.); this will be called "medium." Cells were grown in plastic Petri dishes of 150 mm diameter (Falcon Plastics, Oxnard, Calif.) in a humidified incubator gassed with 5% CO₂ in air at 37°C. In a typical experiment, dishes containing about 10⁶ cells were washed three times in Dulbecco's phosphate-buffered saline (10) without Ca²⁺ and Mg²⁺ (PBS) and incubated for 10 min in 2.5 ml (per dish) 0.05% trypsin in PBS at 37°C. The cells were suspended in medium and a portion, diluted in physiological saline, was counted in a Cellozone particle counter (Particle Data, Inc., Elmhurst, Ill.). Cells were then seeded in 150-mm plastic Petri dishes at a density of 2 x 10⁵ cells per cm² and incubated at 37°C for 24-36 h. To initiate the experiment, the growth medium was aspirated from the dish and replaced with medium containing the desired experimental compound. The cells were then incubated for different periods of time, usually 12 h. At the end of that time, the degree of morphological alteration was photographed with a Leitz inverted microscope at a magnification of 200. The cells were harvested for enzyme assay and glycolipid analysis as follows. The experimental medium was aspirated and the cell sheet washed three times with warm (37°C) PBS. The cells were detached as stated above for enzyme assays, or by mechanical scraping for glycolipid analysis, immediately suspended in 10 vol of ice-cold PBS, and centrifuged at 200 g for 3 min at 4°C. The cells were suspended in (10 ml per original dish) ice-cold PBS and centrifuged again. The cell pellet was maintained on ice for early glycolipid extraction and enzyme assay.

Glycolipid Isolation and Analysis

Glycosphingolipids were isolated from washed cell pellets and partitioned into neutral and sialic acid-containing classes as previously described (9). The neutral glycosphingolipids were further purified by saponification (9) and separated by thin-layer chromatography on silica gel G plates (E. Merck, Darmstadt, West Germany) with the solvent system chloroform-methanol-water (65:25:4, vol/vol/vol). The glycosphingolipids were detected by spraying the plates with 50% ammonium bisulfate and heating at 180°C for 15 min. They were identified by comparing their migration to authentic standards and quantified by densitometric scanning. The ganglioside fraction was separated by thin-layer chromatography (23), detected with resorcinol reagent, and quantified by densitometric scanning as previously described (22).

Incorporation of radioactively labeled precursors was measured in cells grown in either [1-¹⁴C]galactose (1.25 μCi/ml, 28 mCi/mmol, International Chemical and Nuclear Corp., Irvine, Calif.) or N-[acetyl-¹¹H]-mannosamine (62.5 μCi/ml, 400 μCi/mmol, Tracerlab, Waltham, Mass.) for 24 h. The radioactive glycosphingolipids were extracted and separated by thin-layer chromatography as above, and the radioactivity in individual glycolipids was detected and quantitated with a Berthold chromatogram scanner (Varian Associates, Walnut Creek, Calif.).

Assay of CMP-Sialic Acid: Lactosylceramide Sialyltransferase

The glycolipid acceptor, lactosylceramide, was obtained from Miles-Yeda, (Rehovoth, Israel) and the sugar nucleotide donor, CMP-[4-¹⁴C]sialic acid (6.5-9.0 mCi/mmol), from New England Nuclear, Boston, Mass. The washed cell pellets were suspended in 2.5 vol of 0.25 M sucrose containing 0.1% 2-mercaptoethanol and disrupted by freezing and thawing four times (12). The assay of sialyltransferase activity was similar to that described by Fishman et al. (13) but modified for optimum results with HeLa cell extracts. The reaction mixture contained the following: 5 n mole of lactosylceramide, 30 n mole of CMP-[¹⁴C]-NAN, 25 μg of bovine cardiolipin (Applied Science Labs, Inc., State College, Pa.), 1.25 μmol of sodium-cacodylate buffer (pH 6.8), 375 μg of Cutscum (Fisher Scientific Co., Fairlawn, N.J.), and 300 μg of cell protein in a final volume of 25 μl. For each assay the same reaction mixture minus lactosylceramide was incubated as a control. The reactions were terminated after 3 h, at which time the activity still increased linearly.

The reaction products were isolated by either of two procedures. The first procedure, using the separation of

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the labeled glycolipid from the precursors on small Sephadex columns, has been described (12). The second procedure took advantage of the lipophilic nature of gangliosides in acidic calcium solutions (3). To each incubation tube, 1.0 ml of chloroform, 0.5 ml methanol, 0.3 ml 2% CaCl₂, and 0.15 ml 1.0 M formic acid were added. After vigorous mixing on a Vortex mixer (Science Industries, Inc., Springfield, Mass.) and centrifugation, the upper phase was discarded. The lower phases were washed twice with 1.0 ml of upper phase solvent and dried in counting vials. Recovery of GM₃ was over 90%. Radioactivity was determined by liquid scintillation counting. Both procedures produced comparable results but the second partitioning method was simpler and faster. Activities are reported as picomoles of product formed from the exogenous acceptor per milligram protein per hour.

The reaction product GM₃ was identified by its migration on thin-layer silica gel and its degradability by purified neuraminidase from Clostridium perfringens (Sigma Chemical Co., St. Louis, Mo.). After complete digestion (16 h with 0.1 U of enzyme in 0.1 ml of 0.05 M sodium-acetate buffer, pH 5.0), all of the radioactivity was recovered as sialic acid.

**Chemicals**

Propionic, butyric, and pentanoic acids (obtained from Sigma Chemical Co.) were prepared as 0.5 M stock solutions in H₂O titrated to pH 7.45 with sodium hydroxide. All other test compounds were added directly to MEM plus 10% calf serum, titrated to pH 7.45 with sodium hydroxide, and sterilized by filtration with a 0.45 µm Nalgene filter unit (Sybron Corp., Rochester, N. Y.). Other compounds tested were acetic, hexanic, octanoic, decanoic, crotonic, α-aminoisobutyric, l-α-amino-n-butyrин, γ-amino-n-butyric, and salicylic acids obtained from Sigma. Also tested (from Sigma) were n-butyric acid methyl ester, n-butanol, methylparabens, propyl parabens, and dimethylsulfoxide. Isobutyric acid was obtained from Fisher Scientific Co., and DL-2-aminobutyric acid, phenethyl alcohol, and phenylacetic acid were obtained from Eastman Kodak Co., Rochester, N. Y. Tertolic acid was obtained from Farhan Research Laboratories, Cleveland, Ohio. Actinomycin D (Calbiochem, San Diego, Calif.) was also utilized.

**RESULTS**

**Effect of Butyrate on the Ganglioside Composition**

Fig. 1 shows the ganglioside pattern of HeLa cells grown for 24 h in normal medium and in medium supplemented with butyrate (5 mM). Only two resorcinol-positive areas were present on the chromatogram. The doublet corresponding to GM₃ was more intensely stained in the glycosphingolipid extract of cells exposed to butyrate than in that of normal cells. The level of the slower-migrating ganglioside, which probably is GM₂, was unaffected by butyrate. The two gangliosides were further identified by their sensitivity to bacterial neuraminidase. The ganglioside extract isolated from cells grown in the presence of both butyrate (5 mM) and small amounts of N-[acetyl-³H]-d-mannosamine was incubated with purified neuraminidase and the products were separated on thin-layer plates (Fig. 2). The resorcinol-positive material and the radioactivity migrated with the sialic acid standard. Only terminal sialic acids are hydrolyzed under these conditions. The other hydrolysis product of GM₃, the lactosylceramide Gl-2, was also observed upon charring the chromatogram. The gangliosides of untreated cells were similarly identified.

The glycolipid content of normal and butyrate-treated HeLa cells is given in Table I. Three neutral glycosphingolipids (the mono-, di-, and trihexosylceramide) and two gangliosides (GM₃ and GD₃) were observed in this cell line. When the cells were exposed for 24 h to 5 mM butyrate in growth medium, the GM₃ content increased 3.5-fold, the Gl-1 content increased about 70%, while the slight changes of the other glycosphingolipids were insignificant. Although N-acetylgalactosaminylgalactosylgalactosylglucosylceramide (globoside) is present in many human tissues, none was detected in HeLa cells. The cell extracts contained some lipid material that migrated on the chromatogram between the globoside and GM₃ standards, but this material was not labeled with [¹⁴C]-galactose, in contrast to the other glycosphingolipids.

The incorporation of radioactive sugars into different glycosphingolipids in the presence and absence of butyrate is shown in Table II. When HeLa cells were grown for 24 h in medium containing N-[acetyl-³H]-d-mannosamine, a specific precursor of sialic acid, tritium incorporation into GM₃ was 6.5 times greater in the presence of butyrate than in the controls and the amount of radioactive material per nanomole of GM₃ was twice as large. Tritium incorporation into GD₃ was similar for both treated and untreated cells. Butyrate-treated cells also showed an almost twofold increase in incorporation of [¹⁴C]-galactose into GM₃ but in this case the amount of radioactive material per nanomole of glycosphingolipid was approximately half that of control cells (Table II).
Induction of Sialyltransferase Activity

The increased amount of \(G_{M3}\) has been correlated with an increase in the specific activity of CMP-sialic acid:lactosyl ceramide sialyltransferase (briefly called sialyltransferase), as a result of HeLa cell exposure to 5 mM butyrate (13). Fig. 3 shows that the specific activity increased upon butyrate exposure after a 4-h lag, reaching within 12 h a level 14 times greater than that of untreated cells. The possibility of sialyltransferase activation or inhibition by some other cell component was excluded by a mixing experiment (Table III). Addition of butyrate (5 mM) to the assay mixture also did not affect the enzyme activity.

The effect of different butyrate concentrations on enzyme induction, measured 24 h after butyrate addition to HeLa, is shown in Fig. 4. Morphologi-
Figure 2: Radioscan of thin-layer chromatogram of HeLa-derived gangliosides before and after neuraminidase treatment. Gangliosides were extracted and isolated from HeLa cells grown for 24 h in medium containing sodium butyrate (5 mM) and N-[acetyl-\textsuperscript{3H}]-D-mannosamine (156 \mu M, 62.5 \mu Ci/ml). One aliquot was untreated (lane 2, in middle) while another aliquot was incubated overnight with 0.1 U neuraminidase (in 0.2 ml of 0.05 M sodium acetate and 0.154 M NaCl, pH 5.0) before chromatography (lane 3). Lane 1 contains the designated standards. The chromatogram was then scanned for radioactivity. The left panel is the scan of lane 2 and the right panel that of lane 3. After the radioactive scan, the lower portion of the chromatogram was stained with the resorcinol reagent, and then the upper portion was charred as described.

Table I: Glycolipid Composition of HeLa Cells Grown in Normal and Butyrate-Supplemented Media

| Glycolipid | Structure | Control | Butyrate | nmoles/mg cell protein |
|------------|-----------|---------|----------|-----------------------|
| GI-1       | cer-glcs* | 0.55    | 0.95     |                       |
| GI-2       | cer-glsc-gal | 0.41    | 0.45     |                       |
| GI-3       | cer-glsc-gal-gal | 1.85   | 2.21     |                       |
| Gs         | cer-glsc-gal-NAN | 0.49 | 1.72     |                       |
| Go         | cer-glsc-gal-NAN-NAN | 0.23 | 0.19     |                       |

HeLa cells were cultured for 24 h at 37°C in normal and sodium butyrate (5 mM)-supplemented media. Glycolipids were extracted, isolated, separated, and quantitated as described in Materials and Methods. Results are the average of two experiments.

* Abbreviations: cer, ceramide; glc, glucose; gal, galactose; NAN, N-acetylneuraminic acid.

0.25 \mu g/ml allowed the production of some enzyme while no morphological alteration was observed. Other glycosyltransferases involved in glycolipid biosynthesis (12) did not increase in the presence of butyrate (Table V).
TABLE II

Incorporation of [14C]Galactose and N-[Acetyl-3H]-D-Mannosamine into Glycosphingolipids of Normal and Butyrate-Treated HeLa Cells

| Glycosphingolipid | Control Cells | Butyrate-Treated Cells |
|-------------------|---------------|------------------------|
|                   | 14C           | 3H                     | 14C           | 3H                     |
|                   | cpm/mg protein | cpm/mmol | cpm/mg protein | cpm/mmol | cpm/mg protein | cpm/mmol |
| Gl-1              | 4,465          | 8,118     | --*            | 2,165      | 2,279         |
| Gl-2              | 2,938          | 7,166     | --             | 1,698      | 3,773         |
| Gl-3              | 17,128         | 9,258     | --             | 9,375      | 4,422         |
| GM1a              | 3,033          | 6,190     | 7,249          | 14,794     | 5,681         | 47,421    |
| GM1b              | 6,957          | 15,124    | 14,983         | 32,572     | 3,274         | 11,646    |

* No radioactivity was observed.

HeLa cells were cultured at 37°C in normal and sodium butyrate (5 mM)-supplemented medium which also contained [1-14C]galactose (45 μM, 1.25 μCi/ml) or N-[acetyl-3H]-D-mannosamine (156 μM, 62.5 μCi/ml). After 24 h, the cells were harvested and the radioactive glycosphingolipids were extracted, separated by thin-layer chromatography, and quantitated by radioscanning as described in Materials and Methods.

FIGURE 3 Increase of sialyltransferase activity in HeLa cells after exposure to butyrate. At time 0, the medium of HeLa cell cultures was replaced with medium containing 5 mM sodium butyrate. At the designated times, some cultures were harvested and assayed for sialyltransferase activity as described in Materials and Methods.

Effect of Cell Cycle Inhibitors on Enzyme Induction

In order to establish whether the increase of sialyltransferase was simply a result of cellular arrest at a particular stage of the cell cycle, the effects of thymidine and Colcemid were examined. Cells incubated for 24 h in medium containing 3 mM thymidine, and thus all arrested in S phase, showed no altered morphology and no enzyme increase. But when their incubation was continued for another 12 h in the presence of thymidine and 5 mM butyrate, their morphology was altered and the enzyme had reached typical elevated levels (Table VI). When Colcemid (0.6 μg/ml) and butyrate were added simultaneously to normally
FIGURE 4 Effect of butyrate and decanoate on sialyltransferase activity in HeLa cells. Cells were grown for 12 h in media containing different concentrations of sodium butyrate (●) or decanoate (○), harvested, and assayed for sialyltransferase activity. Activity in untreated cells was 7 pmol/mg protein/h (-----).

Growing HeLa cells, sialyltransferase activity increased but no process formation was seen.

Since serum withdrawal causes process formation in neuroblastoma cells (27), its effect on HeLa cells was investigated. When these cells were incubated for 12 h in serum-free medium, no alteration in morphology or enzyme level was seen (Table VI). However, when the cells were incubated without serum but with 5 mM butyrate, the typical cell processes and the characteristic enzyme increase were observed.

Effects of Lipophilic Agents on Sialyltransferase Induction and Morphological Alterations

To determine the specificity of sialyltransferase induction and the correlation between this induction and the morphological changes, the effect of several other compounds was measured. As Table VII shows, all fatty acids that produced the typical process formation also caused enzyme induction. The absence of enzyme induction with decanoate is shown for different decanoate concentrations in Fig. 4. The tight grouping of the C6, C8, and C10 saturated fatty acids as the only compounds producing these effects is particularly noteworthy. Acetate and octanoate, ineffective morphologically, produced only small increases in enzyme

TABLE IV

Actinomycin D Inhibition of Butyrate-Induced Sialyltransferase Increase and Cell Shape Changes

| Actinomycin D | Sialyltransferase Activity | Morphology (+Butyrate) |
|---------------|---------------------------|------------------------|
| µg/ml pmol/h/mg protein | Control + Butyrate | + |
| 0 | 5 | 46 |
| 0.03 | 0 | 61 |
| 0.06 | 0 | 56 |
| 0.25 | 8 | 28 |
| 0.50 | 0 | 3 |

HeLa cells were grown for 12 h in normal butterate (5 mM)-supplemented media containing different amounts of actinomycin D. The cells were then analyzed for process formation (+) and sialyltransferase activity.
TABLE V
Activities of Different Glycosyltransferases in HeLa Cells Grown in Normal and Butyrate-Supplemented Media

| Glycosyltransferase | Reaction | Control | Butyrate |
|---------------------|----------|---------|----------|
|                     |          | pmol/mg | protein h |
| Glucosyltransferase* | Cer + UDP-Glc → | 1071 | 1041 |
|                     | Gl-1 + UDP |        |          |
| Galactosyltransferase | Gl-2 + UDP-Gal → | 154 | 193 |
|                     | Gl-3 + UDP |        |          |
| Sialyltransferase   | Gl-2 + CMP-NAN → | 5 | 69 |
|                     | G\textsubscript{M3} + CMP | | |

Cells were grown for 24 h in normal or sodium-butyrate (5 mM)-supplemented medium, harvested, and the enzymes were assayed in cell homogenates. It has been shown previously that UDP-Gal:Gl-1 galactosyltransferase activity is not increased in butyrate-treated cells (13).

* Glucosyltransferase activity was assayed by the method of Basu et al (1).† Glucosyltransferase activities are given in cpm/1.5 h/mg protein.

† Galactosyltransferase activity was assayed by the method of Kijimoto and Hakomori (21).

§ With saturating concentrations of CMP-NAN, sialyltransferase activity was 220 pmol/mg protein/h in butyrate-treated cells.

TABLE VI
Effect of Thymidine, Colcemid, and Serum-Deprivation on the Induction of Sialytransferase Activity by Butyrate

| Inhibitor          | Sialytransferase activity |
|--------------------|---------------------------|
|                    | Control | Butyrate |
|                    | pmol/mg protein/h |
| Experiment 1       |         |         |
| None               | 8.2 ± 1.6 | 67.2 ± 7.8 |
| Thymidine          | 6.5 ± 0.9 | 93.8 ± 7.7 |
| Colcemid           | 7.5 ± 0.8 | 90.5 ± 8.2 |
| Experiment 2       | 3.0 | 62 |
| None               | 2.0 | 52 |

In Experiment 1, HeLa cells were grown for 24 h in normal or 3 mM thymidine-containing media. Normal medium was then replaced with normal or 5 mM butyrate-supplemented medium containing either 0.6 μg/ml Colcemid or no further addition. Cells grown with thymidine were maintained in thymidine-containing medium with and without added butyrate (5 mM). After 12 h the cells were harvested and the enzyme was assayed. Stated activities are averages of two separate determinations. In Experiment 2, HeLa cells were grown for 12 h in normal and serum-free media with and without butyrate (5 mM).

TABLE VII
Induction of Sialyltransferase by Chain Fatty Acids

| Fatty acid (sodium salt) | Sialyltransferase activity pmol/mg protein/h |
|--------------------------|---------------------------------------------|
| None                     | 7                                           |
| Acetate (20 mM)          | 18                                          |
| Propionate (10 mM)       | 63                                          |
| Butyrate (1 mM)          | 46                                          |
| Butyrate (5 mM)          | 65                                          |
| Crotonate (2 mM)         | 0                                           |
| Tretrolate (2 mM)        | 3                                           |
| Pentanoate (5 mM)        | 48                                          |
| Hexanoate (5 mM)         | 7                                           |
| Octanoate (2 mM)         | 15                                          |
| Decanoate (0.9 mM)       | 7                                           |

HeLa cells were grown for 24 h in medium supplemented with the above fatty acids (sodium salts). Morphological changes were determined microscopically: (+) indicates process formation, (−) no process formation.

DL-2-aminobutyric, and isobutyric acids as well as butyric acid methylester and n-butanol) were tested (at concentrations up to 5 mM) for their effect on morphology alone and were found ineffective.

Other compounds, some of which were reported to cause some morphological alterations (14) were also tested for enzyme induction (Table VIII). However, they did not produce the characteristic smooth, elongated processes caused by propionate,


### TABLE VIII

**Absence of Sialyltransferase Induction by Other Compounds**

| Compound                | Sialyltransferase activity | Morphology  |
|-------------------------|----------------------------|-------------|
|                         | pmol/mg protein/h          |             |
| None                    | 9                          | -           |
| 5 mM butyrate           | 63                         | +           |
| 1% DMSO                 | 0                          | -           |
| 3 mM methylparabens     | 5                          | *           |
| 1 mM propylparabens     | 14                         | *           |
| 5 mM 2-phenylethanol    | 2                          | -           |
| 5 mM salicylate         | 0                          | -           |
| 5 mM phenylacetate      | 7                          | *           |

Conditions as in Table VII.

* Cell processes found which are distinctly shorter, more branched, and less smooth than those produced by butyrate.

butyrate, and pentanoate, nor did they induce sialyltransferase activity.

**DISCUSSION**

The alteration of glycosphingolipid biosynthesis by butyrate observed earlier (13) was investigated in detail. The cellular content of G\textsubscript{M3} is increased 3.5-fold without significant increase in the other membrane glycosphingolipids. G\textsubscript{M3} has been identified by its characteristic chromatographic migration, its susceptibility to neuraminidase treatment, and by the incorporation of a labeled sialic acid precursor, N-acetyl-mannosamine, which was clearly higher, per nanomole of G\textsubscript{M3}, than the incorporation into control cells. Interestingly, the incorporation of labeled galactose per nanomole of G\textsubscript{M3}, as well as the other glycosphingolipids, was less in the presence of butyrate than in its absence. This effect could have resulted from the more extended growth of the control than the butyrate exposed cells (14), or from a decreased sugar transport in the presence of butyrate. Increased sugar transport has been reported after viral transformation of chick embryo (31) and baby hamster kidney (20) cells. Sugar transport is also higher in growing compared to nongrowing cells.

The increased synthesis of G\textsubscript{M3} results from the increase in the specific activity of CMP-sialic acid:galactosylceramide sialyltransferase activity in cells incubated in the presence of butyrate. Other glycosyltransferases involved in glycolipid biosynthesis are not elevated.\(^1\) The increase in sialyltransferase activity appears to be due to induction of enzyme synthesis as it is blocked by inhibitors of RNA and protein synthesis. The induced sialyltransferase turns over within 16-24 h as indicated by the reduction in enzyme activity upon removal of butyrate; thus the continued presence of butyrate is required for enzyme induction. These results as well as those from experiments in which the mixture of cell homogenates from induced and uninduced cells is used seem to rule out the possibility that butyrate causes the increase of sialyltransferase by the activation of the enzyme or the removal of an enzyme inhibitor.

Butyrate induces the sialyltransferase activity and morphological changes in HeLa cells even in the absence of serum. In contrast, neuroblastoma cells rapidly form cell processes upon removal of serum from the medium by a cycloheximide-independent process (29). Interestingly, butyrate can slowly produce cell processes also in human neuroblastoma cells in the presence of serum (26).\(^1\)

Butyrate appears to arrest HeLa cells in the G\textsubscript{1} phase of the cell cycle\(^2\) and glycolipid biosynthesis appears to be maximal during G\textsubscript{1} (4). However, the arrest of the cell cycle by butyrate does not seem to be the sole source of elevated enzyme levels, because cells blocked by thymidine in the S phase do not show morphological or sialyltransferase alterations but they develop these characteristic alterations when butyrate is also added. Similarly, Colcemid-treated cells, blocked in the M phase, do not show any enzyme increase, but the enzyme is still induced by butyrate (although there is no evidence of process formation). This result suggests that microtubular assembly may play an essential part in process formation (18, 16, 29).

It is clear from this work that only the unmodified, straight-chain, saturated fatty acids (C\textsubscript{3}, C\textsubscript{4}, and C\textsubscript{5}) are effective in producing both the type of cell shape alteration and the sialyltransferase induction described for butyrate. The shorter and longer fatty acids are much less effective or are ineffective in enzyme induction and they produce no shape changes. The mechanism by which the short-chain fatty acids cause these specific effects is unknown.

Simmons and Breuer\(^3\) have shown that process elongation of HeLa cells starts 6–7 h after addition

\(^{1}\) In addition, G\textsubscript{M3}-sialidase activity is the same in control and butyrate-treated HeLa cells. (J. Tallman, P. H. Fishman, and R. C. Henneberry, unpublished observations.)
of butyrate. The increase in sialyltransferase activity demonstrated here begins earlier, 4 h after butyrate addition. It is therefore possible that the change of the glycosphingolipid pattern at the cell surface is necessary for the development of cell processes. For example, the surface change may be required to allow cell processes to attach to the surface of the culture dish, counteracting the tendency of the cell surface to reduce its surface area. This assumption agrees with the observation that, on butyrate removal, enzyme activity decreases well before morphological reversion occurs.

The effect of butyrate, and presumably of propionate and pentanoate, is not limited to HeLa cells. Butyrate-induced morphological alterations have been observed with CHO cells (32), with Chang liver, L-132, and Intestine 40 cells (14), and with neuroblastoma cells, but not mouse neuroblastoma cells (26).

Morphological changes were also found associated with exposure to mono- or dibutyl cyclic AMP and inhibitors of phosphodiesterase in Chinese hamster ovary (16), transformed fibroblast (19) and neuroblastoma (25) cell lines. In contrast, cAMP or mono- or dibutyl cyclic AMP were ineffective in altering the morphology of HeLa cell lines (14) or inducing sialyltransferase activity. When butyrate-treated and control HeLa cells were acid extracted at 4, 8, 12, and 24 h after butyrate (5 mM) addition, no difference in the cAMP content per milligram protein was found (as kindly measured by Dr. A. Peterkofsky). While work is being continued in this area, we have at this time no evidence that the butyrate effect is related to cAMP.

Finally, there is a direct temporal correlation between changes in cell morphology and glycolipid metabolism which may be relevant to the altered glycolipid metabolism observed in many, but not all transformed cells (for a recent review see reference 2). As indicated in the introductory paragraphs, previous studies with phenotypic revertants (22, 8, 6) or mass transformation of normal cells with RNA tumor viruses (23, 15, 27) have not clarified this association. Since reduced glycolipid glycosyltransferase activities are observed in transformed cells with altered glycolipid compositions (2, 5, 8, 21), the modulation of sialyltransferase activity (and thus G_{M_3} levels) in HeLa cells by butyrate may provide a convenient model to explore the role of these complex carbohydrates in malignant processes.

Received for publication 15 October 1974, and in revised form 22 February 1975.

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