ULTRASTRUCTURAL AND ENZYMIC MODULATION OF HELA CELLS INDUCED BY SODIUM BUTYRATE AND THE EFFECTS OF CYTOCHALASIN B AND COLCEMID

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SUMMARY

Sodium butyrate causes HeLa cells to assume an elongated and jagged shape. Ultrastructurally this change is associated with the formation of bundles of microfilaments. Desmosomes were present between adjacent cells. No increase in microtubules was observed in the butyrate-treated cells.

Butyrate induces an increase in the activity of 2 membrane-bound enzymes, alkaline phosphatase and 5'-nucleotidase; however, the activity of a third membrane enzyme, acetylcholine esterase, is reduced. The activities of the several other enzymes with different subcellular localizations are not significantly increased. Colcemid and cytochalasin B prevent or reverse the butyrate-mediated change in HeLa cell morphology and also partially inhibit the induction of alkaline phosphatase activity in these cells. The effect of cytochalasin B on alkaline phosphatase induction may be caused by a reduction in protein synthesis produced by this fungal metabolite.

INTRODUCTION

Recently it has been reported that certain aliphatic saturated acids alter the shape of HeLa and Chinese hamster ovary (CHO) cells in culture, causing them to assume a spindle shape (Ginsburg, Salomon, Sreevasan & Freese, 1973; Ghosh, Deutsch, Griffin & Cox, 1975; Wright, 1973). These effects are specific in that only butyrate, pentanoate and 2-methyl butyrate are capable of producing the morphological modulations, while other short-chain aliphatic acids and analogues are inactive (Ginsburg et al. 1973; Ghosh et al. 1975). In HeLa cells these 4- and 5-carbon saturated acids increase the activity of 2-membrane-associated enzymes, sialyltransferase (Fishman, Simmons, Brady & Freese, 1974) and alkaline phosphatase (Griffin, Price, Bazzell, Cox & Ghosh, 1974). The alteration in cell shape and increase in enzyme activities require protein synthesis. The kinetics and characteristics of sodium butyrate induction of sialyl transferase (Fishman et al. 1974) and alkaline phosphatase (Griffin et al. 1974) have been previously described.

In the present study the effects of sodium butyrate on the morphology of HeLa cells was studied by electron microscopy. The increase in the activity of membrane-
associated sialytransferase and alkaline phosphatase prompted an investigation of the effects of butyrate on other enzymes with various subcellular localization. The possible relationship between butyrate-mediated alterations in cell shape and increase in the activity of certain enzymes was studied using Colcemid and cytochalasin B which prevent or reverse the butyrate-mediated change in HeLa cell shape.

MATERIALS AND METHODS

Chemicals

Butyric acid was obtained from Fisher Scientific Co. and was neutralized with stoichiometric amounts of sodium hydroxide to produce sodium butyrate. Colcemid and cytochalasin B were purchased from Grand Island Biologic Co. and Aldrich Chemical Co. respectively.

Cell culture

HeLa cells (Bottomley, Trainer & Griffin, 1969) were grown as monolayer cultures in plastic T18 flasks (Falcon Co.) or in flint glass bottles in Waymouth medium (Grand Island Biologic Co.) supplemented with 10% foetal calf serum containing 50 U./ml penicillin, 50 μg/ml streptomycin and 30 μg kanamycin/ml. Cultures were examined daily with an inverted microscope and representative flasks were fixed with Kähles solution and stained with Giemsa for cytology. Methods for subculturing using 0.04% trypsin and 0.05 mM ethylenediaminetetra-acetate have been described (Cox & MacLeod, 1962). All chemicals were added to cultures 18 h after subculturing and were dissolved in complete medium, except for cytochalasin B which was dissolved in dimethylsulphoxide (DMSO) and then diluted with complete medium. Equivalent amounts of DMSO were added to controls. In all experiments the maximum cell density was approximately 15 x 10^4 cells/cm². Cell counts were made with a Neubauer Hemocytometer on trypsinized cells suspended in complete medium.

Electron microscopy

HeLa cells were subcultured on Lab Tek Chambers. At specified times control and butyric acid-treated cultures were washed free of media with phosphate-buffered saline, and fixed in situ for 30 min in 4% glutaraldehyde buffered with sodium cacodylate, pH 7.4. The monolayers were rinsed 3 times with cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanols, and embedded by the addition of Marglas resin to the chambers of the Lab Tek culture system. Following polymerization, the cells were separated from the glass slide by immersion in liquid nitrogen. Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with a Siemens A-1 electron microscope.

Enzyme assays

Alkaline phosphatase activity was measured with 72 mM phenylphosphate as substrate in 0.05 M carbonate-bicarbonate buffer with 10 mM MgCl₂, pH 10.7. Phenol released from substrate was measured by a diazo-coupling technique (Fishman & Ghoah, 1967).

Acid phosphatase activity was determined at pH 4.4, using 8 mM p-nitrophenylphosphate in 0.05 M acetate buffer (Bingham & Zittle, 1963). p-Nitrophenol released was measured in a Beckman DU spectrophotometer at 410 nm.

5'-Nucleotidase activity was determined by a modification of the method of Lopes, Zucker-Franklin & Silber (1973), using 20 mM β-glycerophosphate to determine non-specific phosphatase activity and 1 mM 5'-adenosine monophosphate as the substrate for 5'-nucleotidase in 0.05 M Tris-HCl as buffer at pH 7.4. The release of inorganic phosphate was measured by the method of Baginski & Zak (1960).

Acetylcholine esterase was measured photometrically by the method of Ellman, Courtney, Andres & Featherstone (1961). Cells were lysed in 0.2% Triton X-100 final concentration, which was found to be optimal for the HeLa acetylcholine esterase. Acetylthiocholine, 20 mM,
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was used as substrate and the thiocholine released was measured by its reaction with the reagent 5,5'-dithiobis-2-nitrobenzoate (DTNB), 0.01 M, to produce the yellow anion of 5-thio-2-nitro-benzoic acid. The reaction was carried out at pH 8.0 in 0.1 M phosphate buffer and the rate of colour production was measured at 412 nm in a Gilford 2000 recording spectrophotometer. The nmol of acetylthiocholine hydrolysed were derived from the formula described by Ellman et al. (1961). A blank consisting of cell lysates, DTNB, and buffer was included in all assays to correct for the release of thiols from the cells.

Lactic acid dehydrogenase was assayed by following the oxidation of 0.14 mM β-DPNH (β-dihydrolipsozyme-pyridine nucleotide) at 340 nm with a Gilford 2000 recording spectrophotometer using sodium pyruvate as substrate in 0.1 M phosphate buffer, pH 7.4 (Wroblewski & LaDue, 1955).

β-Glucuronidase activity was determined at pH 5.2 using 1.2 mM phenolphthalein glucuronide as substrate in 0.075 M acetic buffer (Kerr & Levy, 1951).

Glycine cleavage enzyme was measured in nmol CO₂ evolved from the carboxyl group of glycine per mg of total cell protein in 90 min at 37 °C (Yoshida & Kikuchi, 1970). The assay was performed using HeLa cells suspended in 1.8 mM glycine in Dulbecco's phosphate-buffered saline, pH 7.4. The evolution of 14CO₂ from ³H-1-glycine was used as a measure of the glycine cleavage enzyme in cells grown with and without 1 mM Na-butyrate for 96 h. The labelled 14CO₂ evolved from the suspension of intact HeLa cells was absorbed in 0.1 M KOH as described for branched chain keto acid decarboxylases by Dancis, Hutzler & Cox (1973) and radioactivity was determined by liquid scintillation counting.

Preparation of cells for enzyme assay

Trypsinized cell suspensions were washed with 0.15 M NaCl solution. The cells were allowed to swell in a hypotonic solution (0.03 M NaCl) and incubated for 2 h at 4 °C and then homogenized in a Ten Broeck glass tissue grinder with approximately 30 strokes of the pestle. Enzyme assays were carried out on the homogenate except in those instances specifically described.

Determination of protein

Protein in cell homogenate was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) using crystalline bovine serum albumin as the standard.

Incorporation of [¹⁴C]leucine

HeLa cell monolayers were pulsed for 3 h with 0.2 μCi/ml L-[¹⁴C]leucine with a specific activity of 54 mCi/mmol (New England Nuclear). For measurement of [¹⁴C]leucine uptake, the flasks were immersed in ice, drained by inverting for 2 min, washed twice with ice-cold 0.15 M NaCl and the monolayer scraped into 1 ml of ice-cold 8% trichloroacetic acid (TCA). The supernatant was separated from the TCA precipitate by centrifuging at 1800 rev/min at 4 °C in an International PR₂ refrigerated centrifuge. The supernatant was decanted and 0.2 ml were counted using a scintillant described by Dancis et al. (1973). The leucine uptake was expressed on the basis of cpm per mg cell protein.

Comparisons between leucine incorporation into TCA-insoluble material and alkaline phosphatase activity were carried out by incubating cells with butyrate and/or cytochalasin B, or Colcemid for various times and then labelling the cultures for 3 h with 0.1 μCi/ml [¹⁴C]leucine. Cells were washed 3 times with 10 ml of 0.15 M NaCl and then lysed with 2 ml of 0.5% sodium deoxycholate. A 1-ml aliquot of the lysate was extracted with 4 ml of ice-cold 5% TCA. The protein precipitates were separated by centrifuging at 1800 rev/min for 40 min in an International PR₂ refrigerated centrifuge. The protein residue was dissolved in 0.2 ml of 4 N NaOH by heating at 60 °C for 4 h. The remaining 1 ml of deoxycholate lysate was used for enzyme assay and protein determinations. ¹⁴C-label was counted with a liquid scintillant in a Packard Tricarb Liquid Scintillation Spectrometer (Dancis et al. 1973). Incorporation of [¹⁴C]leucine into protein is expressed as counts per min per mg protein.
RESULTS

Effect of sodium butyrate on HeLa cell shape and ultrastructure

HeLa<sub>6b</sub> cells ordinarily grow as clusters of rounded cells as shown in Fig. 1A. In the presence of 1 mM sodium butyrate the cells assume a jagged elongate configuration (Fig. 1B). At the ultrastructural level this change is associated with a striking increase in the number of microfilaments that are arranged in conspicuous bundles, similar to the bundles of tonofilaments found in squamous epithelium as seen in Fig. 2A. Fig. 2B demonstrates the well developed desmosomes that are occasionally found connecting adjacent butyrate-treated cells. In Fig. 2C cells grown without sodium butyrate did not show these well developed desmosomes, although intercellular attachment zones were observed. HeLa cells grown in the presence of sodium butyrate do not show "cytopathic" or "toxic" changes. Cell membranes are intact. Myelin figures are absent. There is no apparent increase in the number of lysosomes and other cell organelles including microtubules, mitochondria, ribosomes, and rough endoplasmic reticulum, which are similar to control cells.

Effect of sodium butyrate on activities of HeLa cell enzymes

Table 1 shows the changes in enzyme activities of HeLa cells grown in medium containing 1 mM sodium butyrate. Sialyltransferase (Fishman et al. 1974) and alkaline phosphatase (Griffin et al. 1974) activity were previously shown to be increased in HeLa cells grown with sodium butyrate. Both of these enzymes are primarily associated with the membrane fractions of cells. As shown in Table 1 a third membrane-bound enzyme, the 5'-nucleotidase also is increased in activity. However, the activity of acetylcholine esterase, another putative membrane-associated enzyme in HeLa cells is moderately reduced.

The activity of several other enzymes, acid phosphatase, β-glucuronidase, glycine cleavage enzyme and lactic acid dehydrogenase were not significantly altered. These enzymes have various putative subcellular localizations that include lysosomes, mitochondria and cytosol. It should be appreciated that the subcellular localization of the above enzymes is tentative, since attempts to obtain pure fractions of cell organelles from cultured cells are only partially successful and cross-contamination of cell fractions is frequently observed (Mahoney, Hart, Steen & Rosenberg, 1975).
Fig. 2. Ultrastructure of HeLa cells grown in complete medium for 24 h with and without 1 mM sodium butyrate. × 22000.

A, butyrate-treated cells showing bundles of microfilaments (arrow).
B, butyrate-treated cells showing desmosome formation (arrow).
C, control HeLa cell showing intercellular attachment zone (arrow).
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Table 1. Effect of sodium butyrate on the activities of HeLa cell enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Putative subcellular localization</th>
<th>Specific activity</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Base-level</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>Membranes</td>
<td>0.084 ± 0.017&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>Membranes</td>
<td>0.176 ± 0.046&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetylcholine esterase</td>
<td>Membranes</td>
<td>1.30 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Lysosomes and nuclei</td>
<td>0.026 ± 0.002&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>Lysosomes and endoplasmic reticulum</td>
<td>0.75&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycine cleavage enzyme</td>
<td>Mitochondria</td>
<td>0.334 ± 0.070&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lactic acid dehydrogenase</td>
<td>Cytosol</td>
<td>0.338 ± 0.105&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Replicate cultures were grown in medium without and with 1 mM sodium butyrate for 72-96 h. All assays were carried out in duplicate on at least 4 replicate cultures of control and butyrate-treated cells. The mean values and standard deviations of enzyme activities per mg of cell protein are shown except for β-glucuronidase.

a Alkaline phosphatase specific activity in μmol of phenol released per min per mg cell protein.
b 5'-Nucleotidase specific activity in μmol PO<sub>4</sub> per h per mg cell protein.
c Acetylcholine esterase specific activity in nmol of acetylthiocholine hydrolysed per min per mg cell protein.
d Acid phosphatase specific activity in μmol p-nitrophenol released per min per mg cell protein.
e β-Glucuronidase specific activity in nmol of phenolphthalein released per min per mg cell protein (means of 2 replicate cultures).
f Glycine cleavage enzyme specific activity in nmol of CO<sub>2</sub> evolved from 1-[<sup>14</sup>C]glycine in 90 min per mg cell protein.
g Lactic acid dehydrogenase specific activity in Δ O.D. 340 per min per mg cell protein.

Effect of cytochalasin B and Colcemid on morphology and butyrate-mediated increase in alkaline phosphatase activity

The alteration in cell shape and the induction of increased activity of several membrane-bound enzymes in HeLa cells grown with sodium butyrate raises the possibility that the alteration in cell shape and the changes in activities of membrane-associated enzymes may be related. Direct demonstration of this relationship is difficult. However, the use of agents which reverse or inhibit butyrate effects on cell shape provide a convenient indirect test for this hypothesis. Figs. 1C and D show that both cytochalasin B and Colcemid prevent and reverse development of the long cytoplasmic processes induced by butyrate. As demonstrated in Fig. 1C, cells treated with cytochalasin B are rounded, with a crinkled cell margin. Colcemid causes the HeLa cells grown in butyrate to assume a rounded shape resembling that of the controls, except the cells remain separated (Fig. 1D).

Fig. 3 shows the kinetics of increase in alkaline phosphatase activity during growth in medium with sodium butyrate. Addition of either Colcemid or cytochalasin B during the linear phase of increase in enzyme activity markedly reduced the magnitude of the increase during the subsequent 24 h. The mechanism of this inhibition was investigated by measuring radioactive leucine incorporation into TCA-soluble and...
Fig. 3. Effect of cytochalasin B and Colcemid on sodium butyrate-induction of alkaline phosphatase activity in HeLa culture. Alkaline phosphatase activity measured as μmol phenol released per min per mg cell protein × 10^3. O, no additions (control); △, sodium butyrate, 1 mM; ■, cytochalasin B, 20 μM; ▲, Colcemid, 0.2 μM.

Table 2. Effect of cytochalasin B on alkaline phosphatase activity and [14C]leucine incorporation in control and butyrate-treated HeLa cells

<table>
<thead>
<tr>
<th>Additions to medium</th>
<th>Duration of incubation with cytochalasin B, h</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>3's</td>
</tr>
<tr>
<td>None (control)</td>
<td>AP</td>
</tr>
<tr>
<td>Na-butyrate (48 h)</td>
<td>40.1</td>
</tr>
<tr>
<td>Cytochalasin B</td>
<td>14.0</td>
</tr>
<tr>
<td>Na-butyrate (48 h)+ cytochalasin B</td>
<td>38.1</td>
</tr>
</tbody>
</table>

AP, mean alkaline phosphatase activity expressed in μmol phenol released from disodium phenyl phosphate per min per mg protein × 10^3 on at least 2 replicate cultures.

[14C]leucine incorporated into TCA-insoluble material following a 3-h labelling with 0.1 μCi/ml. Values are mean cpm per mg cell protein × 10^3 on at least 2 replicate cultures.

Cytochalasin B final concentration, 20 μM.

Sodium butyrate-treated cultures were grown in the presence of 1 mM concentrations of the compound for 48 h before adding cytochalasin B to some of the replicate cultures.
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TCA-insoluble cellular material. Since cytochalasin B has been reported to inhibit the uptake of monosaccharides (Cohn, Banerjee, Shelton & Bernfield, 1972; Estensen & Plagemann, 1972), it was important to measure TCA-soluble [14C]leucine as an index of intracellular amino acid uptake. Neither cytochalasin B, nor sodium butyrate, nor a combination of both agents affected TCA-soluble radioactivity expressed per mg of cell protein. Therefore, any reduction in incorporation of radioactive label into TCA-insoluble material probably represents reduced protein synthesis. Table 2 shows that in HeLa cells both sodium butyrate and cytochalasin B inhibit leucine incorporation into TCA-insoluble material by approximately 30–50%. When both sodium butyrate and cytochalasin B are added together to HeLa cultures, protein synthesis is reduced by 70–90%. Despite the reduced protein synthesis in butyrate-treated cultures, the alkaline phosphatase activity is increased about 3:5-fold during
77 h of growth. In Table 2, the addition of cytochalasin B to cultures grown in medium with sodium butyrate for the preceding 48 h further reduced protein synthesis and markedly inhibited the subsequent increase in enzyme activity. The rise in alkaline phosphatase activity observed in control cultures has been previously shown to be the result of depletion of cystine from the medium (Cox & MacLeod, 1964). Cytochalasin B effects on leucine incorporation are dosage-dependent, as seen in Fig. 4 (open bars) and the inhibition is reversible after removing the substance, as is also shown in Fig. 4 (cross-hatched bars).

**Table 3. Effect of Colcemid on [¹⁴C]leucine incorporation in HeLa cells**

<table>
<thead>
<tr>
<th>Additions to medium</th>
<th>Incorporation of [¹⁴C]leucine, cpm/mg cell protein × 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>16.0</td>
</tr>
<tr>
<td>Colcemid (7 h)</td>
<td>16.3</td>
</tr>
<tr>
<td>0.04 µM</td>
<td>16.3</td>
</tr>
<tr>
<td>0.2 µM</td>
<td>16.4</td>
</tr>
<tr>
<td>1.0 µM</td>
<td>15.7</td>
</tr>
<tr>
<td>Exp. 2</td>
<td></td>
</tr>
<tr>
<td>None (control)</td>
<td>32.4</td>
</tr>
<tr>
<td>Na-butyrate (48 h)</td>
<td>25.2</td>
</tr>
<tr>
<td>Colcemid (7.5 h)</td>
<td>31.3</td>
</tr>
<tr>
<td>Na-butyrate (48 h)</td>
<td>27.6</td>
</tr>
<tr>
<td>+ Colcemid (7.5 h)</td>
<td></td>
</tr>
</tbody>
</table>

Na-butyrate concentration 1 mM.
Colcemid concentration 0.2 µM in Exp. 2.

Colcemid also inhibits the butyrate-mediated increase of alkaline phosphatase activity when added during the linear phase of the induction (50 h), as shown in Fig. 3. This inhibition is not quite as marked as that observed with cytochalasin B. The mechanism of the inhibition may be different, since Colcemid in concentrations used for cellular studies does not inhibit protein synthesis, as shown in Table 3.

**DISCUSSION**

Alteration of HeLa cell morphology by short-chain aliphatic acids is selective in that only butyric, pentanoic, and 2-methylbutyric acids mediate these effects (Ginsburg et al. 1973; Ghosh et al. 1975). Other fatty acids and analogues are ineffective. The mechanism of the morphological changes induced by butyrate in HeLa cells is not known. HeLa cells grown for 24 h with sodium butyrate lose their epithelioid morphology and develop extensive cytoplasmic processes. The change in cell shape has been attributed, in part, to assembly of microtubules, since the morphological effects are prevented or reversed by colchicine (Ginsburg et al. 1973) and by increased intracellular calcium (Henneberry, Fishman & Freese, 1975). Changes of cell shape are frequently attributed to alterations in the assembly of microtubules.
However, as shown in the present study, the microtubules of butyrate-treated HeLa cells are not dramatically affected when studied by electron microscopy. Our findings suggest that butyrate affects primarily the microfilament system resulting in an increase in the number of filament bundles. These are arranged in a fashion similar to the tonofilaments found in cells of squamous epithelial origin. Moreover, butyrate promotes the association of filaments with plasma membrane allowing formation of desmosomal attachments between adjacent cells. These ultrastructural findings are of considerable interest because of controversy concerning the nature of the cervical carcinoma from which HeLa cells were derived (Gey, Coffman & Kubicek, 1952). Originally the carcinoma was classified as being of squamous origin but review of the original histologic preparations were interpreted as an adenocarcinoma (Jones, McKusick, Harper & Wu, 1971). Our findings of bundles of microfilaments and well developed desmosomes in butyrate-treated HeLa cell cultures support an epithelial origin for these cells.

Butyrate apparently interacts with HeLa cells to modulate membranes. This interaction is associated with an increase in the activity of at least 3 membrane-bound enzymes, sialyltransferase (Fishman et al. 1974), alkaline phosphatase and 5'-nucleotidase. Two other membrane-associated enzymes, galactosyltransferase (Henneberry, Fishman & Freese, 1975) and acetylcholine esterase, exhibit either no change or a reduction in their enzymic activity. The activities of certain other enzymes with different putative subcellular localizations are not significantly altered by growth in medium with sodium butyrate. The mechanism of enhanced activity in membrane-bound enzymes is not known, although cycloheximide will prevent the increase in sialyltransferase (Fishman et al. 1974) and alkaline phosphatase (Griffin et al. 1974). It is noteworthy that butyrate mediates an increase in the activity of 3 membrane-associated enzymes in HeLa cells despite its 30–50 % inhibition of protein synthesis. In previous studies the concentration of cycloheximide used to block the induction of alkaline phosphatase and sialyltransferase inhibited protein synthesis by 85–90 %.

The relationship between the butyrate-mediated alteration in cell morphology and the modification in membrane-associated enzyme activities was approached indirectly by investigating the effects of compounds that prevent and reverse the changes in cell shape. Both Colcemid, which inhibits the polymerization of tubulin and prevents the formation of microtubules, and cytochalasin B, which inhibits contractile processes that depend upon the integrity of the actinomyosin microfilaments, reverse the butyrate-induced alteration in HeLa morphology. Both of these compounds also reduce the extent of increase in alkaline phosphatase activity during the linear phase of butyrate ‘induction’. Cytochalasin B curtails the increase of enzyme activity more effectively than Colcemid. The mechanism through which cytochalasin B inhibits the increase in alkaline phosphatase may be related to a reduction in protein synthesis. Cytochalasin B inhibits protein synthesis by 30–50 % in HeLa cell cultures and when it is added to cells grown with sodium butyrate for 48 h the inhibition of protein synthesis approximates 70–90 %. To our knowledge, there is only one other study reporting that cytochalasin B reversibly inhibits protein synthesis in mammalian cell cultures. Koch & Oppermann (1975) have shown in HeLa cells infected
with polio virus that cytochalasin B initiates a partial breakdown of polyribosomes, suggesting that it inhibits initiation of protein synthesis. Cytochalasin B has been used extensively to study a wide variety of contractile processes and its effects have been attributed to interaction with microfilaments. Interpretation of some of these observations may require re-examination in view of the inhibition of protein synthesis by this fungal metabolite.

It is surprising that the alkaline phosphatase activity of butyrate-treated cultures does increase slightly in the presence of cytochalasin B, despite the marked inhibition of protein synthesis. Moreover, the moderate reduction in the butyrate-mediated increase of alkaline phosphatase activity observed with Colcemid cannot be explained by effects on protein synthesis. It is known that the increase of alkaline phosphatase activity in HeLa cells induced by growth in medium with hydrocortisone is caused by an increase in the catalytic efficiency of the enzyme, rather than an increase in the alkaline phosphatase content of cells (Griffin & Cox, 1966; Cox, Elson, Tu & Griffin, 1971). This enhanced catalytic efficiency is apparently the result of a modification of alkaline phosphatase prior to its incorporation into the membrane mosaic (Cox, Ghosh, Bazzell & Griffin, 1975). The induced form of the enzyme has approximately one-half the phosphate residues found in base-level alkaline phosphatase (Griffin, Price & Tu, 1973). The dephosphorylation of the enzyme is believed to alter the energy of activation of the enzyme-substrate complex, leading to an increase in catalytic efficiency. Preliminary studies by Griffin and his associates (unpublished data) indicate that sodium butyrate mediates similar modification of alkaline phosphatase and thereby increases its catalytic activity. Perhaps Colcemid interferes with the modification of the enzyme by affecting membrane conformation or function. It should be noted that Colcemid does not inhibit the butyrate-mediated increase of sialyltransferase activity in HeLa cell cultures (Simmons, Fishman, Freese & Brady, 1975). This suggests that the mechanism of ‘induction’ of this enzyme may differ from alkaline phosphatase. This speculation is supported by major differences between these enzymes in the kinetics of ‘induction’ (Fishman et al. 1974; Griffin et al. 1974).

The mechanism of butyrate-mediated modulation of HeLa cells is not known. The restricted structural and steric specificity required of aliphatic saturated acids in order to produce the effects on cell shape and membrane-associated enzyme activities suggests that these molecules may interact with cell membranes to perturb their structure and functions. These compounds may therefore constitute useful probes for altering the membrane mosaic and for further elucidating structure and function relationships in cell membranes.

Supported by a research grant from the USPHS National Institutes of Health. Stephen I. Deutsch is a Medical Scientist Trainee. We appreciate the excellent technical assistance of Adrianna Rukenstein. Support for these studies was also provided by the Berger Foundation and the Polly Annenberg Levee Charitable Trust.
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(Received 27 November 1975)