EFFECTS OF SODIUM BUTYRATE
ON THE MEMBRANE GLYCOCONJUGATES OF MURINE SARCOMA VIRUS-TRANSFORMED RAT CELLS

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ABSTRACT

The temporal relationship between butyrate-induced cellular flattening of murine sarcoma virus-transformed rat cells (MSV-NRK) and alterations in certain surface-associated biochemical markers of transformation, e.g., surface glycopeptides, glycolipids, fibronectin, hexose uptake, and cell-substrate adhesion was examined. The induction of elevated levels of the ganglioside GM₃ and of a GD₁₆-like ganglioside were observed to precede or to parallel cellular flattening. Likewise, enhanced incorporation of radioisotopically labeled fucose into a novel fucose-containing component, i.e., glucopyranosyl (1 → 3) fucopyranosyl-threonine, was also observed to occur at an early stage of cellular flattening. In contrast, a shift in the molecular weight distribution of trypsin-sensitive, surface fucoproteins was observed to occur at a late stage of cellular flattening. Moreover, surface fibronectin was not detectable in the butyrate-flattened MSV-NRK cells despite the fact that the cells manifested significantly enhanced cell-substrate adhesion. Thus, butyrate appears to be a useful tool for understanding the sequential changes associated with expression of the transformed phenotype of MSV-NRK cells.

KEY WORDS surface membrane glycoconjugates virus transformation butyrate

It has been demonstrated that addition of short-chain fatty acids, e.g., butyric acid, to HeLa cells results in the development of long cellular processes and a change in morphology from a typical epithelial appearance to a more fibroblastic one (13, 16, 17, 36). This morphological change was associated with increased CMP-sialic acid:lactosylceramide sialyltransferase activity and elevated levels of the glycolipid hematoside (12, 42).

Prompted by these observations, we undertook studies to examine the effects of butyrate on the morphologic features and membrane components of murine sarcoma virus-transformed rat cells (MSV-NRK). MSV-NRK cells grown in medium supplemented with 2 mM sodium butyrate changed in morphology from the round appear-
The morphological phenotype of MSV-NRK cells. In addition, a relationship between the time of induction of cellular flattening and the elaboration of cytoskeletal elements, i.e., microtubules and microfilaments, was observed. Thus, butyrate was demonstrated to be useful in the modulation of the morphological phenotype of MSV-NRK cells.

In addition to a change in cell shape, sarcoma virus transformation is accompanied by a variety of surface-related alterations, e.g., enhanced hexose uptake, decreased cellular adhesion, and changes in glycolipid and glycopeptide composition. Specifically, many virus-transformed cells are relatively enriched in higher molecular weight, trypsin-sensitive, surface glycopeptides as compared to control cells [52]. The loss of reduction of a high-molecular-weight external glycoprotein (fibronectin) [25, 51], thought to be associated with cell-cell (4, 9, 23, 53, 55) or cell-substrate adhesion (2, 22, 35, 54), also has been reported in transformed cells. Moreover, studies in this laboratory [43-47] have demonstrated a marked decrease in the level of isotopically labeled fucose incorporated into a component designated FL4, which has recently been putatively identified as glucopyranosyl(1 → 3)fucopyranosyl-threonine [29, 47]. In contrast, a closely related component, i.e., fucosyl-threonine, designated FL3, has not been found to substantially change in transformed cells [47]. Changes have also been noted in cell glycolipid composition after viral transformation. Often the transformed cells manifest a significant decrease in more complex glycolipids (6, 19, 20, 40).

In the current study, we have examined the relationship between butyrate-induced morphological changes in MSV-NRK cells and alterations in a number of surface membrane parameters often associated with sarcoma virus transformation.

MATERIALS AND METHODS

Cell Culture

Seed cultures of the newborn rat kidney (NRK) cells (clone 2) and of MSV-transformed cells were kindly supplied by Dr. K. Somers (Eastern Virginia Medical School). Cells were routinely cultivated in Eagle’s minimum essential medium containing 10% fetal calf serum, i.e., control medium, and were examined periodically for mycoplasma. The sodium salts of short-chain fatty acids (Sigma Chemical Co., St. Louis, Mo.) were prepared by titration to pH 7.4 with NaOH. Solutions were filter-sterilized and added to the medium in the appropriate concentration just before use.

Analysis of Glucosyl-Fucosyl-Threonine (FL4)

Medium containing [14C]fucose (2.5 μCi/ml, 1.4 Ci/mmole; New England Nuclear, Boston, Mass.) was added to rapidly growing cells. When the cells were confluent (~48 h) the monolayers were washed three times with phosphate-buffered saline (PBS), pH 7.2, and scraped directly into 9 ml of 60% ethanol (vol/vol). The cell suspension was extracted for 5 min in a boiling water bath, pelleted at 1,000 g, and the insoluble residue was reextracted with 3 ml of 60% ethanol as before. The combined extracts were taken to dryness, resuspended in 2.0 ml of H2O, and centrifuged at 15,000 g to remove insoluble material. The supernatant fraction was applied to a thoroughly washed 2.5-cm high column of Ag 50 (H+) in a Pasteur pipet, washed with 5 ml of H2O, and eluted with 10 column volumes of 0.5N NH4OH. Essentially all of the FL4 in the initial extract is recoverable in the basic eluate as judged by studies with labeled standards. The basic eluate was taken to dryness, resuspended in methanol-H2O (1:1, vol/vol), and an aliquot was chromatographed in one dimension on silicic acid thin-layer plates in CHCl3-CH30H-concn NH4OH (40:80:25, by volume). The plates were scraped in 0.5-cm bands from origin to solvent front, and radioactivity was measured by scintillation spectrometry. A portion of the basic eluate was chromatographed in CHCl3-CH3OH-H2O (60:35:8, by volume) to exclude the possibility that radioactivity from residual-free fucose was contributing to that ascribed to FL4. In the latter system, FL4 is readily separable from fucose. When examining the level of FL4 in whole cell ethanol extracts, it is not advisable to utilize the one-dimensional sequential double-solvent system described by Larriba [31]. Using this system, we have observed that at least two fucose-labeled components co-migrate with FL4. In contrast, the double-run system seems to be satisfactory for examining FL4 of washed pellet preparations [44, 45, 46] because the contaminating compounds appear to be localized largely in the cytosol.

Ganglioside Analysis

Confluent monolayers were washed three times with PBS, scraped into PBS, and pelleted by centrifugation at 500 g for ~10 min. The pellet was extracted and partitioned against an aqueous phase according to the method of Folch et al. [14]. The combined upper aqueous phases were reduced to 1/3 their original volume and dialyzed against 1,000 vol of cold distilled H2O for 48 h. The dialysate was lyophilized, resuspended in CHCl3-CH3OH (2:1, by volume), and an aliquot was chromatographed on silicic acid thin-layer plates in CHCl3-CH3OH-concn NH4OH-H2O (60:35:1:7, by volume). Gangliosides were visualized by resorcinol spray [49] and quantitated by scanning densitometry. Authentic GM2, GM3, and GD1a, were obtained from Supelco, Inc. (Belleville, Pa.). Authentic GM3 was prepared from BHK-21 cells. Bovine brain ganglioside mixture was obtained from Sigma Chemical Co., and its contents were identified by gas-liquid chromatography (kindly performed by Martin Klinger) and by thin-layer chromatographic comparison with standard gangliosides. Quantitation of total sialic acid in ganglioside preparations was done essentially as described by Miettinen and Takki-Luukkainen [34].

Surface Glycopeptide Preparation

Exponentially growing cultures were labeled for 48 h in medium supplemented with [14C]fucose (5 μCi/ml, 1.4 mmole; New England Nuclear, or [14C]fucose (0.5 μCi/ml, 60.9 Ci/mole;
New England Nuclear). Surface glycopeptides were prepared by a modification of the method Buck et al. (8) (S. Richards and M. Steiner, manuscript in preparation). Briefly, cells were subjected to trypsin treatment (1 mg/ml) at 37°C for 15 min followed by addition of soybean trypsin inhibitor. The trypsinate, obtained after a low-speed centrifugation and a further 100,000 g centrifugation, was dialyzed against H2O and lyophilized. After reconstitution in buffer (0.01 M Tris, 0.15 M NaCl, pH 7.6), trypsinites containing alternative isotopic forms of fucose (14C or 3H) were mixed and subjected to pronase digestion (0.1 mg/ml) for 96 h. With addition of fresh pronase every 24 h. Pronsate-digested samples were lyophilized, resuspended in a minimal volume of 0.1 M Tris-acetate, pH 9.0, containing 0.1% SDS (wt/vol), 0.01% EDTA (wt/vol), and 0.1% beta-mercaptoethanol (vol/vol), and analyzed on a Sephadex G-50 fine column (0.6 x 150 cm) equilibrated with the same buffer. The column fractions (~0.35 ml each) were analyzed by scintillation spectroscopy.

In some experiments, co-dialysis of trypsinates containing alternative isotopic forms was performed followed by pronase digestion and gel filtration. Profiles comparable to those obtained by mixing samples after dialysis were obtained.

**Cell Protein/Glycoprotein Analysis**

**EXTERNAL LABELING:** Confluent monolayers in 60-mm tissue culture dishes were washed three times with PBS, pH 7.2. Iodination was carried out in PBS containing 250 μCi of carrier-free 125I (New England Nuclear), 5 mM glucose, 0.2 U of glucose oxidase (Sigma, type V), and 20 μg of laccoperoxidase (grade B, Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.) (24). The reaction was allowed to proceed for 10 min at room temperature with frequent agitation and was terminated by the addition of ice-cold 0.17 M NaI in PBS containing 2 mM phenylmethylsulfonyl fluoride. Cells were disrupted at 100°C for 3 min in 1% SDS-1% beta-mercaptoethanol-0.5 M urea. Samples were stored at -30°C if not analyzed immediately.

**METABOLIC LABELING:** Exponentially growing cells were labeled for 48 h in growth medium supplemented with either 1 μCi/ml [3H]glucosamine (45-60 Ci/mmol; New England Nuclear) or 1 μCi/ml [3H]fucose (50 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Monolayers were washed twice with cold PBS, scraped from the growth surface, and pelleted. Cell pellets were disrupted at 100°C in 1% SDS and stored at -30°C until analyzed by polyacrylamide gel electrophoresis.

**Polyacrylamide Gel Electrophoresis**

Gel electrophoresis was performed according to the procedure of Laemmli (30). Coomassie blue-stained gels which contained 14C-labeled material were further processed for fluorography (3), or for radioautography. Radioautography of dried gels was performed with Kodak No-screen X-ray or Kodak Blue M film for fluorography for 1-4 wk.

**Cell-Substrate Adhesion Assay**

Cells were seeded into 60-mm tissue culture dishes at a cell density of 1.0 x 10⁶ cells/plate and incubated for 24 h. Plates were washed twice with PBS, and 2 ml of trypsin in PBS (150 μg/ml) was added. Cells were incubated at 37°C and at various times released cells were counted in a hemocytometer.

**Hexose Transport Assay**

Cells were plated at 4 x 10⁴ cell/60-mm tissue culture dish and were incubated for 24 h. Plates were rinsed with 37°C PBS and floated in a 37°C water bath. 3 ml of [3H]-2-deoxyglucose (1.0 μCi/ml, 30-60 Ci/mmol; New England Nuclear) in 37°C PBS was added to each dish, and the plates were incubated for 8 min. After removal of the isotope by aspiration, the plates were washed twice with ice-cold PBS, scraped into 1 ml of the same, and adjusted to 1% SDS. After brief sonication, aliquots were taken for protein determination and total radioactivity.

**Protein Determination**

Protein determinations were carried out by the method of Lowry et al. (33). Experimental results expressed as milligram protein refer to whole cell protein. Butyrate-treated MSV-NRK and NRK cells contain somewhat more protein per cell than their control counterparts, e.g., control NRK cells, 0.26 ng protein/cell; NRK cells after a single passage in butyrate-supplemented medium (BSM), 0.32 ng protein/cell; control MSV-NRK cells, 0.16 ng protein/cell; MSV-NRK cells after two passages in BSM, 0.23 ng protein/cell.

**RESULTS**

**Effects on Low Molecular Weight Fucose-containing Components**

Within 96 h after the addition of BSM to MSV-NRK cells, there was a three- to fourfold increase in the incorporation of [3H]fucose into FL4 (Fig. 1). Passage of MSV-NRK cells for 30 passages in BSM did not further alter the level of incorporation of labeled fucose into FL4 (Table I). However, the rate of reversion to control levels of FL4 differed for short-term (96 h) vs. long-term (30 passages) butyrate-treated cells. The former required one passage whereas the latter required three passages. In a similar manner the rate of morphological reversion of short- and long-term butyrate-treated cells has previously been shown to be approximately one and three passages, respectively (3).

Examination of several additional short-chain fatty acids, i.e., propionic, isobutyric, pentanoic, hexanoic, heptanoic, and acetonic acid, at concentrations of 2 and 5 mM, for their effect on cellular morphology and FL4 metabolism revealed that only pentanoic acid affected the MSV-NRK phenotype with respect to these parameters.

**Effects on Ganglioside Composition**

Passage of MSV-NRK cells in BSM resulted in elevation of the amount of the ganglioside GM4 to a level comparable to that of NRK cells (Table II). Moreover, a second ganglioside, similar in chromatographic mobility to bovine GD1a, was observed to increase approximately twofold, within 96 h, in butyrate-treated MSV-NRK cells. In contrast, growth of the normal cells in BSM for 96 h
Effects on Trypsin-sensitive Surface Glycopeptides

As has been found in other transformed cells (7) we have observed that MSV-NRK cells contain relatively more of the rapidly eluting, presumably higher molecular weight, fucose-labeled glycopeptides than do normal NRK cells (Fig. 2A). After two passages in BSM (Fig. 2B), there was only a very minor shift in the glycopeptide profile toward the less rapidly eluting glycopeptides. However, after 5 and 19 passages (Fig. 2C and D), there was a marked increase in the more slowly eluting fucoglycopeptides. Moreover, the profile of the trypsin-sensitive surface fucoglycopeptides of long-term butyrate-treated MSV-NRK cells was comparable to the profile of NRK cells (Fig. 3). The butyrate-induced change in fucoglycopeptides was fully reversible after shift to control medium, although the rate of reversion was slower than was observed for FL4 or for GM3 and GD1a (Table II). For example, after 19 passages of MSV-NRK cells in BSM, five to six passages in control medium were required to revert the fucoglycopeptide size distribution to that of control MSV-NRK cells.

Effects on Fibronectin and Cell-substrate Adhesion

The butyrate-flattened cells did not have a demonstrable level of fibronectin as measured either by external labeling with $^{125}$I (Fig. 4) or by metabolic labeling with $[^{14}$C]glucosamine (Fig. 5) or $[^{14}$C]fucose. Despite the fact that fibronectin was not observed, the butyrate-treated MSV-NRK cells were more adhesive to substrate than control MSV-NRK cells as judged by a trypsinization assay (6.8 min to release one-half of the butyrate-treated MSV-NRK cells vs. 4.2 min for the control MSV-NRK cells). NRK cells were the most adhesive (8.5 min to release one-half of the cells).

Effects on Hexose Uptake

The markedly elevated hexose uptake associated with MSV-NRK cells as compared to NRK cells (Table III) does not appear to be significantly changed after butyrate treatment. Hence, sugar uptake does not appear to be affected by cellular shape change.

DISCUSSION

A role for gangliosides in cell shape determination has been suggested by others (13, 21, 28, 42). In this regard, we observed a butyrate-mediated in-
**TABLE I**

**Effect of Sodium Butyrate on Incorporation of [3H]Fucose into FL3* and FL4 of MSV-NRK Cells.**

| Cell line | No. of passages in sodium butyrate | FL4 cpm/mg protein | FL3 cpm/mg protein | FL4/FL3 |
|-----------|-----------------------------------|--------------------|--------------------|---------|
| NRK       | 0                                 | 5,320              | 3,276              | 1.6 (±0.34)$§$ |
| NRK       | 1                                 | 8,473              | 3,198              | 2.6 (±0.21)$|| |
| MSV-NRK   | 0                                 | 1,006              | 3,062              | 0.3 (±0.04)$§$ |
| MSV-NRK   | 1                                 | 3,732              | 4,334              | 0.9 (±0.1)$§$ |
| MSV-NRK   | 30                                | 3,690              | 4,167              | 0.9 (±0.04)$§$ |
| MSV-NRK   | Reversal (3 passages no sodium butyrate) after 30 passages in butyrate | 999                | 3,342              | 0.3     |

* FL3 has been putatively identified (29) as fucosyl-threonine. Average of two experiments in duplicate.
§ Cells were labeled with [3H]fucose, and FL3 and FL4 were extracted and analyzed as detailed in Materials and Methods and in the legend to Fig. 1. (Fig. 1 represents a separate experiment from those shown above.)
|| The standard deviation of FL4/FL3 for seven experiments done in duplicate.

Protein determination was done on the whole cell suspension. The protein content per cell was somewhat higher, i.e., ~30% in both NRK cells and MSV-NRK cells grown in medium supplemented with 2 mM sodium butyrate.

**TABLE II**

**Effect of Sodium Butyrate on the Ganglioside Composition of NRK and MSV-NRK Cells**

| Cell line | No. of passages in sodium butyrate | GM3 | GM3 | GM1 | GDI-like | GDN2 | nmoI sialic acid/10 mg cell protein |
|-----------|-----------------------------------|-----|-----|-----|----------|------|----------------------------------|
| NRK       | 0                                 | 16.8| 2.4 | ND  | ND       | ND   | ND                               |
| NRK       | 1                                 | 16.0| 3.0 | ND  | ND       | ND   | ND                               |
| MSV-NRK   | 0                                 | 7.8 | 6.7 | Trace | 1.2     | 0.4  | ND                               |
| MSV-NRK   | 1                                 | 11.2| 6.5 | Trace | 2.2     | 0.3  | ND                               |
| MSV-NRK   | 19                                | 16.1| 6.2 | Trace | 2.4     | 0.2  | ND                               |
| MSV-NRK   | Reversal (3 passages no sodium butyrate) after 19 passages in sodium butyrate | 7.4 | 6.3 | Trace | 1.4     | 0.3  | ND                               |

Gangliosides were extracted and the ganglioside fraction was isolated and chromatographed as detailed in Materials and Methods. Gangliosides are designated according to the nomenclature of Svennerholm (48): GM3, N-acetylneuraminylgalactosylglucosylceramide; GM3, N-acetylgalactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GM1, galactosyl-N-acetylglactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GM1, GM1, galactosyl-N-acetylglactosaminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GD1a, N-acetylneuraminylgalactosyl-N-acetylneuraminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide; GD1b, galactosyl-N-acetylneuraminyl-N-acetylneuraminyl-[N-acetyleneuraminyl]-galactosyglucosylceramide; GD1c, N-acetylneuraminyl-galactosyl-N-acetylneuraminyl-[N-acetyleneuraminyl]-galactosylglucosylceramide. Data represent the average of duplicate determinations from three experiments.

ND, not detectable. (Levels of ganglioside of 0.2 nmoI or higher could be detected in the experiments shown above.)

Increase in the ganglioside GM3 in MSV-NRK cells that were undergoing cell-shape alterations from round and highly refractile to flat and poorly refractile. The nontransformed counterpart NRK cells manifested neither a detectable cell shape change nor an increase in GM3. Moreover, both the increase in the level of GM3 and the cell shape change were readily reversible upon shift of the cells to control medium. Hence, the results with virus-transformed cells would be consistent with the suggestion that this ganglioside plays a role in modulation of cell shape. In addition to GM3, a GD1a-like ganglioside (chromatographically similar to standard GD1a) was increased twofold in butyrate-treated MSV-NRK cells and markedly increased in NRK cells. Because the increase in the GD1a-like ganglioside occurs in the butyrate-treated NRK cells as well as the MSV-NRK cells, it would not appear to be associated with the process of cellular flattening. However, we did...
FIGURE 2  Sephadex G-50 glycopeptide profile of 2 mM butyrate-treated MSV-NRK cells. Exponentially growing cells labeled with radioactive fucose for 48 h were used in all experiments. For details of preparation and analysis of glycopeptides, see Materials and Methods. (A) 3H-labeled (○) NRK cells vs. 14C-labeled (●) control MSV-NRK cells. (B) 3H-labeled (○) MSV-NRK cells passaged twice in butyrate medium vs. 14C-labeled (●) control MSV-NRK cells. (C) 3H-labeled (○) MSV-NRK cells passaged six times in butyrate medium vs. 14C-labeled (●) control MSV-NRK cells. (D) 3H-labeled (○) MSV-NRK cells passaged 19 times in butyrate medium vs. 14C-labeled (●) control MSV-NRK cells. The phenol red dye marker eluted at fractions 136-146 in column A, fractions 123-133 in column B, fractions 122-132 in column C, and fractions 125-135 in column D. Radioactivity was not detected in fractions beyond those shown in the figure. Reversal of the isotopic form of fucose did not influence the elution patterns. The profiles shown above are typical of three separate experiments.

observe that the increase in the GD1α-like ganglioside was accompanied by a change in the appearance of the cytoplasmic microfilaments, from a largely random distribution to a mostly parallel one (using immunofluorescence with antibody against chicken actin; unpublished observations). The parallel distribution was very similar to the arrangement of microfilaments previously observed in the MSV-NRK cells (3). Moreover, the parallel appearance is like the appearance of sheath fibers recently reported by Zigmond et al. (56). Further studies are planned using double immunofluorescence with antibody prepared against the GD1α-like component and against actin to ascertain whether the ganglioside is associated with the organization of sheath fibers in NRK and MSV-NRK cells.

Another feature of the butyrate-treated MSV-NRK cells is the enhanced incorporation of radioisotopically labeled fucose into a novel fucose-containing component, i.e., FL4. The enhanced incorporation of fucose into FL4, similar to the increase in GM3, occurs concomitant with the cell shape change. The elevated level of FL4 in short-
FIGURE 3  Sephadex G-50 glycopeptide profile of butyrate-treated MSV-NRK cells vs. NRK cells. For experimental details, see Fig. 2. NRK cells were labeled with $^3$H-fucose (●) and MSV-NRK cells grown for 18 passages in BSM were labeled with $[^{14}]$C-fucose (○). The phenol red dye marker eluted in fractions 133-143. The profile shown above is typical of three separate experiments.

Long-term butyrate-treated MSV-NRK cells rapidly return to that of untreated MSV-NRK cells upon shift to control medium, whereas the reversion of FL4 and the reversion of the cellular morphology in long-term butyrate-treated cells was somewhat slower. It should be noted that NRK cells have markedly higher levels of FL4 than do MSV-NRK cells. Hence, the effects of butyrate on MSV-NRK cells with regard to FL4 parallel the effects observed with GM3 in that both glycoconjugates are rapidly shifted to a more “normal” composition.

MSV-NRK cells grown in butyrate-supplemented medium also display a shift in the size distribution of trypsin-sensitive surface glycopeptides to a lower molecular weight range that is comparable to that of normal NRK cells. The butyrate-induced alteration in surface glycopeptide and its reversal is most evident after the cell shape change is complete. This is in contrast to the alterations in gangliosides and in FL4, which parallel the cell shape change. Because the cellular doubling time of butyrate-treated cells is reduced as compared to untreated MSV-NRK cells (3), it might be argued that the alteration in glycopeptides is related to a decreased rate of growth. In this regard, it has been demonstrated that oncornavirus-transformed cells with a reduced growth rate have a glycopeptide distribution similar to that of normal growing cells (7).

FIGURE 4  Autoradiograph of an SDS polyacrylamide gel of $^{125}$I-labeled cell surface components. Cells were iodinated using the $^{125}$I-lactoperoxidase method, as described in Materials and Methods and analyzed on 7% SDS polyacrylamide slab gels. (A) NRK cells, (B) MSV-NRK cells grown in medium without added butyrate, and (C) MSV-NRK cells after 19 passages in medium supplemented with 2 mM butyrate. The numbers to the right indicate the molecular weight and position of marker proteins: myosin, 210,000; beta-galactosidase, 130,000; bovine serum albumin, 68,000; and ovalbumin, 45,000. After mild trypsin treatment (10 μg/ml, 10 min, room temperature), the $^{125}$I-labeled protein of ~250,000 mol wt (arrow) was quantitatively and preferentially removed. The gel profile shown above typifies the results of eight separate experiments.

The major shift in the molecular weight distribution of surface glycopeptides is observed.

MSV-NRK cells grown in BSM show an enhancement in cell-substrate adhesion. This is consistent with our previous electron microscope observation of a dramatic increase in the number of cell-substrate attachment plaques (3). Because fibronectin is thought to have a functional role in cell-substrate attachment, it was of interest to de-
FIGURE 5 Fluorogram of an SDS polyacrylamide gel of $^{14}$Clglucosamine-labeled cell proteins. Cultures were grown for 48 h in medium containing $^{14}$Clglucosamine. Cells were harvested by scraping, disrupted in 1% SDS, and aliquots were analyzed on a 6% polyacrylamide slab gel as described in Materials and Methods. (A) Chicken embryo fibroblasts, (B) control NRK cells, (C) control MSV-NRK cells, (D) MSV-NRK cells passaged 30 times in BSM. The position of fibronectin is indicated by an arrow, and the position of the molecular weight markers is indicated by the arrowheads: myosin, mol wt 210,000; phosphorylase A, 93,000; bovine serum albumin, 68,000; ovalbumin, 45,000. The open arrow denotes the stacking gel/separating gel interface. Labeled material migrating just below this region was variable in amount, did not stain with Coomassie blue, was not labeled with fucose, and was not readily trypsin-sensitive. The results shown above typify the findings of three separate experiments.

To determine whether the enhanced substrate adhesiveness was associated with an increase in cell-surface fibronectin, fibronectin was not observed in butyrate-flattened MSV-NRK cells by either lactoperoxidase-catalyzed iodination or metabolic labeling with radioisotopically labeled sugars. In contrast, NRK cells which are also quite flat and well-adhered to the substrate have prominent levels of fibronectin. These observations are consistent with the idea (10, 37–39) that other protein(s) and/or glycosaminoglycans are intimately involved in the mediation of cell-substrate adhesion of butyrate-treated MSV-NRK cells.

Fibronectin has also been postulated to participate in cell-to-cell adhesion (4, 9, 23, 53, 55) and to be associated with the cellular microfilament network (1, 27). Butyrate-treated MSV-NRK cells, unlike NRK cells, manifest extensive cell-to-cell overlap and lack cell-to-cell adherens junctions (3). Such cellular overlap and the absence of adherens junctions might be interpreted to indicate that the lack of fibronectin results in decreased cell-to-cell adhesion. Add-back experiments (2, 54) are in progress to test this hypothesis. The well-developed microfilament network in MSV-NRK cells grown in BSM in the absence of demonstrable fibronectin would suggest that this protein may not have a central role in the organization of this type of microfilament assembly system in butyrate-treated MSV-NRK cells.

Butyrate has been shown by others to be a useful tool in the study of various membrane-bound enzymes (11, 18, 26), hormones and hormone receptors (15, 50), and control of cell differentiation (32, 41). This study illustrates the potential usefulness of butyrate as a tool in the study of the role of glycoconjugates in membrane structure and function.

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### Table III

| Cell line | No. of passages in sodium butyrate | cpm/mg protein/8 min | Hexose transport relative to control NRK cells |
|-----------|---------------------------------|----------------------|---------------------------------------------|
| NRK       | 0                               | 45,846               | 1.00                                        |
| NRK       | 1                               | 63,492               | 1.38                                        |
| MSV-NRK   | 0                               | 325,501              | 7.12                                        |
| MSV-NRK   | 1                               | 450,916              | 9.83                                        |
| MSV-NRK   | 30                              | 453,447              | 9.92                                        |

* Cells were plated at a density of $4 \times 10^4$ cells/60-mm tissue culture dish and were assayed 24 h later for hexose uptake in medium containing $[^3H]$deoxyglucose. The results shown above represent the average of two separate experiments done in duplicate with a standard deviation of <5%. For details, see Materials and Methods. The presence or absence of sodium butyrate in the assay medium did not influence the results.
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