Sodium Butyrate Induces Histone Hyperacetylation and Differentiation of Murine Embryonal Carcinoma Cells

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ABSTRACT Cells from embryonal carcinoma (EC) lines 6050A and PCC4.aza1R differentiate in response to treatment with sodium butyrate as well as retinoic acid (RA) or hexamethylenebisacetamide (HMBA). Murine 6050A EC cells exposed to sodium butyrate possess hyperacetylated forms of histones H4 and altered forms of histones H2a and H2b, whereas histones from cells treated with other inducers appear to be unaffected. These results might indicate that the mechanism by which sodium butyrate promotes differentiation of EC cells is different from the ways in which RA and HMBA act. Differentiation-defective PCC4(RA)-1 EC cells fail to respond to RA, presumably because they possess minimal amounts of active binding protein for RA (cRABP). Sodium butyrate treatment of these cells results in only a modest level of differentiation. On the other hand, exposure to sodium butyrate plus RA leads to extensive differentiation. As is the case with 6050A cells, PCC4(RA)-1 cells treated with sodium butyrate also contain hyperacetylated histones. Furthermore, these cells now possess high levels of cRABP. The latter observations suggest that sodium butyrate has the ability to reactivate a silent cRABP gene in PCC4(RA)-1 cells and thereby lead to extensive differentiation via the retinoid pathway when RA is added.

Regions within the chromosome that are being actively transcribed appear to undergo alterations in chromatin structure (1-5). These alterations might be necessary to facilitate accessibility of the nucleosomal DNA to RNA polymerase binding or function (6-8). Structural changes have been variously ascribed to differential interactions of nonhistone chromosomal proteins with mononucleosomes (9, 10); alterations in methylation of specific regions of the DNA (11, 12); and posttranslational modifications of core-particle histones due to phosphorylation or hyperacetylation (6, 8, 13). The accumulation of hyperacetylated core particle histones is one of many changes occurring during rapid cell growth, maturation, and cell differentiation. When cell cultures are exposed to millimolar concentrations of sodium butyrate they accumulate hyperacetylated core-particle histones, notably histones H3 and H4, due to the inhibition of activity of histone deacetylase(s) (14, 15). Such treatments can lead to the initiation of new gene expression resulting in, for example, the production of alphafetoprotein in rat hepatoma cells (16), induction of thymidine kinase in thymidine kinase-minus CHO cells (17), and the differentiation of murine erythroleukemia and human neuroblastoma cells (18, 19).

Embryonal carcinoma (EC) cell lines have proven to be useful in studies of physical and chemical stimuli leading to differentiation (e.g. 20, 21). Retinoic acid (RA) and hexamethylenebisacetamide (HMBA) are particularly effective promoters of EC cell differentiation (21-23). The induction of EC cell differentiation by the former compound appears to be mediated by its interaction with a specific cellular binding protein (cRABP) (24, 25). HMBA does not compete with RA for cRABP sites, although evidence has been presented to suggest that its effectiveness is also related in some way to cRABP function: mutant EC lines that lack detectable levels of cRABP fail to differentiate in response to either RA or HMBA (25, 26). Finally, it has been proposed that the RA-cRABP complex acts by translocating to the nucleus and

1 Abbreviations used in this paper: EC, embryonal carcinoma; HMBA, hexamethylenebisacetamide; RA, retinoic acid; and SSEA-1, stage-specific embryonic antigen.
influencing gene expression by interaction with chromatin (24). The alterations in the pattern of gene expression would presumably be specific, involving suppression of genes characteristic of undifferentiated cells and activation of other genes that lead to a differentiated phenotype.

To determine whether sodium butyrate, an agent that appears to cause random gene activation (27), is capable of promoting differentiation of EC cells, we added this chemical to three cultured EC lines and compared its effects with those of RA and HMBA. One of the cell lines studied lacks cRABP activity and is defective for differentiation. The results indicate that sodium butyrate can cause dramatic alterations in the phenotype of EC cells and suggest that the differentiation-defective mutant under study possesses one or more intact cRABP structural gene(s) reactivated by sodium butyrate.

**MATERIALS AND METHODS**

**Cell Lines and Culture Techniques:** Sources, derivations, and properties of EC cell lines 6050AJ, PCC4.aza1R, and PCC4(RA)−1 have been described previously (20, 25). Cells were grown at 37°C (5% CO₂ in air) in Dulbecco-modified Eagle's medium supplemented with 10% fetal calf serum, antibiotics, and extra glucose and glutamine (20). Cells were exposed as indicated to 10⁻⁶ M RA; 3 mM HMBA; 1-3 mM sodium butyrate or a combination of RA and sodium butyrate for up to 10 d. Procedures involving RA were carried out in a room in which light of wavelengths <550 nm was filtered out.

**Detection of SSEA-1 Antigen:** The presence or absence of cell surface stage-specific embryonic antigen (SSEA-1), whose expression is characteristic of undifferentiated EC cells (28), was determined by immunofluorescence analysis: briefly, after culture for 5 d in plastic tissue culture dishes containing medium with or without chemical effectors, cells were rinsed with Hank's balanced salt solution and fixed with cold methanol for 5 min. The fixed cells were rinsed with phosphate-buffered saline and incubated for 45 min with a monoclonal antibody raised against SSEA-1 (28) and diluted 1:300. The cells were washed with phosphate-buffered saline and exposed to a fluorescein-conjugated secondary antibody (Litton Bionetics, Frederick, MD; 1:20) for 45 min. After further wash, the stained cells were evaluated with a Leitz Orthoplan microscope equipped with Plomé illumination.

**Assays for Plasminogen Activator:** Cells were assayed for secretion of plasminogen activator by the fibrin-agar overlay assay as described by Beets et al. (29). Sparse cultures were incubated under the overlay for 6 h. Fibronectin zones were identified and counted following Coomasie Brilliant Blue staining. A total of 300 single cells were scored for each experimental condition.

**Isolation of Histones:** Histones were isolated by a modification of the procedures of Vidalí et al. (30) and Candido et al. (31). Briefly, monolayers were disrupted by a brief trypsinization and cells were pelleted and washed in a phosphate-buffered saline. The cell pellet was resuspended in reticulocyte suspension buffer containing 0.5% Nonidet P-40 for 5 min at 0°C. After centrifugation at 8,000 g the supernate was made 0.2 N in H₂SO₄ and mixed gently for 15 min at 0°C. Acid-soluble proteins were collected in 0.1 M acetic acid, and lyophilized. Protein determinations were by Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, CA) (32).

**Acid-Urea Polyacrylamide Analysis of Histones:** Histones were analyzed by acid-urea gel electrophoresis on 15% acrylamide: 0.8% bisacrylamide slab gels containing 6.25 M urea (33). 30 μg of total histones in 2.5 M urea/5% acetic acid buffer were electrophoresed 24 h at 200 V with cooling. Gels were stained 1 h in 7% acetic acid containing 1% Amido black and 1 h in 0.1% Coomassie Blue in 7% acetic acid: 5% methanol. Gels were destained in 7% acetic acid: 5% methanol.

**Measurement of Cellular Retinoic Acid-binding Protein:** cRABP activity was measured as detailed by Schindler et al. (25) and Sherman et al. (34). Cytosolic extracts were prepared by disrupting cells and collecting the supernates after high speed centrifugation. Extracts were incubated with [³H]RA (2.5 x 10⁻⁷ M; 30 Ci/mM) plus or minus a 200-fold excess of unlabeled RA at 0°C for 4 h; unbound RA was subsequently removed with charcoal-coated dextran. Reaction mixtures were then subjected to sucrose density gradient centrifugation (25) or Sephadex G25 chromatography (34) and fractions were collected and counted. Specific binding activity is calculated as the difference in the labeled RA associated with the excluded volume of the Sephadex column or at the 28 region of the gradient in the absence and presence of excess unlabeled RA.

**RESULTS**

**Effects of Sodium Butyrate on EC Cell Morphology**

Under normal culture conditions, 6050AJ and PCC4.aza1R EC cells are characteristically small and round with scant cytoplasm and large nuclei containing prominent nucleoli (e.g., Figs. 1 A and 2 A). After exposure to 10⁻⁶ M RA or 3 mM HMBA, cells from lines 6050AJ and PCC4.aza1R assume a differentiated phenotype, i.e., flat, polygonal cells with increased cytoplasmic volume and a dispersed chromatin pattern (Fig. 1, B and C, and references 25, 26). Treatment of the same cells with 1 mM sodium butyrate induces similar morphological differentiation of a substantial fraction of the cells (Figs. 1 D and 2 B) without evidence of toxicity or notable effects on proliferation (Fig. 3 A). As Fig. 2 C illustrates, PCC4.aza1R cells with altered morphology retained this phenotype for at least 72 h after removal of sodium butyrate.

Under standard culture conditions, PCC4(RA)−1 cells appear small, rounded and birefringent (Fig. 4 A; references 25, 26). After exposure to 1 mM sodium butyrate, the cells flatten somewhat and acquire a more typical EC morphology (Fig. 4 B). The proliferation rate is only slightly affected (Fig. 3 B). There is no sign of cells with a non-EC phenotype. When PCC4(RA)−1 cells are exposed to 3 mM sodium butyrate, extensive toxicity is observed. However, if cells are cultured for 1 d in 1 mM sodium butyrate before exposure to the inducer at 3 mM, some toxicity is observed but there is no net loss of cells (Fig. 3 B; parental PCC4.aza1R cells behave similarly [Fig. 3 A]). Of the surviving PCC4(RA)−1 cells, ~10% possess a fibroblastic morphology. When 10⁻⁶ M RA is added concomitantly with the sodium butyrate, there is no further effect upon viability (Fig. 3 B) but many more of the treated PCC4(RA)−1 cells assume an altered morphology (Fig. 4 A).

![FIGURE 1 Changes in morphology of 6050AJ EC cells in response to RA, HMBA, and sodium butyrate. (A) cells in control medium; (B) cells exposed to 3 mM HMBA for 5 d; (C) cells exposed to 10⁻⁶ M RA for 5 d; (D) cells exposed to 1 mM sodium butyrate for 5 d. All figures are phase-contrast micrographs. Bar, 50 μm.](image-url)
FIGURE 2 Effects of sodium butyrate upon PCC4.aza1R cells. (A) PCC4.aza1R cells in control medium; (B) PCC4.aza1R cells exposed to 1 mM sodium butyrate for 4 d; (C) PCC4.aza1R cells after 5 d of exposure to 1 mM sodium butyrate followed by 3 d in the absence of this compound. Bar, 50 μm.

In fact, within 10 d of culture, about half of the cells are non-EC-like in appearance with the monolayer containing discrete areas of both fibroblastic and epithelioid cells (Fig. 5). It is unlikely that the latter result is due to selection of a minor population of differentiated cells in the original PCC4(RA)-1 cultures because such cells have never been observed in these cultures under other conditions. Also, cell numbers are similar in cultures treated with sodium butyrate plus or minus RA, whereas the proportion of non-EC cells is much greater in the former case.

Analysis of Differentiation by Loss of SSEA-1

Analysis of undifferentiated cells from lines 6050AJ, PCC4.aza1R and PCC4(RA)-1 by indirect immunofluorescence demonstrated the presence of SSEA-1 on almost all cells (e.g., Fig. 6, B and F). Expression of SSEA-1 is lost by 6050AJ and PCC4.aza1R cells after exposure to sodium butyrate (Fig. 6 D; and reference 26), as is observed when these cells are treated with RA or HMBA (26). PCC4(RA)-1 cells retain SSEA-1 when treated with RA (Fig. 6 F) or HMBA (26). In the presence of sodium butyrate, some cells lose reactivity to SSEA-1 antibody (not shown). After treatment with sodium butyrate plus RA, the population that loses reactivity is substantial and the remaining fluorescence does not appear to associate characteristically with cell surfaces (Fig. 6 H).

Production of Plasminogen Activator

Measurement of plasminogen activator secretion by the fibrin-agar overlay assay demonstrated low basal levels of secretion in 6050AJ, PCC4.aza1R, and PCC4(RA)-1 cells when maintained in the absence of inducers. Exposure to 10^{-6} M RA causes a marked increase in the number of lysis zones surrounding single 6050AJ and PCC4.aza1R cells but has little effect on PCC4(RA)-1 mutant cells (Table I). In the presence of sodium butyrate 6050AJ and PCC4.aza1R cells also show large increases in the number of plasminogen activator-secreting cells, although the values are not as great as those seen after treatment with RA. Plasminogen activator secretion by PCC4(RA)-1 cells is essentially unaffected by exposure to sodium butyrate. However, when these cells are treated with sodium butyrate plus RA, there is a large increase in the number of secreting cells (Table I).

Effects of RA, HMBA, and Sodium Butyrate on Histone Profiles

Upon PAGE, total histones isolated from untreated 6050AJ or PCC4(RA)-1 cells exhibit a distinct distribution of histone subspecies. Three histone H3 subspecies (non-, mono-, and diacetylated forms denoted H3o, H31, and H32, respectively) and three forms of histone H4 (H4o, H41, H42) are observed

FIGURE 3 Effects of sodium butyrate upon growth of PCC4.aza1R and PCC4(RA)-1 cells. Cells are seeded at 2 x 10^4/60-mm tissue culture dish. On the day after seeding (first arrows), some of the cells were maintained in control medium (O), whereas others received 1 mM butyrate (O). 1 d later (second arrows) cells from some of the latter dishes were exposed to 3 mM butyrate alone (O) or together with 10^{-6} M RA (O). Culture media in all dishes were changed every 2 d. Cells were removed from dishes with trypsin-EDTA solution and counted with a hemocytometer. Values shown are averaged from triplicate determinations. (A) 6050AJ cells; (B) PCC4(RA)-1 cells.
FIGURE 4 Effects of sodium butyrate upon PCC4(RA)-1 cells. (A) PCC4(RA)-1 cells in control medium; (B) PCC4(RA)-1 cells following 4 d of exposure to 1 mM sodium butyrate; (C) PCC4(RA)-1 cells after treatment for 1 d with 1 mM sodium butyrate and 3 d with 3 mM sodium butyrate plus 10^-6 M RA. Bar, 50 μm.

Effects of Sodium Butyrate on Cellular RA-binding Protein in Mutant Cells

In the absence of treatment with inducers of differentiation, 6050AJ (35) and PCC4.aza1R (35; Table II) cells possess readily detectable levels of cRABP, whereas values for differentiation-defective PCC4(RA)-1 cells are at or barely above background (25; Fig. 8B and Table II). Levels of cRABP often change when EC cells are induced to differentiate; the values reached appear to reflect the nature of the differentiated progeny (35). When PCC4.aza1R cells are treated for several days with RA, cRABP values rise about threefold (Table II). A more modest increase is observed following exposure of the cells to sodium butyrate.

The very low levels of cRABP activity in PCC4(RA)-1 cells do not change following treatment of the cells with RA (Table II). However, when PCC4(RA)-1 cells are exposed to sodium butyrate with or without RA, and are tested for the presence of binding protein, cytosolic extracts show levels of binding activity which are elevated more than 10-fold (Table II). Binding of [3H]RA can be dramatically reduced with an excess of unlabeled RA and resides in the 2S region following sucrose gradient sedimentation (Fig. 8A). In these respects, the activity resembles the cRABP activity found in other EC cells (24, 25).

DISCUSSION

After treatment with sodium butyrate, 6050AJ and PCC4.aza1R cells undergo dramatic morphological alterations, secrete markedly increased amounts of plasminogen activator and lose SSEA-1 surface antigen. Taken together,
these phenotypic alterations demonstrate clearly that differentiation has occurred (20, 25, 26, 28, 34). Histone H4 from sodium butyrate-treated EC cells is hyperacetylated as has been observed with other cells (13). Multiple forms of histones H2a and/or H2b are also observed. These might represent acetylated subspecies as well, although it is possible with these histone types that the variation is due to phosphorylation (6, 31). As has been suggested previously (1, 6), resultant modifications at the nucleosome level might be partially or fully responsible for the alterations in gene expression that yield the observed phenotypic changes (18, 19). If so, then the mechanism by which butyrate promotes differentiation of EC cells is different from the one(s) operative with RA or HMBA since these agents did not induce alterations in histone profiles.

In a previous report (23) it was claimed that F9 EC cells are unresponsive to sodium butyrate (concentration unspecified). In that study and others, dibutyryl cAMP, when added along with RA, was shown to enhance the phenotypic differences between EC cells and the ultimate differentiated derivatives (23, 36-39). However, the enhancing effect was presumably due to the cAMP rather than the butyrate moiety since agents such as cholera toxin elicit the same response (23). It remains to be determined by a systematic analysis whether F9 and other EC cells not used in the present investigation are responsive or unresponsive to sodium butyrate.

In various studies involving new gene expression in the presence of sodium butyrate, the effects appeared to be reversed when the chemical was removed (19, 27). Our preliminary observations with PCC4.aza1R cells (see Fig. 2C) suggest that the described effects of butyrate upon these cells is irreversible. This is consistent with the view that butyrate has triggered the permanent differentiation of EC cells rather than some transient alteration.

Three notable points emerge from the response of the differentiation-defective cell line PCC4(RA)-1 to sodium butyrate: (a) these cells are less responsive to the inductive effects of sodium butyrate than the parental line (PCC4.aza1R) and a second, unrelated EC line (6050AJ); (b) although RA fails to induce differentiation of PCC4(RA)-1 cells and sodium butyrate alone has only a modest effect, the combination of sodium butyrate plus RA promotes extensive differentiation; and (c) although PCC4(RA)-1 cells possess little, if any, detectable cRABP activity, butyrate-treated cells possess high levels of cRABP. The large increase of cRABP activity in PCC4(RA)-1 cells is unlikely to be due only to differentiated forms in these cultures because activity levels are similar whether cells are treated with butyrate alone (in which case there is little differentiation) or with butyrate plus RA (a condition promoting substantial differentiation). Two possibilities are suggested by these observations. The first is that the paucity of cRABP activity in PCC4(RA)-1 cells is not due to structural mutations in the cRABP genes but rather due to a trans-acting regulatory block which can be reversed by butyrate. Alternatively, assuming that the cRABP gene is not X-linked, it is conceivable that in PCC4(RA)-1 cells, one of the two genes for cRABP is mutated whereas the homolog is intact but epigenetically silenced (see reference 17). Sodium butyrate treatment might reactivate the latter cRABP gene by altering chromatin structure. Whichever alternative is correct, our results suggest that sodium butyrate restores the ability of PCC4(RA)-1 cells to differentiate by reestablishing the retinoid-cRABP pathway; thus, a maximum effect is seen only when RA is added along with the sodium butyrate.

Finally, the fact that nonmutagenized cells are more responsive than PCC4(RA)-1 cells to butyrate alone might indicate that alternate pathways are potentially operative in these cells.

Table I

| Cell line | Untreated | RA | Sodium butyrate | RA + Sodium butyrate |
|-----------|-----------|----|-----------------|----------------------|
| 6050AJ    | 5         | 51 | 37              | ND†                  |
| PCC4.aza1R| 1         | 63 | 23              | ND                   |
| PCC4(RA)-1| 2         | 5  | 3               | 27                   |

* The assay was performed on sparsely seeded cultures using the fibrin-agar overlay procedure. Cells were exposed to the chemical effectors for 2 d and then overlaid with the assay reagents. The cultures were scored for the presence of lysis zones after 6.5 h. Percentages are based upon counts of 300 single cells for each treatment.

† RA was used at a concentration of 10⁻⁶ M.

‡ Sodium butyrate was used at a concentration of 1 mM for 6050AJ and PCC4.aza1R cells and at 3 mM (after 1 d at 1 mM) for PCC4(RA)-1 cells.

§ Not done.
Sucrose density gradient analysis of RA binding by cytosolic extracts of PCC4(RA)−1 cells treated with inducers of differentiation. Extracts were prepared, incubated with [3H]RA alone (p) or together with a 200-fold excess of unlabeled R (9) and analyzed for cRABP activity by centrifugation as described in Materials and Methods. Extracts were obtained from cells treated either with (A) 1 mM sodium butyrate for 10 d or with (B) 10−6 M RA for 8 d (followed by culture in the absence of RA for 2 d to diminish the intracellular concentration of unlabeled RA). T and B refer to the top and bottom of the gradient, respectively. The sedimentation coefficient of the peak in A is approximately 2S.

**TABLE II**

| Cell line          | Treatment          | cRABP levels (fmol/mg protein) |
|--------------------|--------------------|-------------------------------|
| PCC4.azaIR         | Control            | 493                           |
|                    | RA                 | 1,387                         |
|                    | Sodium butyrate    | 754                           |
| PCC4(RA)−1         | Control            | 106                           |
|                    | RA                 | 81                            |
|                    | Sodium butyrate    | 1,294                         |
|                    | Sodium butyrate and RA | 1,245                      |

*EC cells were cultured, cytosolic extracts were prepared, and binding protein activity was assayed as described in Materials and Methods. RA treatment was carried out at a concentration of 10−6 M over 8 d. The cells were kept in control medium for 2 d to dilute out the added RA. Sodium butyrate was used at a concentration of 1 mM for a period of 10 d.

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REFERENCES

1. Allfrey, V. G. 1977. Post-synthetic modifications of histone structure—mechanism for control of chromosome structure by modulation of histone-DNA interactions. In Chromatin and Chromosome Structure. H. J. Li and R. A. Eckhardt, editors. Academic Press, Inc., New York. 167-191.

2. Franke, W. U., Scheer, M. Trendelenburg, H. Zentgraf, and H. Spring. 1977. Morphology of transcriptionally active chromatin. Cold Spring Harbor Sympos. Quant. Biol. 42:755-772.

3. McKnight, S. L., M. Bustin, and O. L. Miller, Sr. 1977. Induction of thymidine kinase in enzyme-deficient Chinese hamster cells. Cold Spring Harbor Symp. Quant. Biol. 42:741-754.

4. Weintraub, H., and M. Groudine. 1976. Chromosomal subunits in active genes have an altered conformation. Science (Wash. DC). 193:846-856.

5. Wu, C., Y.-C. Weng, and S. C. R. Elgin. 1979. The chromatin structure of specific genes. II. Disruption of chromatin structure during gene activity. Cell. 16:807-814.

6. Boffa, L. C., G. Vidali, R. S. Mann, and V. G. Allfrey. 1978. Nucleosome structure. Annu. Rev. Biochem. 49:1115-1156.

7. Perry, M., and R. Chalkley. 1982. Histone acetylation increases the solubility of chromatin and occurs sequentially over most of the chromatin. J. Biol. Chem. 257:7336-7347.

8. Shewmaker, C. K., and T. E. Wagner. 1980. Analysis of binding interactions between histone core complexes and Simian virus 40 DNA. Eur. J. Biochem. 107:505-510.

9. Weintraub, S., and H. Weintraub. 1979. Isolation of a subclass of nuclear proteins responsible for conferring a DNase I-sensitive structure on globin chromatin. Proc. Natl. Acad. Sci. USA. 76:630-634.

10. Weintraub, S., and H. Weintraub. 1979. Conservative segregation of histones during replication in the presence of cycloheximide. Proc. Natl. Acad. Sci. USA. 76:328-332.

11. Compere, S. J., and R. D. Palmiter. 1981. DNA methylation controls the inducibility of the mouse metallothionein-I gene in lymphoid cells. Cell. 25:233-240.

12. Weintraub, H., A. Laren, and M. Groudine. 1981. a-Globin-gene switching during the development of chicken embryos: expression and chromosomal structure. Cell. 24:333-344.

13. Kato, K. J., M. Colavito-Shapanski, and M. A. Gorovsky. 1982. Histone acetylation and the deoxyribonuclease I sensitivity of the Tetrahymena ribosomal gene. Biochem. Soc. 21:1772-1781.

14. Boffa, L. C., G. Vidali, R. S. Mann, and V. G. Allfrey. 1978. Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. J. Biol. Chem. 253:3364-3366.

15. Vidali, G., L. C. Boffa, E. M. Bradbury, and V. G. Allfrey. 1978. Butyrate suppression of histone deacetylation leads to accumulation of multicycled forms of histones H3 and H4 and increased DNAse I sensitivity of the associated DNA sequences. Proc. Natl. Acad. Sci. USA. 75:2239-2243.

16. Schütz, H. A., L. H. Hughes, and S. S. Thorgrimsson. 1981. Differential effects of dimethyl sulfoxide and sodium butyrate on o- fetoprotein, albumin, and transferrin production by rat hepatoma cells in culture. In Vitro. 17:275-283.

17. Harris, M. 1982. Function of thymidine kinase in enzyme-deficient Chinese hamster cells. Cell. 29:483-492.

18. Leder, A., and P. Leder. 1975. Butyric acid, a potent inductor of erythroid differentiation in cultured erythroleukemic cells. Cell 12:119-122.

19. Prasad, K. N., and P. K. Sinha. 1976. Effect of sodium butyrate on mammalian cells in culture: a review. In Vitro. 12:125-132.

20. Jetten, A. M., M. E. R. Jetten, and M. I. Sherman. 1979. Stimulation of differentiation of several murine embryonal carcinoma cell lines by retinoic acid. Exp. Cell Res. 124:381-391.

21. Speers, W. C., C. R. Reddell, and J. F. Dixon. 1979. Chemically induced bidirectional differentiation of embryonal carcinoma cells in vitro. Am. J. Pathol. 97:563-577.

22. Jakob, H., P. Dubois, H. Eisen, and F. Jacob. 1978. Effects of the hexamethylenebisacetamide on the differentiation of cells of embryonic embryoam. C. R. Acad. Sci. Ser. D 288:109-111.

23. Strickland, S., and V. Mahdavi. 1978. The induction of differentiation in teratocarcinoma cells by retinoic acid. Cell. 15:393-403.

24. Jetten, A. M., and M. E. R. Jetten. 1979. Possible role of retinoic acid binding protein in retinoid stimulation of embryonal carcinoma cell differentiation. Nature (Lond.). 278:180-182.

25. Schindler, J. K. I. Matthaue, and M. I. Sherman. 1981. Isolation and characterization of mouse mutant embryonal carcinoma cells which fail to differentiate in response to retinoic acid. Proc. Natl. Acad. Sci. USA. 78:1077-1080.

26. McCue, P. A., K. I. Matthaue, M. Taketo, and M. I. Sherman. 1983. Differentiation-defective mutants of mouse embryonal carcinoma cells: response to hexamethylenebisacetamide and retinoic acid. Dev. Biol. 96:416-426.

27. Kruh, J. 1982. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. Mol. Cell. Biochem. 42:85-82.

28. Schier, D., and B. D. Knowles. 1978. Monoclonal antibody defining a stage-specific mouse embryonic antigen (SEEA-1). Proc. Natl. Acad. Sci. USA. 75:5565-5569.

29. Beers, W. H., S. Strickland, and E. Reich. 1975. Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. Cell. 6:387-394.

30. Vidali, G., L. C. Boffa, and V. G. Allfrey. 1977. Selective release of chromosomal proteins during limited DNase I digestion of avian erythrocyte chromatin. Cell. 12:409-415.

31. Candido, E. P. M., R. Reeves, and J. R. Davies. 1978. Sodium butyrate inhibits histone deacetylation in cultured cells. Cell. 14:103-113.

32. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

33. Kuff, E. L., and J. W. Fewell. 1980. Induction of neural-like cells and acetylcholinesterase activity in cultured teratocarcinoma cells. Cell. 21:347-355.