Am J Hum Genet 36:1225–1238, 1984

K562 Human Erythroleukemia Cell Variants Resistant to Growth Inhibition by Butyrate Have Deficient Histone Acetylation

BETSY M. OHLSSON-WILHELM,[1] BARBARA A. FARLEY,[2]
BARBARA KOSCIOLEK,[2] SANDRA LA BELLA,[2] AND PETER T. ROWLEY[2]

SUMMARY

K562 is an established human erythroleukemia cell line, inducible for hemoglobin synthesis by a variety of compounds including *n*-butyrate. To elucidate the role of butyrate–induced histone acetylation in the regulation of gene expression in K562 cells, we isolated 20 variants resistant to the growth inhibitory effect of butyrate. Four variants having different degrees of resistance were selected for detailed study. All four were found to be resistant to the hemoglobin-inducing effect of butyrate, suggesting that the two aspects of butyrate response, restriction of growth and induction of hemoglobin synthesis, are coupled. Further, after (5 days) culture with butyrate, two of the four variants exhibit less acetylation of H3 and H4 histones than does the butyrate-treated parent. Analysis of histone deacetylases from the variants indicated that each variant was distinct and that butyrate resistance may be accounted for by decreased affinity of the variant enzymes for butyrate, increased affinity of the enzymes for acetylated histone, or both. The fact that variants selected for resistance to growth inhibition by butyrate are also deficient in butyrate-induced hemoglobin synthesis and have abnormal histone deacetylase activity argues for butyrate inducing K562 cells to synthesize hemoglobin and restrict growth via histone acetylation.

INTRODUCTION

Among inducers of differentiation, *n*-butyrate is of special interest. The discovery that butyrate induces Friend murine erythroleukemia cells [1] led to the recognition

Received March 6, 1984; revised June 8, 1984.
This study was supported by grants CH-204 and CH-250 from the American Cancer Society.
[1] Department of Microbiology and Division of Genetics, University of Rochester School of Medicine, Rochester, NY 14642.
[2] Department of Medicine and Division of Genetics, University of Rochester School of Medicine.
 0002-9297/84/3606-0007S02.00

of the association of increased acetylation of histones, especially of H4, with activation of gene expression [2]. Butyrate causes increased histone acetylation by inhibiting histone deacetylase [3–5]. Such inhibition correlates with increased gene expression since Friend cells treated with sodium butyrate express many species of protein not detected in control cells [6].

The suggestion that acetylation of histones may favor RNA transcription was made nearly 20 years ago [7]. More recently, it has been found that genes associated with acetylated histones are more readily digested by DNAse I [8–10]. The extensive correlation between DNAse I sensitivity and the transcriptional competence of genes [11] makes attractive further analysis of the relationship between acetylation of histones and gene expression.

Butyrate also inhibits cell proliferation [12]. In Friend cells, butyrate arrests the cell cycle in G1 [13]. It also inhibits DNA synthesis [14]. Like histone acetylation, the effect on cell proliferation is reversible upon butyrate removal. The biological effects of butyrate have been reviewed [15, 16].

Because mammalian erythroid cells lose their nuclei during differentiation, the effects of butyrate on erythroid differentiation have been studied primarily in avian species. Acetylation and phosphorylation of histones have been extensively studied [17]. Histone deacetylase activity was found to be higher in the mature erythrocyte than in immature erythrocytes from anemic ducks [18]. Histone acetylation also occurs in a variety of nonerythroid cells [16]. In early trout testis, acetylated histone H4 is differentially associated with template-active chromatin [19].

Another approach to unraveling the effects of histone hyperacetylation on erythroid differentiation is to use inducible erythroleukemia cell lines. Like the Friend cell line, the K562 cell line is an established erythroleukemia line inducible for hemoglobin synthesis. Butyrate [20–30] and 18 other inducers of Friend cells [31] have significant inducing activity for K562 cells. K562 is a human cell line, derived from a patient with chronic myelogenous leukemia in blast crisis [32], which synthesizes hemoglobins of exclusively embryonic and fetal types [33].

We have used K562 cells to answer the question: Is induction of hemoglobin synthesis in K562 by butyrate causally related to acetylation of histones? Butyrate restricts growth and induces acetylation of all core histones in K562 cells in a dose-dependent manner [34]. Here, we show that variants selected for resistance to butyrate-induced growth inhibition are resistant to butyrate-induced hemoglobin synthesis and manifest deficient butyrate-induced histone acetylation, most likely due to structural alterations of histone deacetylases in these variants.

MATERIALS AND METHODS

Chemicals, Serum, and Cells

[^{3}H]L-leucine (142 Ci/mmol) and the sodium salt of [^{14}C]butyric acid (56 mCi/mmol) were purchased from Amersham, Arlington Heights, Ill., and the sodium salt of [^{3}H]acetic acid (2.8 Ci/mmol) from New England Nuclear, Boston, Mass. Butyric acid and thymidine were obtained from Sigma, St. Louis, Mo. Hemin was purchased from Eastman Organic Chemicals, Rochester, N.Y., and prepared as described [35].

K562 cells, the gift of B. and C. Lozzio, University of Tennessee, Knoxville, Tenn., were cultured in RPMI 1640 (Gibco, Grand Island, N.Y.) containing 10% fetal bovine serum (Flow Laboratories, McLean, Va.) in flasks at 37°C in a humidified 5% CO_2 atmosphere. Cell concentration was maintained between 5 and 80 $\times$ 10^4 cells/ml. A clone, LA4, with induction properties similar to previously described K562 cells [31] served as the parental line in these studies. Benzidine staining was performed as described [31]. The interpretation of increased benzidine staining as representing increased hemoglobin concentration was confirmed by absorption spectroscopy on lysates of butyrate-induced cells.

Mutagenesis

The mutagen ICR 191 was the gift of Dr. H. J. Creech, Institute for Cancer Research, Fox Chase, Pa. Log-phase cells (1×10^5 cells/ml, 20 ml) were incubated for 1 hr in complete medium at 37°C with ICR 191 (7.5 μg/ml), which gave 10% viable cells as determined by a growth curve. Following exposure to the mutagen, cells were washed twice, diluted to 20×10^4 cells/ml, and cultured without inducer. At the end of 1 week, butyrate was added at 0.6 mM. Two weeks later, cells were plated in 0.8% methylcellulose containing 0.6 mM butyrate in the same medium at 1,500 cells/ml. The plating efficiency was about 20%. Twenty-four colonies, each containing 128–256 cells, were transferred to fresh medium containing 0.6 mM butyrate in 96-well plates, and 20 clones survived. Variants ("butyrate-resistant" or BR variants) were maintained on medium containing 0.6 mM butyrate. The term "variant" rather than "mutant" is used because all the formal criteria for mutant status have not been fulfilled [36].

Histone Analysis

Histones were prepared from 5-day-old cultures and analyzed by acid urea polyacrylamide gel electrophoresis (PAGE) [37]. Butyrate (5 mM) was present during isolation [38]. Slab gels (1.5 mm) were run at 170 V for 20–30 hrs at 4°C [3]. Gels stained with 0.25% Coomassie Blue R [39] were scanned using a Kipp and Zonen model BD40 recording densitometer.

Histone Deacetylase

Histone deacetylase was measured by the method of Hay and Candido [40]. Labeled histones were prepared by resuspending 8×10^8 log-phase K562 cells in 20 ml of complete medium containing 10 mM sodium butyrate and shaking at 37°C for 15 min. [^{3}H]acetic acid (2.8 Ci/mmol) was added to 150 μCi/ml and incubated with shaking at 37°C for 2 hrs. Cells were centrifuged, resuspended in 200 ml of fresh complete medium containing 10 mM sodium butyrate and 8 mM sodium acetate, incubated 4 hrs at 37°C, centrifuged, and stored at −80°C. Total histones were purified as described above and lyophilized. Prior to use, they were dissolved in 15 parts 5 mM acetic acid:85 parts TMN (10 mM Tris-Cl, pH 7.8, 3 mM $MgCl_2$, 20 mM NaCl).

K562 cells to be assayed (1×10^7) were washed in PBS, resuspended in TMN, homogenized by 20 strokes of a Dounce homogenizer at 4°C, centrifuged at 300 *g* at 4°C for 10 min, and resuspended in TMN at 4×10^7 nuclei/ml. Each assay contained 30 μl of nuclei and 65 μl [^{3}H]histones (a 14-fold excess of substrate over enzyme in cell-equivalents) or ⅓, ⅕, ⅐, or ⅑ this amount of histones, in TMN in a total volume of 100 μl with 0, 0.2, or 0.5 mM butyrate. Reactions were terminated by addition of 10 μl concentrated HCl and boiling. Released [^{3}H]acetate was measured by extraction into ethyl acetate as described [40].

Pilot studies with parental nuclei revealed activity for over 16 hrs. For comparison of the variants with the parent, only the linear portion, the first hour, constituting only 20% of the complete reaction, was utilized.

Two-Dimensional Protein Electrophoresis

Proteins of lysates from parental K562 cells, uninduced or induced with 0.6 mM butyrate for 5 days, were analyzed by two-dimensional PAGE. Separation in the first dimension was produced by Triton X-100 acid urea [41]. Separation in the second dimension used SDS [42].

Globin Synthesis

Globin synthesis was measured by [^{3}H]leucine incorporation, PAGE in Triton X-100, and fluorography, as described [35].

RESULTS

BR Variants Are Not Inducible by Butyrate

Butyrate both induces hemoglobin synthesis and restricts cell growth of the parental K562 cells. The percentage of benzidine-positive cells in butyrate-treated cultures peaks from days 4–6 (fig. 1*A*). It returns to baseline about day 10 despite the continued presence of butyrate in the culture, probably reflecting a combination of growth and lysis of cells induced to differentiate and continued growth of uninduced cells. The higher the butyrate concentration, the higher the maximum percentage of benzidine-positive cells.

The effect of butyrate on parental cell growth is shown in figure 1*B*. The higher the concentration of butyrate, the greater the reduction in growth rate. Butyrate at 1.2 mM permits about four doublings and at 1.8 mM permits about two doublings. For subsequent experiments involving a single concentration, 0.6 mM was chosen as it appeared to be high enough to give good induction but low enough to avoid undesirable toxic side effects apparent at higher concentrations.

Twenty clones resistant to growth inhibition by butyrate were isolated from mutagen-treated cultures containing 0.6 mM butyrate. Four variants (BR 4, 5, 10, and 12) were selected for further study on the basis of differing degrees of resistance to growth inhibition by butyrate. At the standard butyrate concentration, 0.6 mM, the rise in cell number was more rapid for the variants than for the parent (fig. 2*A*). Further, these variants manifested a lower percentage of benzidine-positive cells at 6 days than did the parental cells, which became 28% benzidine positive (fig. 2*B*).

The decreased response to butyrate was not accounted for by reduced permeability to butyrate. [^{14}C]butyrate was taken up rapidly by all four variant lines (data not shown). Taken together, the above findings suggest that the two aspects of butyrate response, restriction of growth and induction of benzidine positivity, are coupled.

No Karyotypic Differences Are Found

The four variants did not differ from the parent in Giemsa-banded karyotype.

Variants Show Altered Patterns of Histone Acetylation

To determine whether the lack of inducibility of the variants by butyrate was associated with an alteration in butyrate-induced histone acetylation, the acetylation patterns of histones from both parents and variant cells were analyzed using acid urea PAGE (fig. 3). In the untreated parent, for both H4 and H3, the nonacetylated

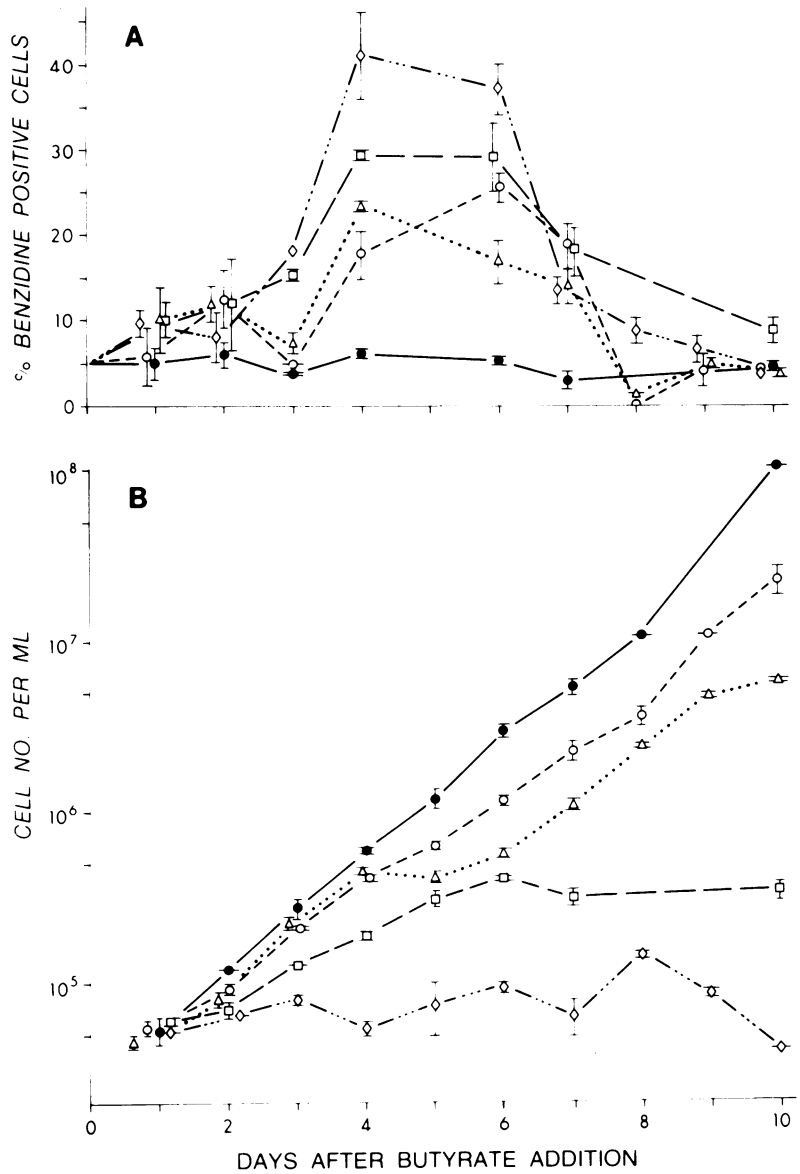

FIG. 1.—Response of parental line to butyrate. K562 cells were cultured at 2×10^4 cells/ml in 0.3, 0.6, 1.2, and 1.8 mM *n*-butyrate and without butyrate. *A*, Percentage of benzidine-positive cells. *B*, Cell concentration, corrected for dilution due to feeding. ●, no butyrate; ○, 0.3 mM; △, 0.6 mM; □, 1.2 mM; and ■, 1.8 mM.

forms of histones H3 and H4 were the most abundant; the monoacetylated, the second; the diacetylated, the third; and the triacetylated, the least. In the parent cultured with butyrate, however, the most abundant form of H3 and H4 was the monoacetylated form, and the proportion of the diacetylated form was increased.

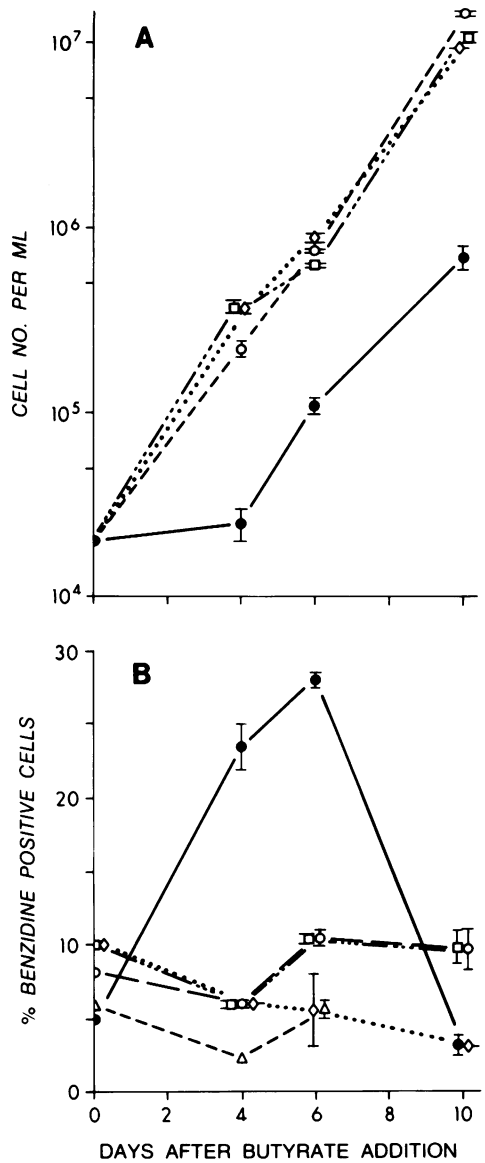

FIG. 2.—Response of variants to butyrate. Parental and variant K562 cells were cultured at 2 × 10^4 cells/ml with *n*-butyrate 0.6 mM. *A*, Cell concentration, corrected for dilution due to feeding. *B*, Percentage of benzidine-positive cells. ●, parent; ○, BR 4; □, BR 5; △, BR 10; ◇, BR 12.

The histone patterns of the variants cultured with butyrate are also shown in figure 3. The overall patterns of histone acetylation are similar to the parent (LA 4 and BA, fig. 3*B*) for BR 4 and BR 5. Surprisingly, this rather insensitive assay revealed differences between histone acetylation patterns of variants BR 10 and BR 12 and the parent, LA 4. Butyrate-treated BR 10 and BR 12 showed an H4

pattern more similar to the untreated parent than to the treated parent. The H3 patterns are intermediate between the uninduced and the induced parent. In summary, after culture with butyrate, two of the four variants showed less histone acetylation than did the parent.

In Friend erythroleukemia cells, butyrate induces the synthesis of a nonhistone chromosomal protein IP_{25} [3]. In Chinese hamster cell nuclei, butyrate also induces a protein called BEP [43], which may be analogous to IP_{25}. We did not detect induction of such proteins in butyrate-treated K562 cells. However, a substantial increase in the number of proteins synthesized in butyrate-treated vs. uninduced cells was observed by two-dimensional PAGE (data not shown), as has been observed in Friend cells [6].

Histone Deacetylase Activity Is Altered in BR Variants

To determine the cause of the decreased butyrate-induced histone acetylation in the variants, histone deacetylase activity was measured at various concentrations

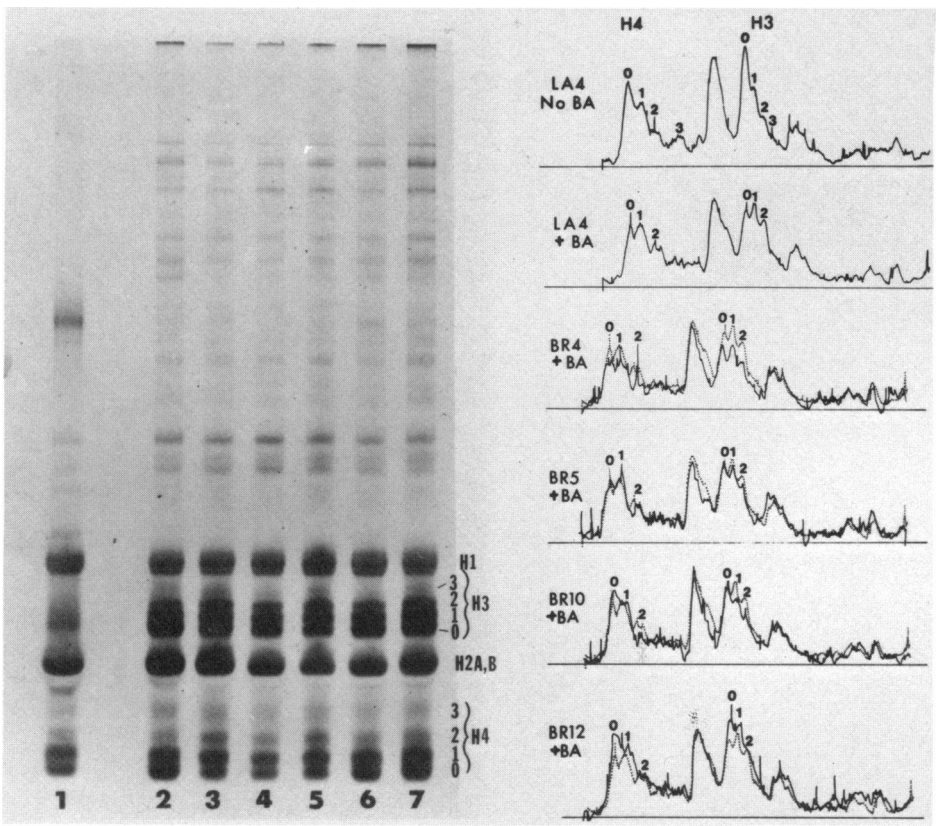

FIG. 3.—Electrophoretic analysis of histone acetylation. K562 cells were cultured with no inducer or with butyrate (0.6 mM) for 5 days. Histones were isolated and analyzed by electrophoresis in acid urea polyacrylamide gels. *A*, Photograph of gel stained with Coomassie Blue. *1*, Calf thymus. *2*, K562 parent cultured without butyrate. *3*, K562 parent with butyrate. *4*, BR 4 with butyrate. *5*, BR 5 with butyrate. *6*, BR 10 with butyrate. *7*, BR 12 with butyrate, *B*, Densitometric scans of gel.

of histone and of butyrate. As expected, the higher the butyrate concentration, the lower the reaction rate at a given substrate concentration. Lineweaver-Burk plots for the parent and four variants were all different. One such comparison, parent vs. BR 10, is shown in figure 4.

Enzyme activity, V_{max}, K_m, and *n*-butyrate K_i for the parent and for each of the four variants are shown in table 1. Each variant has a reduced V_{max} and a reduced K_m. BR 5 and BR 12 have a lower V_{max} than do the other variants. The K_i of the variants differ from that of the parent in both directions: reduced for BR 4 and BR 5 and increased for BR 10 and BR 12, the highest being BR 10.

Some BR Variants Are Hyperresponsive to the Inducer Thymidine

If histone deacetylase is involved in globin-gene activation in K562 cells, it is possible that the response to other inducers of hemoglobin synthesis will also be altered in BR variants. Thymidine is one of the more active inducers of benzidine positivity in K562 cells [44]. Response of the BR variants to culture in various concentrations of thymidine was analyzed and found to be altered in some cases. Benzidine positivity was assessed after 5 days in culture at various doses of thymidine (fig. 5*A*). The higher the dose, the higher the percent of benzidine-positive cells. BR 4 and BR 5 differed little from the parent. However, BR 10 and BR 12 were markedly hyperresponsive.

The effect of thymidine on growth of the variants expressed as a percentage of the cell number at that dose without thymidine is shown in figure 5*B*. BR 10 and BR 12 may show less thymidine-induced growth reduction than did the parent, whereas BR 4 and BR 5 show greater growth reduction than did the parent.

Globin Synthetic Pattern

We also investigated the globin synthetic pattern of the variants by incubation with [^{3}H]leucine and analysis of [^{3}H]globins by Triton X-urea PAGE. Figure 6

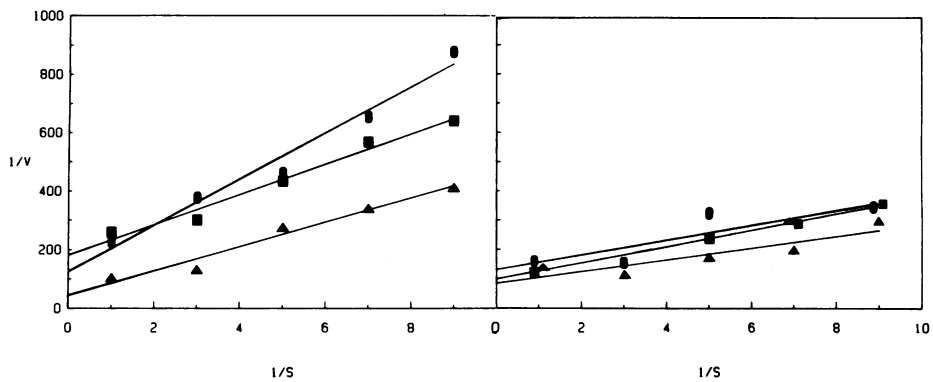

FIG. 4.—Histone deacetylase activity of variants. Histone deacetylase was measured at a variety of concentrations of histones and butyrate as described in MATERIALS AND METHODS. Lineweaver-Burk analyses are shown. All lines were fitted by linear regression. S = histone concentration in terms of dilution of histone preparation described. V = observed activity in terms of [^{3}H]acetate released/hr/ng protein. *Left*, parent. *Right*, BR 10.

TABLE 1

HISTONE DEACETYLASE IN BR VARIANTS

	Activity (cpm/hr/μg protein)	Apparent V_{max}* (cpm/hr/ng protein)	Apparent K_m*	Apparent K_i for *n*-butyrate†(M)
Parent	9.7	0.0220	0.910	8.0×10^{-4}
BR 4	9.6	0.0114	0.212	2.9×10^{-4}
BR 5	4.9	0.0068	0.238	2.8×10^{-4}
BR 10	7.3	0.0122	0.239	20.7×10^{-4}
BR 12	4.1	0.0066	0.287	13.9×10^{-4}

NOTE: See MATERIALS AND METHODS for details. Two replicate experiments gave similar results.

* K_m and V_{max} were calculated as ($-1/x$ intercept) and ($1/y$ intercept), respectively, from the data obtained of the type shown in figure 4 using linear regression. 1 K_m unit = a concentration of histones equal to the total histones derived from 1.7×10^8 cells in 1 ml.

† K_i for *n*-butyrate was calculated as $(1 + i/K_i)/V$, assuming noncompetitive inhibition.

shows analyses of cells induced with thymidine or hemin, a classical K562 inducer. Compared to the parent, BR 10 and, to a lesser extent, BR 12 showed increased induction of epsilon globin in response to thymidine.

DISCUSSION

Butyrate induces benzidine positivity and hemoglobin accumulation in K562 cells as it does in Friend cells. The response is transient, peaking at 4–6 days and disappearing by 10 days (fig. 1*A*), and is accompanied by growth restriction (fig. 1*B*). Both the percentage of benzidine-positive cells and the degree of growth restriction are dose-related.

Four variants, selected for resistance to growth inhibition by butyrate, are also resistant to the induction of benzidine positivity by butyrate (fig. 2). This finding suggests that in this inducible erythroleukemia cell line a common mechanism exists to effect both butyrate-induced hemoglobin synthesis and butyrate-induced growth restriction. We also have evidence based upon examination of colony sizes following induction [45] that growth restriction is coupled to induction of hemoglobin synthesis by a variety of inducers and is not merely the effect of drug toxicity. The butyrate concentration that we have chosen for these studies (0.6 mM) is lower than that used by many others (e.g., 5 mM) [34].

Since butyrate at high concentrations is known to inhibit the deacetylation of histones, we examined this process at two levels. First, we examined overall histone acetylation patterns; subsequently, we probed a specific enzyme: histone deacetylase. Two of the four variants selected for resistance to growth inhibition by butyrate have less butyrate-induced histone acetylation than does the parent (fig. 3). This deficient response is evident upon examination of both H3 and H4 histones. The degree of deficiency is admittedly not marked. This is to be expected for two reasons. First, since K562 is hyperdiploid (pseudotriploid?), one expects at least two and possibly three alleles of histone deacetylase. The effects of a single mutated allele are presumably muted by the presence of one or two normal alleles. Second, we have chosen to work at a low concentration of butyrate, sufficient to induce hemoglobin synthesis but without severely inhibiting growth

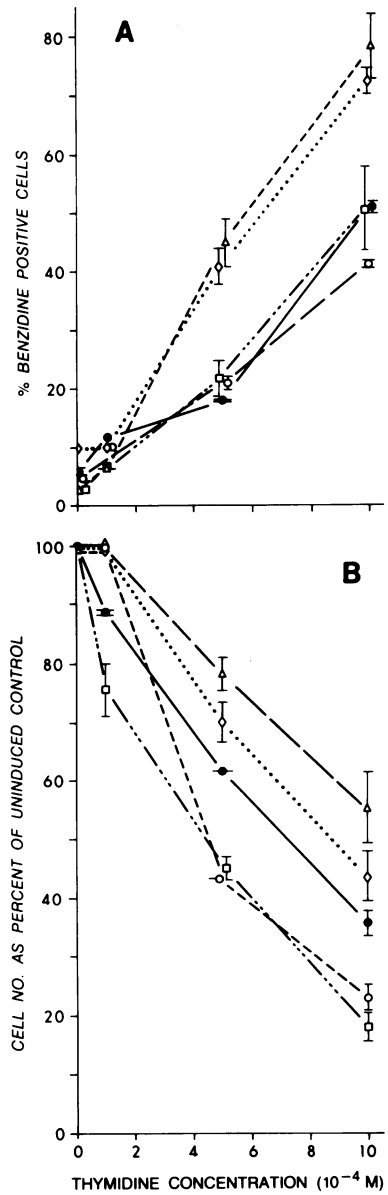

FIG. 5.—Response of variants to various concentrations of thymidine. Cells were cultured at 3×10^4 cells/ml with 1, 5, or 10×10^{-4} M thymidine or no thymidine for 5 days. *A*, Percentage of benzidine-positive cells as a function of thymidine concentration. *B*, Cell no. as a function of thymidine concentration. ●, parent; ○, BR 4; □, BR 5; △, BR 10; ◇, BR 12.

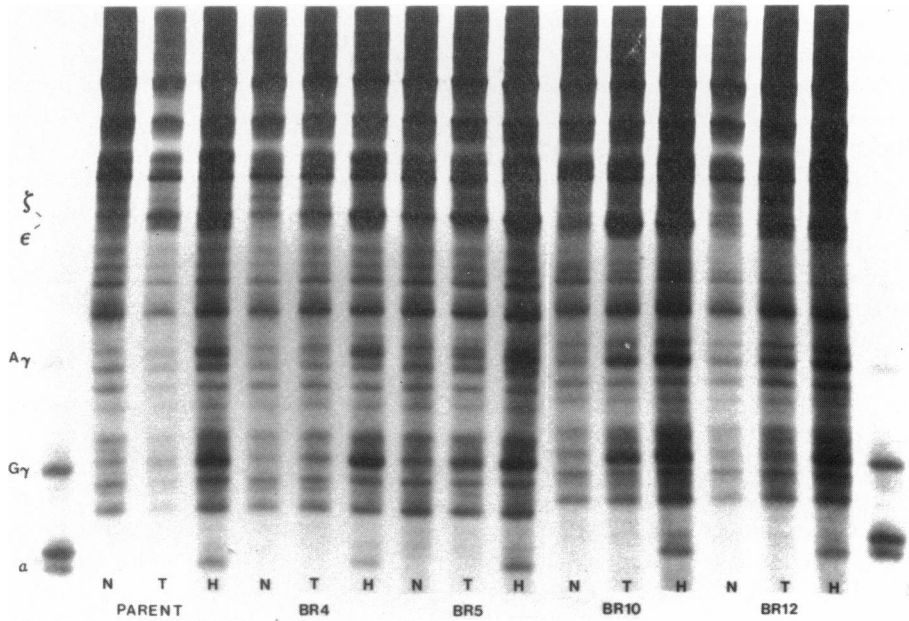

FIG. 6.—Globin-synthetic pattern of variants induced with thymidine or hemin. K562 cells were cultured at 2×10^4 cells/ml with no inducer, with 100 μM thymidine, or with 50 μM hemin. After 4 days, 10^6 cells were incubated at the concentration of 10^5 cells/ml with [^{3}H]leucine. After 16 hrs, cells were lysed and [^{3}H]globins analyzed by polyacrylamide gel electrophoresis in Triton X-100 followed by fluorography. *The first* and *last lanes* contain globin-chain markers. *N* = no inducer, *T* = thymidine, *H* = hemin.

of the entire population. Thus, we expect to affect histones near the globin genes preferentially. These affected histones may form only a small part of the histones that we are analyzing.

We also examined the histone deacetylase activity of the variants. Resistance to butyrate could be explained by an increased amount of normal deacetylase, a catalytically more efficient deacetylase, reduced butyrate inhibitability of the deacetylase, or a combination thereof. The data summarized in table 1 show that deacetylase activity is not increased in any of the variants. V_{max} is reduced, not increased, for each variant, especially for BR 5 and BR 12. However, K_m values are reduced to 23%–32% of the parental value, suggesting increased affinity of the enzyme for acetylated histones. The values for butyrate K_i are especially instructive. The two variants with the most deficient butyrate-induced histone acetylation, BR 10 and BR 12, are the two with the increased butyrate K_i. An increased K_i represents decreased affinity for butyrate, which can explain resistance to deacetylase inhibition by butyrate. The decreased K_i values for the other two variants are puzzling, but further document that the four variants differ.

Taken together, these kinetic parameters support the idea that the histone deactylases of the variants are physically different from that of the parent, presumably due to mutation at one of the histone deacetylase alleles. Proof of this would

require separation of the altered enzymes from the unaltered products of the unmutated allele(s).

Alteration of histone acetylation, in general, would be expected to influence the degree of expression of many genes. This expectation is confirmed by two-dimensional gel analysis of proteins from butyrate-treated parental cells. Many additional proteins are synthesized following butyrate treatment. Thus, it is clear that we are affecting histones on many genes. This response has been found also in Friend cells [6].

Properties other than that selected for were found, in fact, to be altered in the variants. Two variants, BR 10 and BR 12, were found to be hyperinducible by thymidine (fig. 5*A*). This was not the result of being more sensitive to the growth-slowing effect of thymidine; in fact, they were less sensitive (fig. 5*B*). Although the dose of mutagen was selected to make one mutation per cell likely, more than one mutation per cell cannot be excluded.

Finally, the pattern of globin synthesis was also altered (fig. 6). BR 10 and BR 12 manifest hyperinducibility by thymidine of epsilon-globin synthesis. Thus, the two variants most deficient in butyrate-induced histone acetylation and most resistant to butyrate inhibition of deacetylase activity are also those with a detectably abnormal globin-synthetic response to induction by thymidine.

The fact that variants selected for resistance to growth inhibition by butyrate are also found to be deficient in butyrate-induced hemoglobin synthesis and histone acetylation argues for a causal relationship among these three properties.

ACKNOWLEDGMENTS

We thank Dr. Ellis Bell for assistance with the enzyme kinetic analysis, Dr. Lawrence Wisniewski for karyotype analysis, and Drs. Patricia C. Hinkle and Shane C. Weber for helpful discussions and for reviewing the manuscript.

REFERENCES

1. LEDER A, LEDER P: Butyric acid, a potent inducer of erythroid differentiation in cultured erythroleukemic cells. *Cell* 5:319–322, 1975
2. RIGGS MG, WHITTAKER RG, NEUMANN JR, INGRAM VM: *n*-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. *Nature* 268:462–464, 1977
3. CANDIDO EPM, REEVES R, DAVIE JR: Sodium butyrate inhibits histone deacetylation in cultured cells. *Cell* 14:105–113, 1978
4. SEALY L, CHALKLEY R: The effect of sodium butyrate on histone modification. *Cell* 14:115–121, 1978
5. BOFFA LC, VIDALI G, MANN RS, ALLFREY VG: Suppression of histone deacetylation in vivo and in vitro by sodium butyrate. *J Biol Chem* 253:3364–3366, 1978
6. REEVES R, CSERJESI P: Sodium butyrate induces new gene expression in Friend erythroleukemic cells. *J Biol Chem* 254:4283–4290, 1979
7. ALLFREY VG, FAULKNER R, MIRSKY AE: Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci USA* 51:786–794, 1964
8. NELSON DA, PERRY M, SEALY L, CHALKLEY R: DNAse I preferentially digests chromatin containing hyperacetylated histones. *Biochem Biophys Res Commun* 82:1346–1353, 1978
9. SIMPSON RT: Structure of chromatin containing extensively acetylated H3 and H4. *Cell* 13:691–699, 1978

10. VIDALI G, BOFFA LC, BRADBURY EM, ALLFREY VG: Butyrate suppression of histone deacetylation leads to accumulation of multiacetylated forms of histones H3 and H4 and increased DNAse I sensitivity of the associated DNA sequences. *Proc Natl Acad Sci USA* 75:2239–2243, 1978
11. WEINTRAUB H, GROUDINE M: Chromosomal subunits in active genes have an altered conformation. *Science* 193:848–856, 1976
12. WRIGHT JA: Morphology and growth rate changes in Chinese hamster cells cultured in presence of sodium butyrate. *Exp Cell Res* 78:456–462, 1973
13. RASTL E, SWETLY P: Expression of poly(adenosine diphosphate-ribose) polymerase activity in erythroleukemic mouse cells during cell cycle and erythropoietic differentiation. *J Biol Chem* 253:4333–4340, 1978
14. HAGOPIAN HK, RIGGS MG, SWARTZ LA, INGRAM VM: Effect of *n*-butyrate on DNA synthesis in chick fibroblasts and HeLa cells. *Cell* 12:855–860, 1977
15. PRASAD KN: Butyric acid: a small fatty acid with diverse biological functions. *Life Sci* 27:1351–1358, 1980
16. KRUH J: Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol Cell Biochem* 42:65–82, 1982
17. RUIZ-CARRILLO A, WANGH LJ, ALLFREY VG: Processing of newly synthesized histone molecules. *Science* 190:117–128, 1975
18. SANDERS LA, SCHECHTER NM, MCCARTY KS: A comparative study of histone acetylation, histone deacetylation, and ribonucleic acid synthesis in avian reticulocytes and erythrocytes. *Biochemistry* 12:783–791, 1973
19. DAVIE JR, CANDIDO EPM: Acetylated histone H4 is preferentially associated with template-active chromatin. *Proc Natl Acad Sci USA* 75:3574–3577, 1978
20. ANDERSSON LC, JOKINEN M, GAHMBERG CG: Induction of erythroid differentiation in the human leukaemia cell line K562. *Nature* 278:364–365, 1979
21. HOFFMAN R, MURNANE MJ, BENZ EJ JR, ET AL.: Induction of erythropoietic colonies in a human chronic myelogenous leukemia cell line. *Blood* 54:1182–1187, 1979
22. KLEIN G, ZEUTHEN J, ERIKSSON I, ET AL.: Hybridization of a myeloid leukemia-derived human cell line (K562) with a human Burkitt's lymphoma line (P3HR-1). *J Natl Cancer Inst* 64:725–738, 1980
23. CIOE L, MCNAB A, HUBBELL HR, MEO P, CURTIS P, ROVERA G: Differential expression of the globin genes in human leukemia K562(S) cells induced to differentiate by hemin or butyric acid. *Cancer Res* 41:237–243, 1981
24. GUERRASIO A, VAINCHENKER W, BRETON-GORIUS J, ET AL.: Embryonic and fetal hemoglobin synthesis in K562 cell line. *Blood Cells* 7:165–176, 1981
25. PANTAZIS P, LAZAROU A, PAPADOPOULOS NM: Isoenzymes of lactate dehydrogenase in human leukemic cells in culture treated with inducers of differentiation. *J Cell Biol* 90:396–401, 1981
26. VAINCHENKER W, TESTA U, GUICHARD J, TITEUX M, BRETON-GORIUS J: Heterogeneity in the cellular commitment of a human leukemic cell line: K 562. *Blood Cells* 7:357–375, 1981
27. DOKHELAR MC, TESTA U, VAINCHENKER W, ET AL.: NK cell sensitivity of the leukemic K 562 cells; effect of sodium butyrate and hemin induction. *J Immunol* 128:211–216, 1982
28. TESTA U, THOMOPOULOS P, VINCI G, ET AL.: Transferrin binding to K562 cell line. Effect of heme and sodium butyrate induction. *Exp Cell Res* 140:251–260, 1982
29. TESTA U, HENRI A, BETTAIEB A, ET AL.: Regulation of i- and I-antigen expression in the K562 cell line. *Cancer Res* 42:4694–4700, 1982
30. GAMBARI R, RASCHELLA G, TRIPODI M, FARACE MG, FANTONI A: Desferrioxamine inhibits induced erythroid differentiation of human leukemic K-562 cells. *Cell Differ* 12:249–255, 1983
31. ROWLEY PT, OHLSSON-WILHELM BM, FARLEY BA, LABELLA S: Inducers of erythroid differentiation in K562 human leukemia cells. *Exp Hematol* 9:32–37, 1981

32. LOZZIO CB, LOZZIO BB: Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. *Blood* 45:321–334, 1975
33. RUTHERFORD TF, CLEGG JB, WEATHERALL DJ: K562 human leukaemic cells synthesize embryonic haemoglobin in response to hemin. *Nature* 280:164–165, 1979
34. PANTAZIS P, BONNER WM: Butyrate-induced histone hyperacetylation in human and mouse cells: estimation of putative sites of histone acetylation in vivo. *J Cell Biochem* 20:225–235, 1982
35. ROWLEY PT, OHLSSON-WILHELM BM, RUDOLPH NS, FARLEY BA, KOSCIOLEK B, LABELLA S: Hemin preferentially stimulates synthesis of alpha-globin in K562 human erythroid leukemia cells. *Blood* 59:1098–1102, 1982
36. THOMPSON LH, BECKER RM: Isolation of mutants of cultured mammalian cells. *Methods Cell Biol* 6:209–281, 1973
37. PANYIM S, CHALKLEY R: High resolution acrylamide gel electrophoresis of histones. *Arch Biochem Biophys* 130:337–346, 1969
38. DAVIE JR, SAUNDERS CA, WALSH JM, WEBER SC: Histone modifications in the yeast *S. cerevisiae*. *Nucleic Acids Res* 9:3205–3216, 1981
39. COUSENS LS, GALLWITZ D, ALBERTS BM: Different accessibilities in chromatin to histone acetylation. *J Biol Chem* 254:1716–1723, 1979
40. HAY CW, CANDIDO EPM: Histone deacetylase: association with a nuclease resistant, high molecular weight fraction of HeLa cell chromatin. *J Biol Chem* 258:3726–3734, 1983
41. ALTER BP, GOFF SC, EFREMOV GD, GRAVELY ME, HUISMAN THJ: Globin chain electrophoresis: a new approach to the determination of the $^{G}\gamma/^{A}\gamma$ ratio in fetal hemoglobin and to studies of globin synthesis. *Br J Haematol* 44:527–534, 1980
42. O'FARRELL PH: High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250:4007–4021, 1975
43. D'ANNA JA, TOBEY RA, GURLEY LR: Concentration-dependent effects of sodium butyrate in Chinese hamster cells: cell-cycle progression, inner-histone acetylation, histone H1 dephosphorylation, and induction of an H1-like protein. *Biochemistry* 19:2656–2671, 1980
44. HOLLIFIELD KL, LUMADUE JA, DIAMOND AP, MURNANE MJ, MAHONEY MJ: Hemoglobin induction in K562 human leukemic cells with hemin, protoporphyrin, and thymidine. *Am J Hum Genet* 33:146A, 1981
45. ROWLEY PT, OHLSSON-WILHELM BM, FARLEY BA: K562 human erythroleukemia cells demonstrate commitment. *Blood*. In press, 1984