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# TURNOVER OF HISTONE ACETYL GROUPS IN CULTURED CELLS IS INHIBITED BY SODIUM BUTYRATE

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## 1. Introduction

Sodium butyrate has been shown to cause hyperacetylation of histones in HeLa cells and in Friend erythroleukemic cells [1] when added in millimolar concentrations to the culture medium. Since histone acetylation may be important in the control of chromatin transcriptional activity [2] and/or chromatin assembly [3], it is of interest to determine the mode of action of sodium butyrate on this process. A variety of vertebrate cell lines respond to butyrate by accumulating acetylated histone species [4]; this fatty acid can inhibit histone de-acetylating enzymes in vitro [4]. We demonstrate here that turnover of histone acetyl groups in vivo is also inhibited by butyrate.

## 2. Materials and methods

### 2.1. *Cell culture*

Friend erythroleukemic cells, clone 745A [5] were maintained and passaged using the techniques in [6]. Butyric acid, where used, was neutralized with concentrated NaOH and added to cultures to final conc. 5 mM. Histones were labelled with [$^3$H]lysine as in [4]. For the experiments in fig.3, isotopes were added directly to normal complete growth medium. Trichloroacetic acid (5% cold) insoluble radioactivity was determined by the standard procedures in [4].

### 2.2. *Preparation of histones*

Histones were isolated from cells as in [4].

### 2.3. *Acrylamide gel electrophoresis*

Discontinuous slab gels containing sodium dodecylsulfate (SDS) were run as in [7], except that 0.6% *N,N'*-diallyltartardiamide was used as crosslinker instead of methylenebisacrylamide [8]. After electrophoresis for 6 h at 130 V, stained histone bands were solubilized by incubation in 2% periodic acid for 48 h, and counted in 20 ml of ACS counting scintillant (Amersham).

## 3. Results and discussion

To measure histone acetyl group turnover, control cells or butyrate-treated (5 mM, 24 h) cells were incubated for 2 h with [$^{14}$C]acetate and [$^3$H]lysine, transferred to isotope-free medium, and the incubation continued for various times. For the butyrate-treated cells, the fatty acid was present both during the labelling period and during the chase to prevent reversal of the hyperacetylation [1]. Histones were isolated from the chromatin of cells taken at various times during the chase, and analyzed by SDS–gel electrophoresis.

Figure 1 shows the gel pattern from such an experiment. It may be noted that a non-histone protein, $IP_{25}$, is present in chromatin from butyrate-treated cells, but not from control cells. This protein appears during induction of hemoglobin synthesis in Friend cells by dimethyl sulfoxide [9], and we also find it in these butyrate-induced cells, which also synthesize hemoglobin. Its presence may be correlated with nuclear condensation during induced differentiation in these cells (unpublished observations).

The histone content of the butyrate-induced cells

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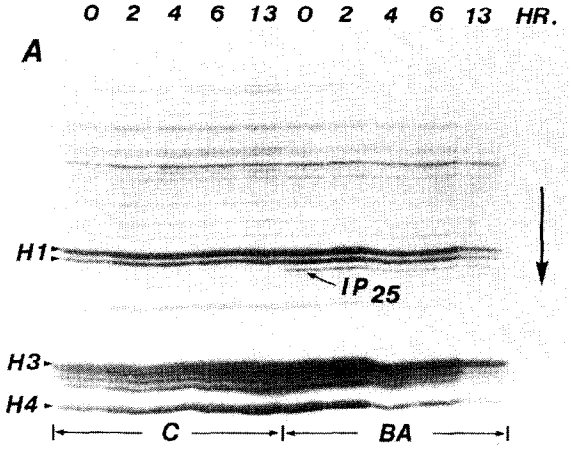


Fig.1. Pulse labelling of Friend Cells with [$^{14}$C]acetate and [$^{3}$H]lysine. Equal numbers of control (C) or butyrate-induced (BA) Friend cells were labelled for 2 h in medium containing [$^{14}$C]acetate (150 $\mu$Ci/ml) and [$^{3}$H]lysine (150 $\mu$Ci/ml) at 37°C. The cells were collected by centrifugation and resuspended in isotope-free medium containing unlabelled acetate (0.1 mM) and lysine (0.5 mM). The cells were then incubated at 37°C, and aliquots taken at 0, 2 h, 4 h, 6 h and 13 h. For the butyrate-induced cells, 5 mM butyrate was present during both the labelling and chase periods. Chromatin was prepared from each sample, and acid-soluble proteins were extracted and analyzed as in [4].

decreases with time, because of the cessation of DNA synthesis in the presence of this agent [10]. This is detailed below.

The gel bands corresponding to the H3, H4 and H2A + H2B regions in fig.1 were excised, solubilized and counted to determine the [$^{3}$H]lysine and [$^{14}$C]-acetate content. The results are shown in fig.2. It is evident that the amount of [$^{14}$C]acetate associated with histones H3 and H4 in untreated control erythroleukemic cells decreases continuously with time during the 'chase' period (relative to the amount of [$^{3}$H]lysine), indicating a rather rapid turnover of the acetyl groups on these histones under normal growth conditions. On the other hand, in cells treated for 24 h with butyrate (5 mM) before the pulse of radioactivity and maintained in the same concentration of the

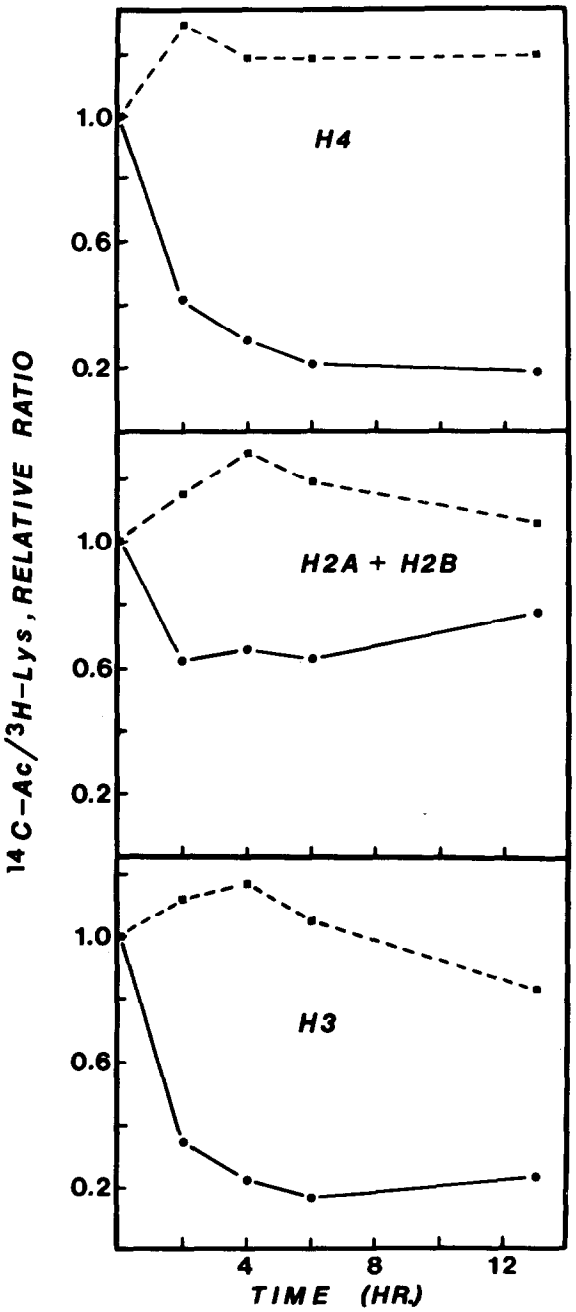


Fig.2. Histone acetyl group turnover in Friend erythroleukemic cells. The histone bands from the gel illustrated in fig.1 were excised, solubilized in 2% periodic acid, and counted in 10 ml of aqueous counting scintillant (ACS, Amersham), using a Nuclear Chicago Isocap counter. The relative [$^{14}$C]acetate/[$^{3}$H]lysine ratio is plotted versus time of chase, the ratio at time zero being set at 1.0. (▪——▪) Butyrate-treated cells; (•——•) control cells.

fatty acid during the chase period, the ratio of [$^{14}$C]-acetate/[$^{3}$H]lysine found in isolated H3 and H4 does not change appreciably during the chase. This indicates that in vivo, cells grown in the presence of butyrate do not deacetylate histones H3 and H4 to any great extent. However, it is known that if butyrate is removed from cell cultures the hyper-acetylation of the histones is entirely reversed [1].

A similar inhibition of deacetylation by butyrate is observed for histones H2A and H2B as shown in fig.2. Again, for these histone species in control cultures deacetylation occurs during the chase period, whereas in the in vivo butyrate treated cells there is little or no turnover of acetyl groups. The rate and extent of removal of acetyl groups is lower for H2A and H2B, because of the lower proportion of $\epsilon$-*N*-acetyl groups relative to $\alpha$-*N*-acetyl groups (of H2A) in this histone pair [2,11,12] since the $\alpha$-*N*-acetyl groups are known to be stable [13]. It is nevertheless clear, however, that in the butyrate-induced cells acetyl group turnover in H2A + H2B is also inhibited.

In control erythroleukemic cells, the half-life of acetyl groups is ~90 min for H4 and 70 min for H3. This is comparable to half-lives of 40 min for duck erythrocyte histone acetyl groups [14], and 20–40 min for those of HTC cells [12].

In order to verify that Friend erythroleukemic cells remained viable during exposure to 5 mM butyrate for the above periods, a sample of cells was incubated in 5 mM butyrate for 34 h, transferred to fresh medium lacking butyrate, and aliquots were labelled with either [$^{3}$H]thymidine, [$^{3}$H]amino acids, or [$^{3}$H]uridine for various times. The incorporation into trichloro-acetic acid-insoluble material was then measured. The results are shown in fig.3, compared to those obtained with control cells run in parallel.

The 12 h lag in the incorporation of all precursors in both butyrate-treated and control cells is due to a period of adaptation to the fresh medium. DNA synthesis is known to be inhibited by butyrate treatment, and the inhibition is reversible [10]. It is seen from fig.3 that 34 h butyrate-treated cells resume DNA synthesis when transferred to fresh medium lacking butyrate (panel C). They also synthesize RNA and protein, though at initial rates somewhat lower than do control cells. Thus they remain viable for at least 34 h in the presence of 5 mM butyrate. This is confirmed by the fact that 99% of both control and

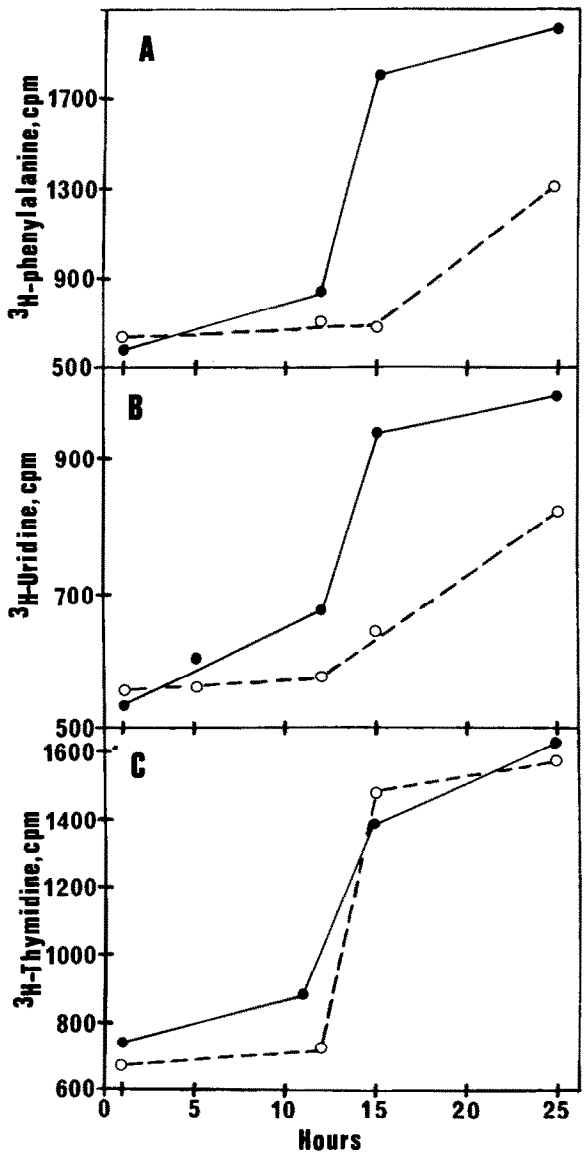


Fig.3. Recovery of Friend leukemia cells from butyrate treatment. Three equal-sized lots of cells were treated for 34 h with 5 mM butyrate and then harvested and resuspended in fresh medium (without butyrate). Starting at time zero of the recovery period, one lot of cells (shown in panel C) was labelled with [$^{3}$H]thymidine (1 $\mu$Ci/ml), a second lot (panel B) with [$^{3}$H]uridine (1 $\mu$Ci/ml), and the third lot (panel A) with [$^{3}$H]phenylalanine (1 $\mu$Ci/ml). At the times indicated in the panels, equal-sized aliquots of cells from each of the cultures were removed and trichloroacetic acid-insoluble cpm determined. Control cell cultures were treated identically except that no butyrate was added. (•——•) Control cells; (o– – – –o), butyrate-treated cells.

treated cells showed exclusion of Trypan blue (data not shown).

Our results thus show that treatment of Friend erythroleukemic cells with sodium butyrate suppresses histone deacetylation in vivo. Butyrate inhibits histone deacetylase activity in vitro [4], without affecting the rate of acetylation. Thus the accumulation of acetylated histones in butyrate-treated cells is due to suppressed turnover of acetyl groups, and suggests that histone deacetylases may be an important metabolic control point for histone acetylation.

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