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Sodium *n*-Butyrate Causes Reversible Decrease in Condensed Chromatin Clumps in HeLa Cells (40593)

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Recently, Riggs *et al.* (1) have reported that nuclei from sodium *n*-butyrate-treated HeLa and Friend erythroleukemia cells contain increased amount of hyperacetylated histone H4. This modification was reversible and the histones reverted to normal within 24 hr after removal of the *n*-butyrate. Subsequently, several laboratories have shown that the hyperacetylation is due to an inhibition of histone deacetylase and that this inhibition is reversible (2–5). Vidali and his associates have recently reported that the reversal is very rapid with most of the acetylated forms of H4 histone converted to the unmodified form within 15 min after removal of the *n*-butyrate (6). It has also been shown that the hyperacetylation can be produced by *n*-butyrate in a variety of vertebrate cell lines and that it may involve H3, H2A, and H2B as well as H4 (2).

In studies of the effect of *n*-butyrate on ectopic secretion of α-hCG in cultures of a human lung cancer cell line (ChaGo), we noted a decrease in the number of condensed chromatin clumps in the treated cells (7). Since it seemed possible that this morphological change was related to changes in acetylation of histones, we examined the chromatin of HeLa cells by light and electron microscopy after treatment with *n*-butyrate and following the removal of the *n*-butyrate.

Materials and methods. Electron microscopy. HeLa cells were grown in 75-cm^2 plastic flasks in Eagle's minimum essential medium with 9% horse serum. Replicates of control cells and cells treated with 5 m*M* sodium *n*-butyrate were incubated for 24 hr and then mechanically removed from the flasks and fixed and processed for light and electron microscopy as previously described (8). Some of the flasks treated for 24 hr with sodium *n*-butyrate were washed three times with phosphate-buffered saline and then incubated in medium free of *n*-butyrate for 15 min, 30 min, 1 hr, 2 hr and 24 hr before fixation. For electron microscopy, sections were double stained with lead citrate and uranyl acetate and examined in a Siemens 1A electron microscope.

Light microscopy. HeLa cells were treated as for electron microscopy and 1-μm sections of the plastic-embedded cell pellets of control cells, 24-hr *n*-butyrate-treated cells, and cells after 24-hr removal of *n*-butyrate were stained with Azure B and basic Fuchsin. Chromatin clumps were counted by light microscopy with an oil immersion objective (magnification 1000 ×). The 900 cells were counted in each group.

Results. Ultrastructurally, the untreated HeLa cell nuclei contained multiple condensed chromatin clumps which were 45 nm to 2.5 μm in greatest diameter (Fig. 1a). After 24-hr treatment with 5 m*M* *n*-butyrate, almost all of the nuclei had diffuse chromatin with only very rare condensed chromatin clumps (Fig. 1b). Cells were examined 15 min, 30 min, 1 hr, 2 hr and 24 hr after removal of the *n*-butyrate. After 15 min, most of the nuclei already contained many small condensed chromatin clumps although the chromatin in these clumps was not as densely aggregated as in untreated cells. After 30 min and 1 hr, the clumps were larger and more dense. In cultures examined 2 and 24 hr after removal of the *n*-butyrate, the nuclei regained the appearance of those in untreated cells (Fig. 1c). There was also a marked decrease in cytoplasmic glycogen in the treated cells with partial reversal 24 hr after removal of the *n*-butyrate.

Although some of the condensed chromatin clumps could not be visible at the resolution of the light microscope (300–400 nm), the larger clumps could be easily seen with an oil immersion objective at 1000 × magnification.

In order to facilitate the quantitation of the clumps, 1-μm sections were examined by light microscopy and the data are present in Table

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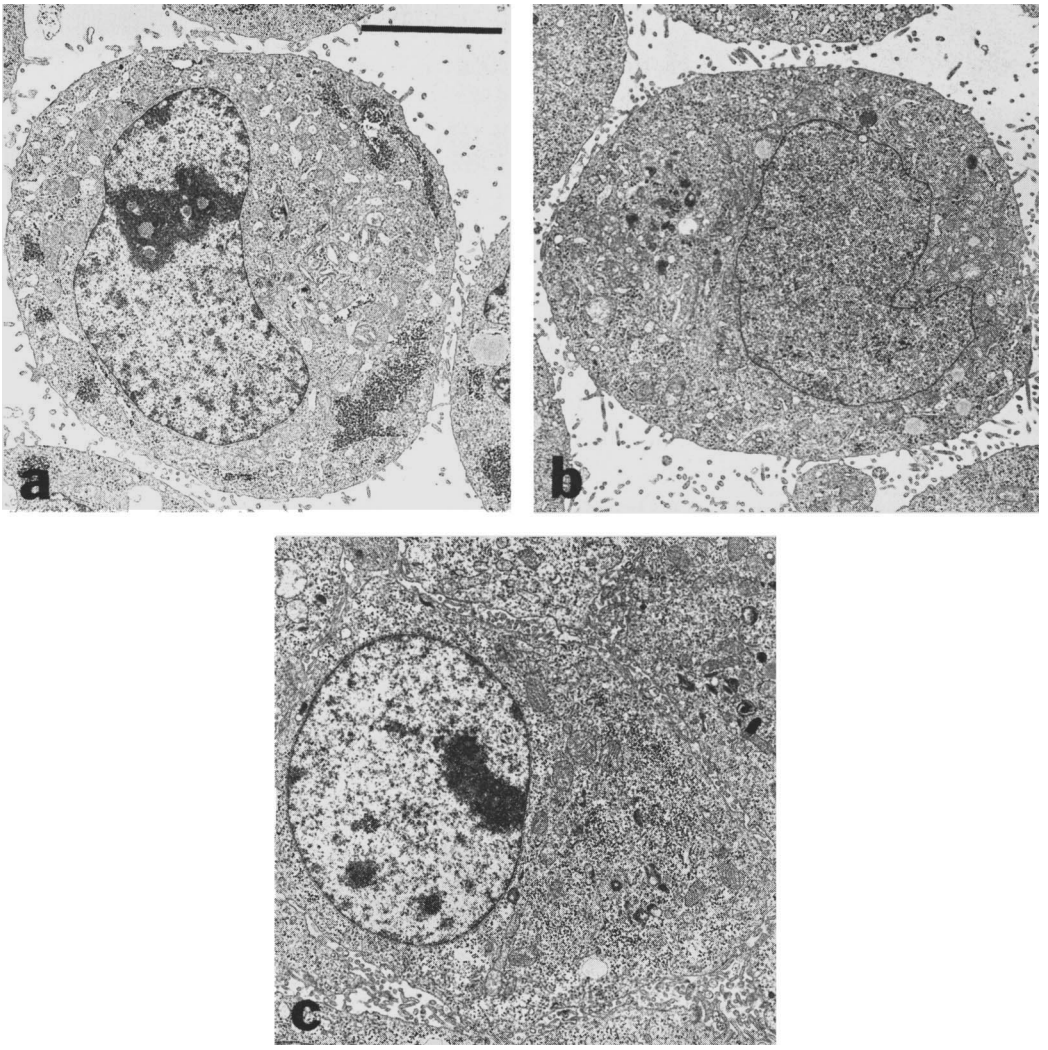

FIG. 1. Effect of sodium *n*-butyrate on HeLa cells: (× 3738) bar = 5 μm. (a) Control HeLa cell showing numerous condensed chromatin clumps in nucleus. (b) HeLa cell showing a marked decrease in condensed chromatin clumps after 24-hr sodium *n*-butyrate treatment. (c) Sodium *n*-butyrate treated HeLa cell after three washings and 24-hr incubation in media free of butyrate showing numerous condensed chromatin clumps.

TABLE I. EFFECT OF SODIUM *n*-BUTYRATE ON CONDENSED CHROMATIN CLUMPS IN HELA CELLS[a]

	Number of condensed chromatin clumps/cell (%)		
	0–4	5–10	>10
Untreated	0	7	93
n-Butyrate treated (24 hr)	42	51	7
n-Butyrate treated (24 hr) followed by 24 hr in butyrate-free media	0	2	98

[a] One-micron plastic sections were examined under oil-immersion objective (magnification 1000 ×. 900 cells were counted in each group.

I. Ninety-three percent of the control cells contained more than 10 clumps of condensed chromatin (>10 usually meaning too many to count). After *n*-butyrate treatment, only 7% of cells had more than 10 chromatin clumps. Twenty-four hours after removal of the *n*-butyrate, 98% of the nuclei again contained more than 10 clumps.

Discussion. Simpson has biochemically studied the structure of HeLa cell chromatin with extensively acetylated H3 and H4 following treatment with *n*-butyrate, and has found that it is more rapidly digested by

DNAase I (4). Vidali and his associates have reported similar findings and have presented evidence for a close association of acetylated histones with the DNAase-I-sensitive sequences (5). Simpson has suggested that modification of the amino terminal regions of H3 and H4 by hyperacetylation might weaken the interactions of adjacent core particles in chromatin and thus reduce the level of compaction of the nucleic acid. It is possible that the hyperacetylation of histones might also lead to the decrease in condensed chromatin clumps that we have observed after *n*-butyrate treatment. Since both the hyperacetylation of H4 and the disaggregation of condensed chromatin clumps are reversible within 15 min after removal of the *n*-butyrate, the association of the morphological changes with the hyperacetylation seems likely.

Summary. There is a marked decrease in condensed chromatin clumps in HeLa cells treated with sodium *n*-butyrate for 24 hr. This change is rapidly reversible, with chromatin clumps reappearing 15 min after removal of the *n*-butyrate. These reversible morphological changes are closely correlated with hyperacetylation of histones in *n*-butyrate-treated cells, and may represent a morphological expression of the biochemical modification of histones.

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