HISTONE ACETYLATION DURING AGING OF HUMAN CELLS IN CULTURE

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Received June 23,1972

SUMMARY:

An age-associated decrease in the rate of histone acetylation has been demonstrated in human diploid cells in culture. There was no change in the histone/DNA ratio with age. It is suggested that the reduction in histone acetylation occurring in old cell populations reflects an accumulation of cells in a stage of the cell cycle not compatible with histone acetylation.

Human diploid fibroblast-like cells in culture undergo a period of rapid proliferation which is followed by a decline in the rate of cell division and the eventual degeneration of the culture. Hayflick (1) has suggested that the basis for this phenomenon is intrinsic to the cell and may represent aging at the cellular level.

Accompanying this decline in proliferative capacity are several morphological and biochemical alterations (2), and such functional alterations could reflect changes occurring at the transcriptional level.

The interaction of basic and acidic proteins with DNA has been suggested as a possible mechanism for the control of gene expression (3,4,5). The chemical modification of these proteins could have a profound influence on this interaction, and indeed modifications such as acetylation, methylation and phosphorylation of histones have been implicated in the control of gene expression (6,7).

In this report we present a survey of the overall apparent net rates of acetylation, methylation and phosphorylation of histones during aging and a more detailed study of the characteristics of histone acetylation.

MATERIALS AND METHODS: The human diploid cell line WI-38 (1) was cultured as previously described (8). Periodically cultures were checked for the presence of mycoplasma by Dr. L. Hayflick of Stanford University. Cells were inoculated into roller bottles at a concentration of 1.2×10^4 cells/cm^2. To survey the rates of acetylation, methylation and phosphorylation fresh medium containing 2-^{14}C-sodium acetate (sp. act. 59 mCi/mM), methionine-methyl ^{3}H (sp. act. 10 μCi/μM) and $H_3{}^{32}PO_4$ (sp. act. 1 μCi/μM) was added to each culture in the logarithmic phase of growth to a final concentration of 0.8 μCi/ml each. Cells were then incubated for an additional 48 hours prior to the isolation of nucleohistone. Detailed studies on the rate of histone acetylation were carried out using ^{3}H-sodium acetate at a specific activity of 8.5 μCi/μM sodium acetate except where noted.

Nuclei were prepared according to the method described by Berkowitz (9), and nucleohistone was isolated essentially according to the method of Shepherd (10). The precipitated non-histone fraction was suspended in 0.5 N H_2SO_4, placed in a boiling water bath for 15 min. and then centrifuged at 10,000 x g for 15 min. This supernatant, containing the hydrolyzed DNA, was saved for determination of DNA content.

Radioactivity in the histone fraction was determined using an Intertechnique SL-30 scintillation counter and Aquasol scintillator (New England Nuclear). All counting data were corrected for channel overlap. The incorporation of the ^{14}C label in the acetyl form was confirmed by hydrolysis of the sample in 6 N HCl and subsequent removal of the label by volatilization at 118°C for 16-20 hours.

Protein content of isolated histone samples was determined by the method of Lowry (11) using bovine serum albumin as a standard. DNA content was determined as described by Burton (12) using calf thymus DNA as a standard.

RESULTS AND DISCUSSION: Initial experiments were conducted to survey possible age-associated changes in the net incorporation of 2-^{14}C-acetate, methyl-^{3}H and $^{32}PO_4$ into histone isolated from cells in the 17th to 52nd population

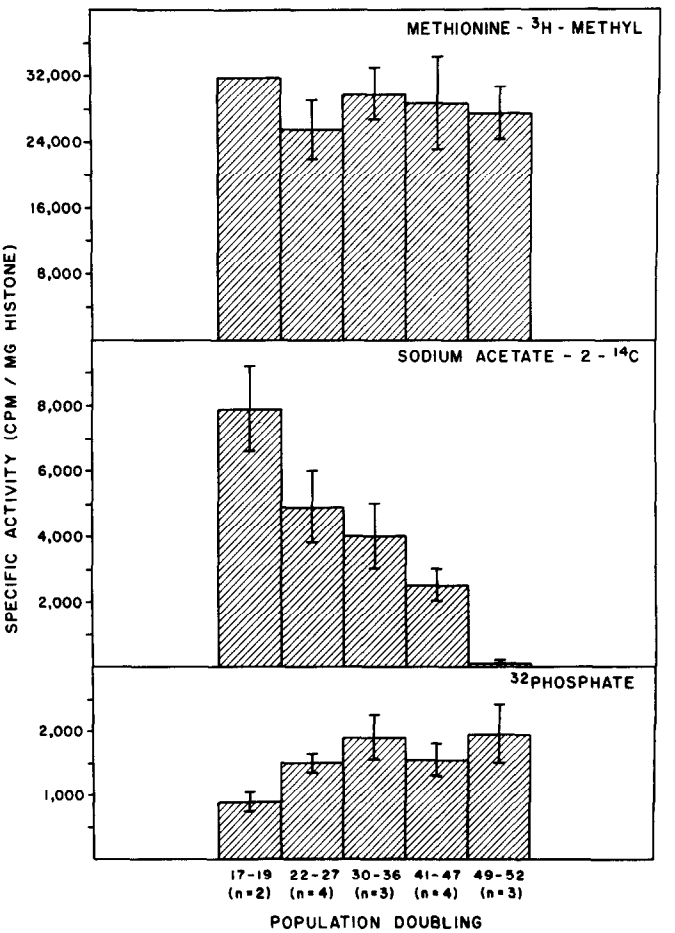

FIGURE 1: Incorporation of 2-^{14}C acetate, ^{3}H-methyl and ^{32}P into histone as a function of age in WI-38 cells. Experimental conditions and isolation of histone are described in Materials and Methods. Bars represent the mean; brackets are ± SE of the mean for each group; N represents the number of determinations.

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doubling (fig. 1). Only the incorporation of acetate into histone changed significantly; there was a gradual decline in acetylation rate which fell essentially to "0" at the end of the life span.

To rule out possible age-associated changes in histone content the histone/DNA ratio was evaluated and was found to remain constant throughout the life span (fig. 2).

Possible age-associated changes in the rate of histone methylation and

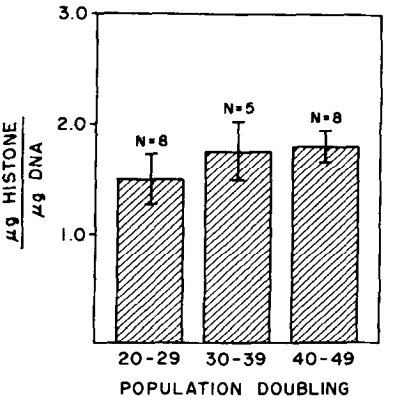

FIGURE 2: The histone/DNA ratio of WI-38 cells as a function of age. Histone and DNA were isolated as described in Materials and Methods.

phosphorylation can not be eliminated by the above experiments since optimum conditions for these reactions were not determined. In addition, the direct incorporation of ^{3}H-methionine into histone could mask any detectable change in histone methylation. Because of the significant decline in histone acetylation however, our further experiments were directed toward substantiating and characterizing this process.

When WI-38 cells were grown in the presence of 15-240 μM ^{3}H-sodium acetate at a constant specific activity of 8.5 μCi/μM, the incorporation of acetate into histone was concentration-dependent between 15 and 120 μM (fig. 3). For both young and old cells the maximum rate of incorporation was attained at approximately 120 μM sodium acetate, a concentration well above that used in preliminary experiments. However, the difference in acetate incorporation between young and old cells was evident over the entire concentration range.

Studies on the time course of incorporation of ^{3}H-acetate (240 μM; sp. act. 8.5 μCi/μM) into histone showed that, for old cell populations, incorporation increased for the first 24 hours and then became relatively constant. For young cell populations, however, incorporation continued to increase throughout the 48 hour period (fig. 4). Differences in the rate of incorporation were evident throughout this period.

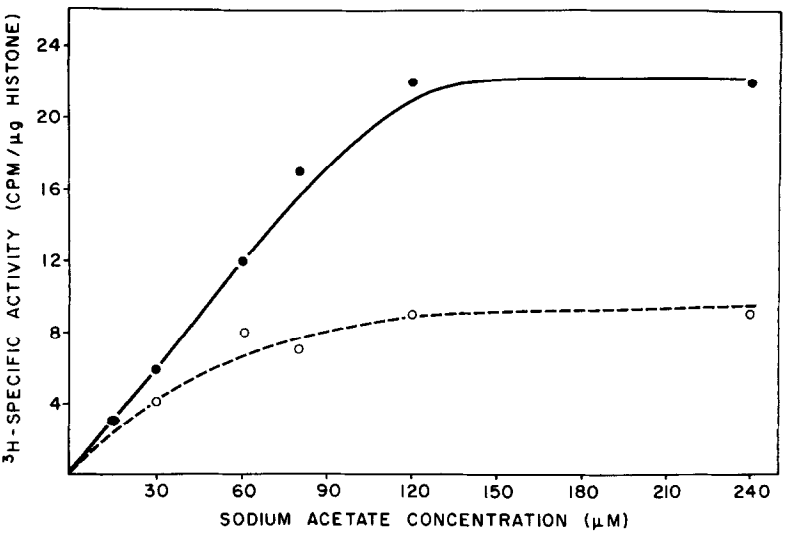

FIGURE 3: Incorporation of ^{3}H-sodium acetate into histone (sp. act. 8.5 µCi/µM) as a function of sodium acetate concentration. Cells were incubated in the presence of the isotope for 48 hours and histone was isolated as described in Materials and Methods. ●—● 25th population doubling; o---o 45th population doubling.

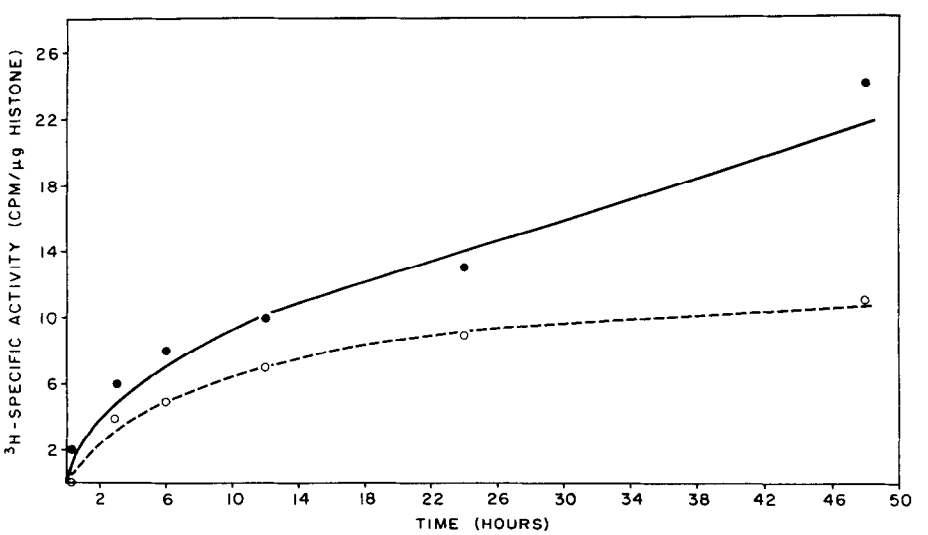

FIGURE 4: Incorporation of ^{3}H-sodium acetate (240 µM; sp. act. 8.5 µCi/µM) into histone as a function of time. Histone was isolated as described in Materials and Methods. ●—● 20th population doubling; o---o 47th population doubling.

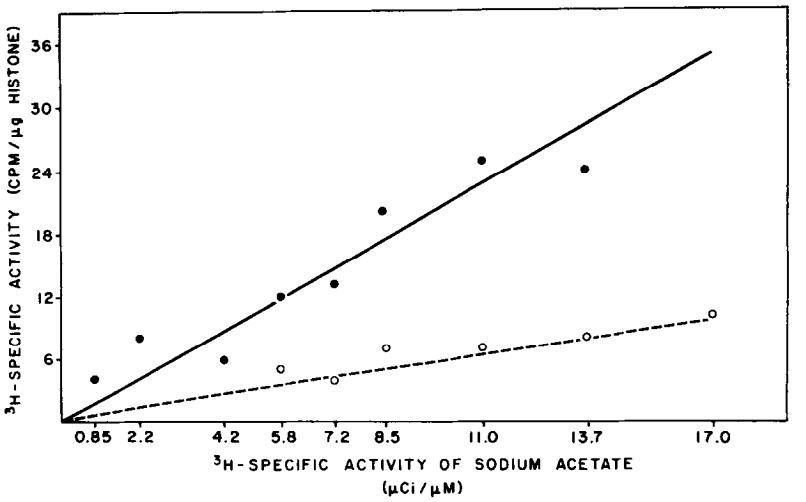

FIGURE 5: Incorporation of ^{3}H-acetate into histone as a function of specific activity. Sodium acetate concentration was 240 µM. Histone was isolated as described in Materials and Methods. ●—● 22nd population doubling; o---o 45th population doubling.

Figure 5 shows that when media containing a constant concentration of ^{3}H-sodium acetate (240 µM) but varying specific activities (0.85-17.0 µCi/µM) were added to replicate cultures the specific activity of histone increased linearly with increasing specific activity of precursor for both young and old cell populations. The rate of ^{3}H-acetate incorporation into histone, however, was considerably slower in old cell populations than that of young cell populations. Thus, possible age-associated changes in acetate pool size and/or acetate metabolism cannot be ruled out.

Table 1 summarizes the results of experiments designed to compare the rate of histone acetylation under optimal conditions of acetate concentration (240 µM) and exposure time (48 hours) in young and old cultures. These data confirmed our earlier findings. Although the age-associated decrease in histone acetylation evident here was not as dramatic as noted previously (fig. 1) there was still a 2-3 fold higher rate of acetylation in younger cells.

The alterations in the apparent net rate of histone acetylation seem firmly documented by these experiments. To what extent possible differences

Table 1

Incorporation of ^{3}H-acetate into histone of young and old cell populations grown in the presence of 240 μM sodium acetate for 48 hours. Specific activity was 8.5 μCi/μM sodium acetate. Histone was isolated as described in Materials and Methods.

INCORPORATION OF ^{3}H-ACETATE INTO HISTONE

OF YOUNG AND OLD CELL POPULATIONS

Population Doubling	N*	^{3}H-Acetate Incorporated into Histone (cpm/μg histone) Mean ± SEM**
19-25	12	21 ± 1.64
45	8	8 ± 1.1

$p < .001$

*N = number of determinations

**SEM = standard error of the mean

in the acetate pool size of young and old populations could influence the results of the above study cannot be assessed at present.

Alterations in the rate of histone acetylation have been observed in other cell systems and have been correlated with changes in gene activation (13) and with the rate of cellular division (14). The occurrence of histone acetylation concurrent with DNA synthesis in many cell types (15) suggests that the reduction observed in old cell populations could reflect an accumulation of cells at a stage in the cell cycle not compatible with histone acetylation.

An age-associated increased fraction of the population delayed or retarded in the G1 phase of the cell cycle has been reported both by Macieira-Coelho *et al.* (16) and by the results of work from our own laboratory (8,17). This accumulation of cells in G1 could be responsible for the gradual decline in histone acetylation observed in this study. Alternatively, the loss of

capacity for histone acetylation could be causally related to the accumulation of cells at a stage prior to DNA synthesis. Additional work is now in progress to determine the nature of the relationship of histone acetylation and aging of WI-38 cells.

Acknowledgements

This work was supported by research grants HD-02721, HD-06323 and GM-00142 from the National Institutes of Health.

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