**N-Acetylation of Arginine-rich Hepatoma Histones**

Paul Byvoet and Harold P. Morris

**SUMMARY**

Previous work in this laboratory has shown that decay rates of histones and DNA isolated from deoxyribonucleoprotein of normal and neoplastic rat tissues were approximately equal, indicating that not only DNA but the nucleohistone complex as a whole is metabolically inert. In contrast, the N-acetyl groups in the combined arginine and slightly lysine-rich histones from these same tissues were found to turn over at much faster rates than whole histones or DNA, with exception of the Novikoff hepatoma, in which this turnover was remarkably slow, approaching that of the histones. Studies were therefore carried out on a number of hepatomas with varying growth rates, which indicated that the half-lives of their histone N-acetyl groups were in general an order of magnitude smaller than that of the whole histones and similar to that of most tissues studied thus far. The data derived from very-slow-growing tumors would seem to indicate that the histone N-acetyl groups from hepatocyte histones exhibit a rapid turnover similar to that in other tissues.

**INTRODUCTION**

It has been suggested that chemical modifications of histones introduce structural changes in the otherwise inert deoxyribonucleoprotein complex which may influence the flow of information from the DNA. One of these reactions concerns the acetylation of amino groups present in the histone molecule. Evidence indicating the presence of 1 acetyl group or less per molecule of histone and the finding that the NH2-terminal amino groups of a number of more or less crude histone fractions were masked to varying degrees when exposed to fluorodinitrobenzene, according to the method of Sanger (9), lead to the general assumption that histones were acetylated only NH2-terminally. Gershey et al. (6) have recently reported, however, that histones also contain ε-amino-acetyllysine. Experiments carried out in this laboratory have shown that the N-acetyl groups in histones turn over much faster than the histones themselves, and it seems therefore conceivable that the amount of N-acetyl groups found in a pure histone species at any given time represents a fraction of the total number of sites available for acetylation (2).

On the other hand, Starbuck et al. (11) have purified histone fractions which were either 100% acetylated at the NH2-terminal end or not at all. In addition, when isolated nuclei were incubated in the presence of radioactive acetate, incorporation of label appeared to occur primarily in ε-N-acetyllysine of histones (6, 12). Thus far, all evidence would therefore lead to the conclusion that the ε-N-acetyl groups on the internal lysine residues are metabolically active, whereas the α-N-acetyl groups at the NH2-terminal ends are stable features of certain histone fractions, similar to those of some other proteins, such as carbonic anhydrase (3). The previously mentioned *in vitro* experiments with isolated nuclei also revealed that the incorporation of labeled acetate was most active in the f2a1 and f3 fractions and was practically negligible in the lysine-rich histones (12). These data indicate that the acetylation of internal lysine residues is not random and definitely rule out the possibility of a spontaneous or artifactual process.

In our previous studies, rats were given injections of alanine-14C to label the histones and tritiated acetate to label the histone N-acetyl groups. At various time points after the injection, groups of animals were killed, and lysine-rich and arginine-rich (combined arginine- and slightly lysine-rich) histone fractions isolated from various tissues and their specific activities with respect to 14C and N-acetyl-3H were determined (2).

As shown in Table 1, the results of these studies indicated that uptake of acetate into N-acetyl groups from lysine-rich histones was much less than that into the arginine-rich histones, which is to be expected in view of the absence of ε-N-acetyl groups in the former (12).

The fact that some uptake of label occurs in the lysine-rich histones is presumably due to incorporation of acetate in the α-N-acetyl position of NH2-terminal residues. Phillips (9) has established that the lysine-rich histone fraction is completely acetylated at the NH2-terminal end. It is therefore not surprising that the specific activity of lysine-rich histones with respect to radioactive acetate decayed at a rate which approached that of the histones themselves. Accurate measurements of this turnover were, however, often hampered by the exceedingly low level of labeling (2).

As was already indicated, the turnover rate of N-acetyl groups in the arginine-rich (combined slightly lysine-rich and arginine-rich) histone fraction was found to be much faster than that of the histones (2). In view of the preceding discussion, it seems that this rapid turnover must be attributed...
f2al and f3 fractions, the feasibility of the study, and the effect on the respective turnover rates. The near absence of the internal e-\(\text{N}\)-acetyl groups and that the \(\alpha\)-TV-acetyl administration of radioactive acetate.

Liver as described previously (1,2). Rats were given i.p. injections of 100 \(\mu\)Ci/kg L-alanine-14C (uniformly labeled) to label the histones and 2 mCi/kg sodium acetate-\(^{3}\)H to label the histone TV-acetyl groups on the NH\(_2\)-terminal residues turn over at the same rate as the histones themselves.

The 2 arginine-rich histone fractions that are acetylated most actively incorporate radioactive acetate into the internal e-N-acetyl positions at approximately similar rates. Thus far, no conditions have been reported that caused a differential effect on the respective turnover rates. The near absence of turnover of N-acetyl groups in Novikoff hepatoma histones encountered in our studies (2) would seem to indicate that in this case both fractions were affected to the same extent. It was this unexpected finding that prompted us to survey a number of hepatoma lines with different growth rates to see whether this slow turnover might be a general property of hepatomas.

In view of the similarity of N-acetylation patterns of the \(\beta\)-al and \(\beta\) fractions, the feasibility of the study, and the absence of e-N-acetyl groups in lysine-rich histones, it was decided to carry out all experiments on the "arginine-rich" histones without further fractionation.

### MATERIALS AND METHODS

The present studies were carried out as described previously (1, 2). Rats were given i.p. injections of 100 \(\mu\)Ci/kg L-alanine-\(^{14}\)C (uniformly labeled) to label the histones and 2 mCi/kg sodium acetate-\(^{3}\)H to label the histone N-acetyl groups. In general, 4 groups of 2 to 3 animals were killed after the injection of radioactive acetate and the arginine-rich (combined slightly lysine-rich and arginine-rich) histone fractions isolated from tumor and liver. All animals received \(^{14}\)C-labeled alanine 24 hr prior to the injection of tritiated acetate. The specific activities of the isolated histone fractions with respect to \(^{14}\)C and N-acetyl(\(^{3}\)H) were determined as described previously (1, 2).

### RESULTS AND DISCUSSION

The results of these experiments are summarized in Table 2. Decay rates were calculated from semilogarithmic plots of specific activity against time by the method of least squares. Each half-life reported was obtained from 9 to 12 experimental points.

As can be seen, the near absence of turnover found in the case of the Novikoff hepatoma did not appear to be a specific feature of hepatomas in general, as the half-lives of histone N-acetyl groups in all tumors studied appeared to be an order of magnitude smaller than those of the whole histones and similar to that of most tissues studied thus far (2).

Because the liver consists of a variety of cell types (4, 5), the half-life found for histones or DNA will differ depending on the time period during which measurement takes place. In the "short-term" studies outlined above, the 1st group of animals was killed 24 hr after injection of the radioactive amino acid. Such experiments will yield an experimental half-life for DNA or histones of 100 to 200 hr. If, however, animals are killed at weekly intervals, after an incubation period of 3 weeks following the administration of the tracers, much longer half-lives will be found, since during the long incubation period the DNA and histones of faster-turning-over cell populations have lost their radioactivity. For assurance of a reasonable uptake of radioactive precursors into DNA and histones of hepatocytes, the animals used in such long-term experiments were 3 weeks old at the time of injection. The decay of the radioactively labeled DNA and histones was therefore due only to dilution with newly synthesized unlabeled material, since in this case no death or migration of cells occurs. The half-life obtained in such studies (Table 3) agreed very well with the growth rate of the liver in rats of that age (1), although it may be expected to become much longer when the animals reach adulthood.2

Because the liver cell population is varied, it was thus far not possible to decide which cell population is responsible for the rapid turnover of N-acetyl groups in liver. The present results indicate that the turnover of histone N-acetyl groups in hepatomas with very slow growth rates \(R_1\) and \(R_2\) appeared to be very similar to that of normal liver. Since it can be assumed that these tumors consist of pure clones of "minimally" malignant hepatocytes, the rapid turnover of N-acetyl groups in liver histones shown in Table 3 can in all probability be assigned to the hepatocytes, although it seems reasonable to assume that the histone N-acetyl groups in littoral cells of normal liver turn over rapidly as well. This instance points out the experimental advantage of these slow-growing tumors, which consist of uniform populations of cells, very similar to hepatocytes, in contrast to normal liver which, besides hepatocytes, contains a multitude of other cell types.

In order to explain the slow turnover of N-acetyl groups in Novikoff hepatoma, in vitro studies have been carried out on histone acetyltransferase activity in chromatin from rat liver and hepatoma (10), which indicated that although the activity in hepatoma chromatin was approximately 70% of that in liver chromatin, this difference did not seem adequate to explain the almost complete absence of histone N-acetyl turnover in this tumor. Meanwhile, in vitro studies by Libby (8) have revealed that the most plausible explanation for this

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Specific activity due to N-acetyl (acetate-(^{3})H) (cpm/mg histone)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;Arginine-rich histones&quot;</td>
</tr>
<tr>
<td>Liver</td>
<td>665 ± 161</td>
</tr>
<tr>
<td>Novikoff hepatoma</td>
<td>71 ± 11</td>
</tr>
</tbody>
</table>

* Averages of 3 experimental values ± S.E. at peak activity 1 hr after administration of radioactive acetate.

\(^2\)In view of low uptake of labeled precursors into histones and DNA, as well as the long half-lives, which generally exceeded the observation period, some spread may be expected in these data. It is, however, obvious that the ratio of histone turnover to that of DNA was close to unity in all cases, which would agree with the notion that histones are metabolically inert and are conserved in a manner similar to that of DNA (1, 7).
Table 2  
Half-lives of arginine-rich histones and their N-acetyl groups from hepatomas and normal liver

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Transplant generation</th>
<th>Growth rate</th>
<th>$t_{1/2}$ (hr) N-acetyl (acetate-$^3$H)</th>
<th>$t_{1/2}$ (hr) histone (L-alanine-$^{14}$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novikoff hepatoma$^a$</td>
<td>Fast</td>
<td>21 (0.28)$^b$</td>
<td>41 ± (0.10)</td>
<td></td>
</tr>
<tr>
<td>Morris hepatomas</td>
<td>Fast</td>
<td>25 (0.39)</td>
<td>52 ± (0.32)</td>
<td></td>
</tr>
<tr>
<td>3924$^e$</td>
<td>250, 261</td>
<td>Fast</td>
<td>1.5 (0.20)</td>
<td>N.D.$^d$</td>
</tr>
<tr>
<td>7777$^e$</td>
<td>50</td>
<td>Fast</td>
<td>3.3 (0.13)</td>
<td>56 (0.34)</td>
</tr>
<tr>
<td>9121$^e$</td>
<td>17, 19</td>
<td>Intermediate</td>
<td>2.4 (0.15)</td>
<td>37 (0.13)</td>
</tr>
<tr>
<td>7800$^e$</td>
<td>28, 29</td>
<td>Intermediate</td>
<td>4.1 (0.41)</td>
<td>34 (0.24)</td>
</tr>
<tr>
<td>9098$^e$</td>
<td>28</td>
<td>Intermediate</td>
<td>3.6 (0.36)</td>
<td>39 (0.16)</td>
</tr>
<tr>
<td>R$^e$, 16, 17</td>
<td>Slow</td>
<td>4.1 (0.10)</td>
<td>43 (0.21)</td>
<td></td>
</tr>
<tr>
<td>R$^e$, 10, 12</td>
<td>Slow</td>
<td>2.5 (0.34)</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>Normal liver</td>
<td>ACI</td>
<td>2.3 (0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Buffalo</td>
<td>1.9 (0.24)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9121 (ACI)</td>
<td>1.9 (0.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sprague-Dawley</td>
<td>2.1 (0.22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.2 (0.15)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sprague-Dawley rats.

$^a$ Ratio S.E. of slope/absolute value of slope (S.E./abs). S.E. derived according to:

$$S.E. = \sqrt{\frac{\sigma^2}{\sum (x - \bar{x})^2}} \text{ where } \sigma^2 = \frac{1}{n-2} \sum (y - \bar{y})^2 - b\sum (x - \bar{x})^2$$

The $t_{1/2}$ for acetate-$^3$H of all hepatomas differed significantly from that of Novikoff hepatoma at $p = 0.05$ for $t_{1/2} < 4$ hr and $p = 0.10$ for $t_{1/2} > 4$ hr.

$^e$ ACI rats.

$^d$ N.D., not determined.

$^a$ Buffalo rats.

Table 3  
Half-lives in days of histones and DNA in rat liver

<table>
<thead>
<tr>
<th>Experiment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>21.6</td>
<td>26.7</td>
<td>28.7</td>
<td>25.6</td>
</tr>
<tr>
<td>Lysine-rich histones</td>
<td>21.7</td>
<td>22.2</td>
<td>17.6</td>
<td>20.5</td>
</tr>
<tr>
<td>Other histones</td>
<td>23.3</td>
<td>17.0</td>
<td>14.2</td>
<td>18.1</td>
</tr>
</tbody>
</table>

phenomenon must be sought in the very low content of a nuclear histone deacetylase in Novikoff hepatoma. In view of these findings, it should be interesting to study the in vitro histone acetyltransferase and deacetylase activities of the various hepatoma lines, in order to see whether any correlations may exist with growth rates or other biochemical properties.

REFERENCES

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