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Histone turnover within nonproliferating cells

(tritium/DNA/brain/liver)

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ABSTRACT **The turnover of DNA and histones in the livers and brains of mice has been determined. These mice had been exposed to constant levels of tritiated water from conception until they were 8 months old. At this point, exposure to tritium was discontinued, and the tritium remaining in DNA and histones was measured at various intervals afterward. The half-lives calculated for these components (with 95% confidence limits given in parentheses) were 117 (85–188) days for liver histone, 318 (241–466) days for liver DNA, 159 (129–208) days for brain histone and 593 (376–1406) days for brain DNA. The difference between histone and DNA turnover is statistically significant for both tissues and indicates that histone turnover within tissues cannot be solely accounted for by cell turnover within the tissue but also must include histone turnover within living cells. The half-life of histone within cells is estimated to be 117 (88–178) days in liver and 223 (187–277) days in brain.**

It is generally agreed that histone synthesis is closely coupled to DNA synthesis in somatic cells and that the turnover of histones within a tissue is very slow compared to that of other nuclear or cytoplasmic proteins (1, 2). Whether this slow tissue turnover is solely due to cell turnover within the tissue or is in part due to histone turnover within intact cells is at present controversial. Piha *et al.* (3) measured the rate of loss of label from brain and liver histones of mice given ^{14}C-labeled lysine in embryo and concluded that all of the observed histone turnover could be accounted for by cell turnover. On the other hand, Hempel *et al.* (4) measured the rate of incorporation of ^{3}H-labeled methionine into the histones of the adult cat kidney and concluded that histone turnover was rapid and almost entirely due to turnover within the living cell.

At the heart of this controversy is the magnitude of cell turnover in these tissues. Piha *et al.* (3) estimated cell turnover from DNA turnover reported by others. Hempel *et al.* (4) considered cell turnover in the adult cat kidney to be negligible because very few labeled cells were seen in autoradiographs of these tissues taken after injection of tritiated thymidine. However, values for DNA turnover reported in the literature are not reliable. They are based on relatively few measurements and vary by orders of magnitude. The finding of negligible cell proliferation in adult cat kidneys conflicts with findings of significant incorporation of DNA precursors in adult mouse kidneys (5) and suggests that the autoradiographic findings might be artifacts.

The question of whether there is histone turnover within the living cell has important implications relating to the stability of the nucleosome, the basic unit of organization of DNA and histone within the chromosome. Complete lack of turnover implies that the nucleosome is rigid and inert, whereas significant turnover suggests a flexible, dynamic structure. To help resolve this question, we have determined the rate of both histone and DNA turnover in mouse brain and liver. These measurements were made on mice that had been exposed to constant levels of tritiated water from conception until 8 months old. They are based on the rate of loss of tritium from DNA and histones after discontinuing exposure of these mice to tritiated water.

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MATERIALS AND METHODS

The procedures used here have been described in detail elsewhere (6). Mice were obtained from litters born to parents maintained on tritiated drinking water (3.0 μCi/ml; 1 Ci = 3.7 $\times$ 10^{10} becquerels) for 4 weeks before mating and were themselves maintained on this until 8 months old. After this time, they were given only tap water to drink. At various times after exposure to tritiated water was discontinued, tissues were removed and nuclei were isolated as before, except that nuclei from the liver were pelleted through one additional layer of 2.3 M sucrose to remove contaminating cytoplasmic material. Chromatin was isolated and fractionated as before. Tritium activity and DNA concentration also were measured as before.

RESULTS

The tritium level in the tissue water of these mice decreased rapidly after exposure to tritium was discontinued, from 2.02 μCi/ml before withdrawal to 0.07, 0.01, and 0.001 μCi/ml 7, 14, and 28 days later, respectively. The data in Fig. 1 compare the rates at which nonexchangeable tritium disappeared from brain histones and from liver histones of these mice. In order to provide a common basis for comparison, tritium content was expressed in terms of the activity (in dpm) found in that amount of isolated histone that was originally associated with 1 μg of DNA in the chromatin. The logarithm of these values was plotted against the interval between discontinuation of tritium exposure and removal of tissue for analysis. The upper line in Fig. 1 represents the best least-squares fit of all data for brain histones and the lower line represents the best least-squares fit of all data for liver histones removed more than 50 days after tritium exposure was discontinued. The slope of these lines indicates an intracellular half-life of 117 days for liver histone and 159 days for brain histone. Their correlation coefficients are -0.92 (liver) and -0.95 (brain), indicating a 95% confidence interval of 85–188 days for liver histone and 129–208 days for brain histone.

The reasons for excluding the first four liver histone data points from the determination of liver histone turnover becomes clear upon examination of Fig. 2. These data represent tritium activity in liver and brain DNA plotted in the same manner as in Fig. 1. The upper line is the best least-squares fit of all brain data points. Its slope and correlation coefficient (-0.83) indicate a half-life of 593 days for brain DNA, with 95% confidence limits of 376–1406 days. The lower line shows pronounced curvature. This results from the presence in liver of two cell populations with distinctly different turnover times (7). The curve drawn through the liver data is the best least-squares

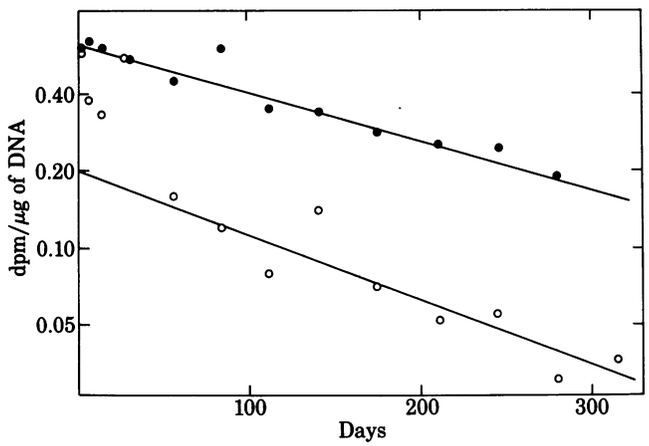

FIG. 1. Tritium activity in histones from the brains (●) and livers (○) of mice after removal of tritium from their drinking water. Tritium activity in histone is expressed as dpm in that amount of isolated histone originally associated with 1 μg of DNA in the chromatin.

fit for the two cell populations. Their half-lives are 12 and 318 days, and they represent 23% and 77% of the total DNA, respectively. The 95% confidence interval for the half-life of the cells with the longer life span is 241–466 days. The initial specific activity in liver DNA was 0.90 dpm/μg. This is in excellent agreement with the value of 0.89 expected on the basis of previous results (6). The initial specific activity in brain DNA was 0.99 dpm/μg, somewhat higher than expected.

Fig. 3 shows the tritium activity remaining in histone after correcting for cell turnover. These data represent the ratio of histone to DNA tritium activity in liver and brain, plotted in the same manner as in Fig. 1. The upper line is the best least-squares fit of the brain data, and the lower line represents the best least-squares fit of all liver data. The slope of these lines indicates a value of 117 days for the intracellular half-life of liver histone and 223 days for brain histone. Their correlation coefficients are −0.92 (liver) and −0.95 (brain), indicating a 95% confidence interval of 88–178 days for liver histone and 187–277 days for brain histone. The use of all of the liver data in these calculations assumes that intracellular histone turnover is essentially the same for both liver cell populations. If, in order to avoid this assumption, the first four data points are excluded, the slope of the best least-squares fit indicates an intracellular half-life of 185 days for liver histone. The correlation coefficient of −0.83 indicates a 95% confidence interval of 115–255 days. However, extrapolating to zero gives an initial histone-to-DNA tritium ratio of 0.30, whereas extensive previous data suggests

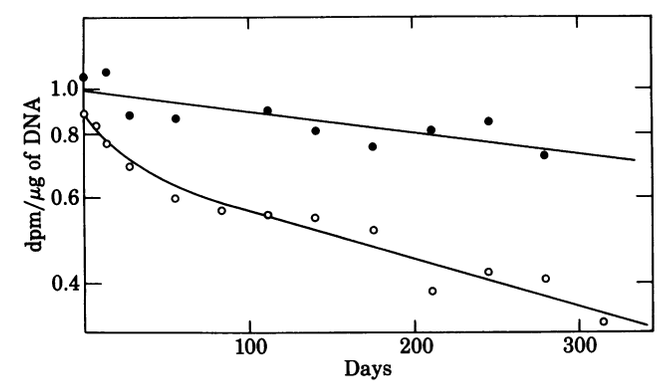

FIG. 2. Tritium activity in DNA from the brains (●) and livers (○) of mice after removal of tritium from their drinking water.

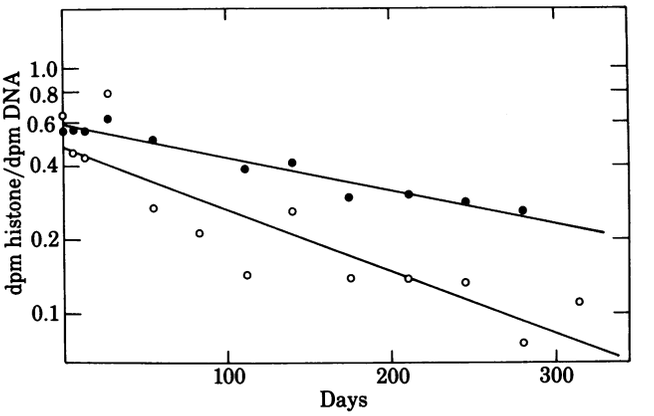

FIG. 3. Specific activity of histone tritium relative to that of DNA in the brains (●) and livers (○) of mice after removal of tritium from their drinking water.

a value of 0.69. Therefore, it seems that the results are more reliable if the early data are not included.

DISCUSSION

The possibility that nonhistone contaminants contribute significantly to the tritium turnover observed in the histone fraction can be rejected on a number of grounds. These histone fractions were isolated from chromatin, which in turn was isolated from nuclei freed of cytoplasmic contaminants by pelleting through 2.3 M sucrose. Even if glycogen or nonhistone nuclear proteins—the most likely contaminants—were present, their very rapid turnover (2, 8) could be easily distinguished from the much slower turnover of histones. That there is no such contamination by these or any other molecules is suggested by the close correspondence between the specific activity found for tritium in the initial histone fraction and that expected. We have shown (6) that all nonexchangeable tritium in the hemoglobin of mice exposed to tritiated water is present only in the nonessential amino acids (excluding tyrosine) and that its specific activity in these positions is 0.9 relative to the specific activity of tritium in tissue water. Assuming that this is true also for histones, the specific activity expected on the basis of the amino acid composition of these histones (9) [and the relative amounts of DNA and histones in the chromatin (10)] is 0.62 dpm/μg of DNA. This is in good agreement with the values actually found for brain histones (0.58 dpm/μg of DNA) and for liver histones (0.57 dpm/μg of DNA).

The half-life of 159 days found for brain histones in these mice is somewhat greater than that of 104 and 117 days reported for mouse brain histones (3) and that of 132 days reported for rat brain histones (2). The half-life of 117 days found for mouse liver histone is not inconsistent with the reported values of 93 and 105 days (3).

Piha *et al.* (3) attributed the histone turnover they observed in brain and liver entirely to cell turnover. They did this on the basis of published data for DNA turnover which suggests half-lives ranging from 115 to 800 days for brain cells and half-lives from 14 to >5000 days for liver cells. In most cases, these half-lives are based on few measurements and are not reliable. We have estimated cell turnover from the turnover of DNA tritium and have shown that it is too slow to account for all of the histone turnover observed in either tissue. This assumes that reutilization of tritium from DNA released by dying cells is not significant, which is not true in all cases. Tritium incorporated into DNA as thymidine can be extensively reutilized, from 35–40% under some circumstances (11), and presumably other DNA

components also can be reutilized. The following considerations show that these circumstances do not apply in the present case. On the basis of their ability to incorporate DNA precursors, mice behave as if they contain three distinct cell populations which take up 89%, 8%, and 3% of the total precursor incorporated into DNA and which have half-lives of 2, 10, and 130 days, respectively (12). If these cell populations were uniformly labeled with tritium and if reutilization of this tritium were 50%, then initially the apparent half-lives of each cell population would be twice their true half-lives. However, solution of the three simultaneous differential equations that describe the tritium content of each population as a function of time shows that the apparent half-life of the cell population with the longest life span decreases rapidly, becoming 151, 139, and 136 days at 10, 20, and 30 days after labeling and asymptotically approaching a value about 2% greater than the true value. The reasons for this behavior become apparent when it is realized that reutilization is not a local process (13). The label released from DNA of dying cells enters the general circulation and can be incorporated into any proliferating cell. Initially, the amount of tritium released by each cell population is in proportion to the amount they reutilize. As time progresses, the tritium content of the cell populations with a short life span drops precipitously and the amount of tritium they make available for reutilization is negligible. At this time, the source of nearly all of the reutilizable tritium is the cells with the longest life span. However, these cells incorporate only 3% of the available tritium. Consequently, their effective rate of reutilization would be only 1.5%.

Clearly, under these circumstances, reutilization of DNA components will have a minor effect on the value calculated for cell turnover. Thus, the disparity between histone turnover and cell turnover is significant and indicates that histone turnover does occur within the intact cell, although at a very slow rate. A number of explanations can be invoked to explain this. Extensive post-synthetic modifications of histones—methylation, acetylation, ubiquitination, ADP-ribosylation, and phosphorylation—are known to occur. Some, if not all, are known to be reversible (4, 14), which could account for some of the observed histone tritium turnover. However, the number of tritium atoms in histones that can be affected by these processes is relatively small. By assuming that only the nonessential amino acids other than tyrosine contain nonexchangeable tritium (6) and expressing the results in terms of nucleosomes, the basic unit of histone organization (10), there are 2113 sites per nucleosome for tritium in histones that would not be affected by postsynthetic modification and only 104 that would.

Histones are conserved during cell replication (15) and all five types of histones in brain turn over, although at somewhat different rates (2). These observations suggest that histone turnover within living cells is a process that affects nucleosomes as a whole at a very slow rate throughout the cell cycle. Perhaps the simplest hypothesis that accounts for this is that although histone synthesis and free nuclear histones cannot be detected in nonproliferating cells (1, 16), both nevertheless occur at levels sufficient to produce the observed turnover by exchange with nucleosomal histones.

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