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Histones of Terminally Differentiated Cells Undergo Continuous Turnover†

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ABSTRACT: In contrast to the widely accepted idea of the nearly absolute metabolic stability of histones, our experiments support the view that the histones of nonproliferating, terminally differentiated cells undergo continuous replacement. This conclusion is based on the incorporation of labeled amino acids into the histones of mouse kidney and liver cells after their intraperitoneal introduction. We have found that the intranuclear uptake of the histones made in the absence of replicative synthesis and their integration into chromatin proceed with striking delay. The metabolic rates of individual histones measured by calculating their half-lives suggest that each histone turns over at a specific rate. With regard to the basic chromatin structure, the nucleosome, such unequal turnover should mean that the histone core does not participate in this process as a single unit but rather as a protein mosaic in which each partner follows its own rate of removal. Additional experiments suggested that intact nucleosomes take part in the replacement, but the relative proportion of the nucleosomes involved should be limited. The nonnucleosomal H1A and H1° histones have been found to undergo faster replacement than the core histones. Moreover, in comparison to each other, these two histone subfractions are also replaced at a different rate. The results of autoradiography of isolated kidney and liver nuclei after continuous labeling with [^{3}H]-thymidine suggest that the histone replacement is not associated with the repair of DNA.

A large body of evidence has indicated that histone synthesis is tightly coupled to DNA synthesis [for reviews, see Elgin & Weintraub (1975) and Tsanev (1980)]. This suggestion has received additional support by the finding that G1 cells possess a reduced amount of histone mRNA (Melli et al., 1977). One of the implications that has been raised from these results considers the metabolic stability of the histones as being almost absolute. A number of reports have suggested, however, that the coupling may not hold true for all systems. Gurley et al. (1972) have demonstrated that histone synthesis occurs in G1-arrested cells at a detectable rate and that this synthesis has been accompanied by a slow turnover of the histones. Adamson & Woodland (1974, 1977) have found that during

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0006-2960/83/0422-4095$01.50/0 © 1983 American Chemical Society

frog oogenesis the intensive histone synthesis is not accompanied by DNA replication. The same was discovered in neuronal cells of newborn rabbits (Brown, 1980) and in resting epithelial lens cells (Herve et al., 1979). Exceptions have been found in proliferating cells by Herve et al. (1979) and Groppi & Coffino (1980); more recently, Wu & Bonner (1981) reported that the histone synthesis in these cells could be separated into basal synthesis continuing throughout the cell cycle and S-phase synthesis linked to DNA replication. Noncoordinate synthesis of the histones in cultured *Drosophila* cells under heat-shock conditions was also established by Sanders (1981). If we, finally, add contributions marking especially the unambiguously high metabolic independence of H1 histones (Gurley et al., 1972; Appels & Ringertz, 1974; Ohba et al., 1975; Tarnowka et al., 1978; Pehrson & Cole, 1982), the list of exceptions will grow impressively.

In the present study, we give evidence in support of an alternative idea of histone metabolism. We believe that the histones of all cell systems undergo replacement not as an exception but regularly and continuously. The following considerations argue for such an assumption. First, it is generally accepted that protein turnover is extensive (Schimke 1975) and that one of its important functions is the "elimination of abnormal and potentially harmful polypeptides, such as result from mutations, biosynthetic errors, or spontaneous denaturations" (Goldberg & John, 1976). So far, we have no reason to exclude histones from this biological regularity. Second, although the current view of the histones is that they are primarily structural proteins, they do not build a static chromatin skeleton. A large degree of microheterogeneity has been observed in the form of postsynthetic modifications or variants whose appearance correlates with the transcriptional activity of the chromatin or developmental stages [for a review, see Sperling & Wachtel (1981)]. The histones could realize easily such a dynamic role through an appropriate replacement. The third consideration favors the "natural" repair as a possible reason for histone replacement.

This paper deals with the metabolic properties of the histones in terminally differentiated cells. We have found that all histones of mouse kidney and liver cells undergo replacement which for each histone type proceeds at a specific rate. Additional autoradiographic experiments have indicated that this replacement should not be related to the repair of DNA.

Materials and Methods

Animals, Radioactive Labeling, Isolation, and Fractionation of Chromatin. Four-month-old C57/BL male mice were intraperitoneally injected with 150 μCi of [^{3}H]lysine (80 Ci/mmol, Amersham). In order to minimize the effect of the variations that occur among different animals, we used two mice for each time point of a given experimental series. At the time intervals indicated, the mice were killed, and the kidney and liver were quickly excised, washed in minimum essential medium (MEM)[1] without serum, transferred to 20 volumes of buffer A [146 mM sucrose, 100 mM KCl, 10 mM Tris-HCl (pH 7.0), 1.5 mM $MgCl_2$, and 0.1 mM PMSF], minced with scissors, and homogenized. The homogenates were filtered through four layers of cheesecloth and the filtrates centrifuged at 500g for 10 min. The crude nuclear material was washed 2 more times and resuspended for 10 min in 15 volumes of the same buffer containing 0.25% NP-40. After centrifugation, the nuclei were washed in buffer B [146 mM sucrose, 100 mM KCl, 10 mM Tris-HCl (pH 7.7), 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 0.1 mM PMSF], resuspended in 2 mL of the same buffer, layered over a 10-mL cushion of 1 M sucrose in 10 mM KCl, 10 mM Tris-HCl (pH 7.7), and 1.0 mM $MgCl_2$, and sedimented at 800g for 10 min. The pellets were washed in buffer C containing 146 mM sucrose, 100 mM KCl, 10 mM Tris-HCl (pH 7.7), and 1.0 mM $MgCl_2$. The pure nuclei were then successively homogenized in 0.075 M NaCl, 0.025 M EDTA (pH 7.7) (once), 0.037 M NaCl, 0.0125 M EDTA (pH 7.7) (once), and finally in deionized water. Chromatin was fractionated by extraction with 125 mM H_2SO_4 at 4 °C for 30 min. The extracted material was precipitated with 8 volumes of cold acetone and stored overnight at −30 °C. The precipitated acid-soluble proteins containing mainly histones were then pelleted at 8000g, air-dried, and dissolved in sample buffer for electrophoresis.

In experiments designed to illustrate the decrease in the specific radioactivity of kidney and liver DNA with the time, as well as for autoradiography, the animals were intraperitoneally injected every second hour with 15 μCi of [^{3}H]thymidine (20 Ci/mmol, Amersham) during a period of 20 h.

Isolation of Short Nucleosome Oligomers. In separate experiments, [^{3}H]lysine-labeled short nucleosome oligomers were prepared according to the procedure of Butler & Thomas (1980). Sixteen hours after the injection of the precursor, the mice were killed, and the kidney nuclei were isolated as described by Hewish & Burgoyne (1973). After digestion with micrococcal nuclease, the released chromatin fragments were fractionated by centrifugation in 5–30% linear sucrose gradients containing 10 mM Tris-HCl (pH 7.0), 1 mM Na_2-EDTA, and 0.1 mM PMSF in a Beckman SW27 rotor at 4 °C for 21 h at 27 000 rpm. The fractions containing up to pentanucleosomes were collected and recentrifuged at 55 000 rpm for 6 h in a Ti60 rotor. The pelleted material was extracted with H_2SO_4, and the histones were recovered as described above, air-dried, and solubilized in sample buffer.

Gel Electrophoresis, Elution, and Recovery of Histones from Gels. The labeled histones were fractionated in 2-mm-thick preparative sodium dodecyl sulfate–polyacrylamide slab gels with an acrylamide to bis(acrylamide) ratio of 300 to 4 and a concentration of 15%. The 5-cm slots were loaded with 300-μL samples containing about 600 μg of protein. The gels were run at 130 V for 7 h. After electrophoresis, they were stained for 30 min with 0.25% Coomassie Blue in 50% methanol–10% acetic acid and destained overnight. The individual histone bands were identified, removed with a scalpel, and treated further as previously described (Djondjurov & Holtzer, 1979). The destained gel homogenates were extracted for 1 h with 1% $NaDodSO_4$ in 0.2 M NaOH. After two extractions with the above solution, the eluates were combined and recentrifuged at 10000g for 20 min. The supernatants were then precipitated with an equal volume of 50% Cl_3CCOOH and left overnight at −30 °C. For removal of $NaDodSO_4$ completely from precipitated histones, they were washed for at least 2 h at −30 °C in 5 mL of acetone, then collected by centrifugation for 20 min at 10000g, and dried.

To check whether the histones recovered in this way were not contaminated with each other, or with some nonhistone proteins, they were reelectrophoresed on acetic acid–urea gels containing 12% acrylamide and 6 M urea (Panyim & Chalkley, 1969).

Calculation of the Histone Half-Lives. For the determination of radioactivity and protein content, the recovered

[1] Abbreviations: MEM, minimum essential medium; PMSF, phenylmethanesulfonyl fluoride; Tris, tris(hydroxymethyl)aminomethane; EDTA, (ethylenedinitrilo)tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Cl_3CCOOH, trichloroacetic acid; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole.

histones were dissolved in 1 mL of 0.1 M NaOH. Of this solution, 0.5 mL was added to 5 mL of toluene–POPOP–PPO scintillator containing 30% Triton X-100; the samples were neutralized with H_2SO_4 and counted in an LKB Ultrobeta spectrometer. The remaining NaOH–histone solutions were used for the quantitation of the protein according to Lowry et al. (1951). The calculated specific radioactivities of the histones (dpm/mg) at each time point after the introduction of [^{3}H]lysine were utilized to construct the curves measuring histone turnover. The half-lives were computed as described by Finlayson (1969). Since the proportion of the cycling cells in kidney and liver tissues of an adult animal is negligible, a correction of the slopes due to the growth was not made.

Flow Microfluorometry. For preparation of a single cell suspension from liver and kidney, the tissues were transferred in MEM and mincned with scissors. After centrifugation, the pellets were resuspended in 0.2 M sodium phosphate buffer (pH 7.5) containing 0.5% pepsin and aggitated for 10 min at 4 °C. The resulting suspensions were washed with sodium phosphate buffer and fixed twice in ethanol (75%) for at least 30 min. The cells were collected at 400*g* for 5 min, then resuspended at 2×10^6 cells/mL in a phosphate buffer containing 1 mg/mL RNase, and kept for 30 min at 37 °C. After a wash with the same buffer, the cells were stained for 15 min with a dye solution (100 mM Tris-HCl, pH 7.4, 12.5 μg of ethidium bromide/mL, and 25 μg of mitramycin/mL). Fluorescence was measured in an ICP II cytophotometer (PHYWE, Gottingen, FRG). Usually 50000 cells were measured at a flow rate of less than 500 cells/s. Analysis of the DNA histograms was made according to Baisch et al. (1975).

Specific Radioactivity of the Amino Acid Pool. For determination of the specific radioactivity of the amino acid pool, the kidney taken from mice injected with [^{3}H]lysine and washed in isotonic-buffered saline was transferred in distilled water and intensively homogenized. An equal volume of Cl_3CCOOH in acetone (10% w/v) was added to the homogenate and the mixture left for 2 h at −10 °C. The mixture was then centrifuged at 75000*g* for 10 min and the supernatant used for measuring the radioactivity and the amount of amino acids. The latter was made according to Stegeman (1960).

Cell Cultures. For in vitro experiments, Friend erythroleukemia cells (clone F4N) were used. They were grown in Dulbecco's MEM supplemented with 10% calf serum. Cells in an active proliferation state were transferred for 1 h in MEM without lysine but containing 1 μCi/mL [^{3}H]lysine. After being labeled, the cells were washed and maintained further in MEM with an excess of unlabeled lysine (250 mg/L). Nuclei and chromatin were isolated and fractionated as previously described (Yancheva & Djondjurov, 1982).

Autoradiography. Four hours after the last intraperitoneal introduction of [^{3}H]thymidine, kidney and liver nuclei were isolated as described in the previous section. The nuclei were fixed twice in glacial acetic acid–methanol (1:3) for 30 min and spread on slides. Autoradiographs were prepared by dipping the slides in Ilford K2 emulsion (Ilford Limited, Basildon Essex, England) diluted (1:1) with deionized water. After the coated slides were dried, they were exposed for 17 days at 0 °C. They were then sequentially washed in xylene, ethanol (100%), ethanol (95%), and distilled water and finally developed and fixed. The nuclei were stained with hematoxylin and eosin as recommended by Humason (1972).

Results and Discussion

Cell System. Terminally differentiated cells from mouse kidney and liver were used in our study. Two different methods were applied to check for the presence of cycling cells. The microfluorometric analysis of initial cell suspensions from both organs is given in Figure 1. The DNA histogram of the kidney cells shows a typical G1-type distribution with no measurable contribution in the S and G2 compartments. Flow microfluorometry of the liver cells reveals two populations with DNA contents corresponding to 2C and 4C, respectively, which confirms the well-known fact that the liver is a settlement for cells with a tetraploid genome. The percentage of the cells undergoing DNA synthesis, calculated on the basis of the histogram, is less than 0.1%.

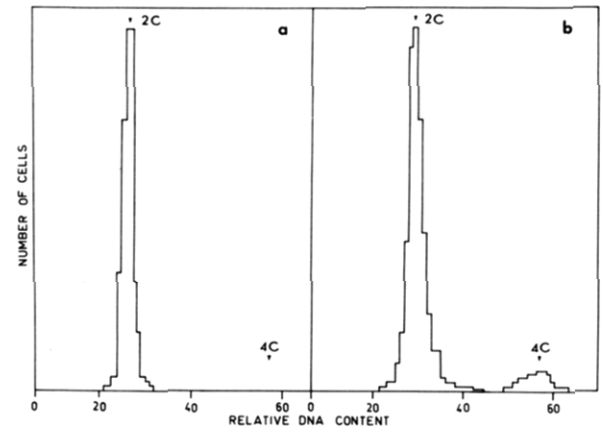

FIGURE 1: Flow cytometry determined DNA histograms of mouse kidney (a) and liver (b) cells. 20000 cells were measured at a flow rate of less than 500 cells/s for each distribution. 2C is the amount of DNA present in G1 phase and 4C the amount in G2 phase.

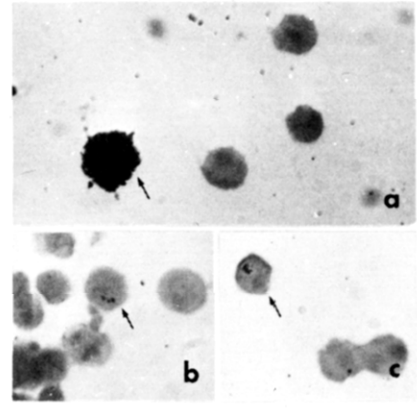

FIGURE 2: Autoradiographs of isolated kidney nuclei after continuous labeling with [^{3}H]thymidine. Two types of labeled nuclei were easily discriminated: heavily labeled, indicating replicative synthesis of DNA (panel a, arrow), and lightly labeled, showing repair synthesis (panels b and c, arrows).

Table I: Autoradiographic Analysis of Kidney and Liver Nuclei

	heavily labeled nuclei (%)[a]	lightly labeled nuclei	
		%	grains/nucleus[b]
kidney nuclei	0.11	0.08	4.6 ± 0.9
liver nuclei	0.17	0.10	5.2 ± 0.9

[a] The percentage of the heavily and the lightly labeled nuclei was determined in 5000 nuclei counted. [b] Only nuclei containing three or more grains were counted.

The autoradiographic determination of the proportion of replicating cells gives comparable results (Table I). In these experiments, we have used continuous [^{3}H]thymidine labeling, which provides good conditions for discrimination between

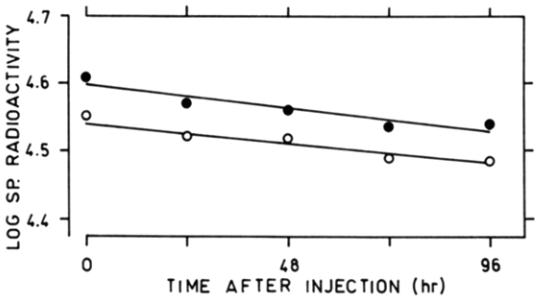

FIGURE 3: Changes of the specific radioactivity of kidney and liver DNA after continuous labeling with [^{3}H]thymidine. The slope of these lines indicates a value of 481 h for the half-life of kidney DNA (○) and 412 h for the liver DNA (●).

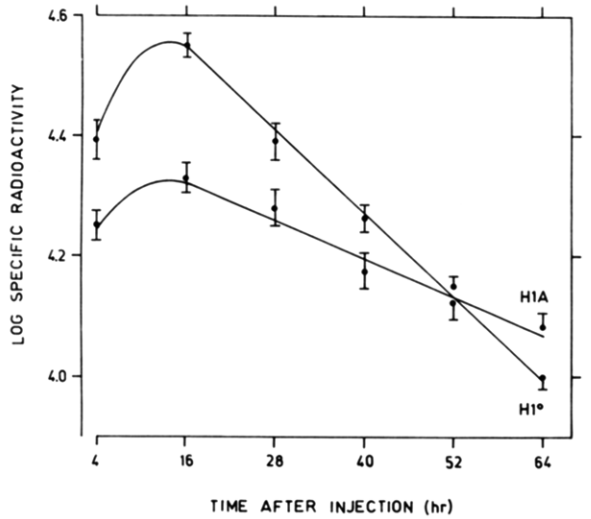

FIGURE 4: Changes of the specific radioactivity (dpm/mg) of mouse kidney H1A and H1° histones with time after intraperitoneal administration of 150 μCi of [^{3}H]lysine. The values are the averages of four experiments; bars indicate the SEM.

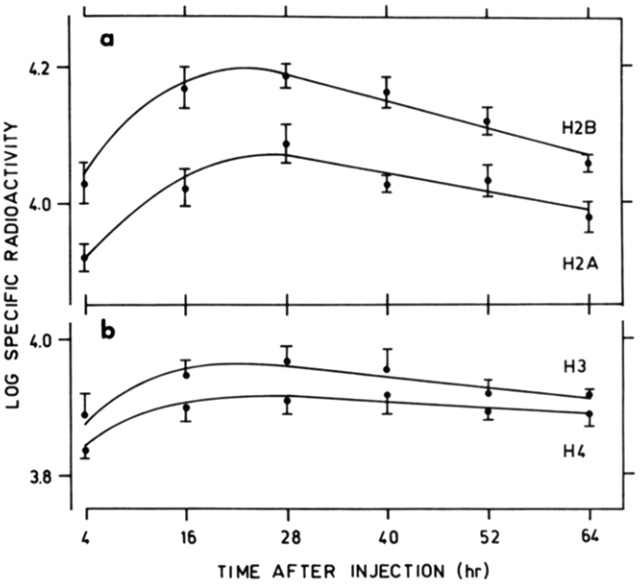

FIGURE 5: Changes of the specific radioactivity (dpm/mg) of mouse kidney H2A, H2B (a) and H3, H4 (b) histones with time after administration of 150 μCi of [^{3}H]lysine. The values are the averages of four separate experiments; bars indicate the SEM.

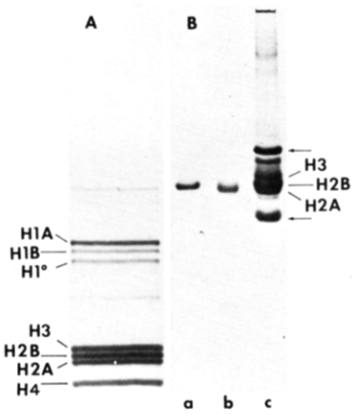

FIGURE 6: (A) Separation of the total nuclear acid extract in a 15%, 2-mm-thick preparative NaDodSO$_4$–polyacrylamide gel. Samples containing about 600 μg of protein were loaded over 5-cm-long slots and run at 130 V for 7 h. After electrophoresis, the gels were stained for 30 min with Coomassie Blue; the histone bands were then excised and the individual histones recovered as described. (B) Standard acetic acid–urea gel electrophoresis of (a) H2B histone recovered from preparative electrophoresis, (b) H2A histone recovered from preparative electrophoresis, and (c) total nuclear extract run as a standard. Arrows indicate the position of H1 and H4.

replicative and repair DNA synthesis. The percentage of replicating cells, appearing in autoradiographs as heavily labeled nuclei (Figure 2), is 0.11% and 0.17%, which is close to the data reported by a number of authors (Grisham, 1969, 1973; Houbrechts & Barbason, 1972; Stocker et al., 1972).

It is clear from the above data that the replicating cells in kidney and liver tissues constitute a negligible proportion, which make them adequately homogeneous for metabolic experiments. Such a conclusion is additionally supported by the observation that the majority of cells actively incorporating [^{3}H]thymidine should not be considered as members of a continuously proliferating population: these cells probably traverse through a limited number of divisions and then enter quiescence. Otherwise, the decrease with time of the specific radioactivity of DNA extracted after labeling should follow regularity typical for an actively growing population (see Figure 3). Although the values for DNA turnover reported in the literature for these cells are not reliable, they all mark extremely long half-life times (Piha et al., 1966; Commerford et al., 1982) which lies in accord with our results. It is thought that these half-lives measure the rate of cell renewal.

Evidence Indicating Histone Replacement. When kidney and liver nuclei were isolated from animals injected with [^{3}H]lysine, radioactivity was found in all histone species (Figures 4 and 5). Since this radioactivity is not essentially associated with the cell renewal (compare the half-life of DNA from Figure 3 with the half-lives of histones listed in Table IV), in the nonproliferating, terminally differentiated cells studied, it could be due either to a contamination of the purified histones with nonhistone proteins whose metabolism has been shown to proceed independently of the cell cycle (Djondjurov & Ivanova, 1979) or to a replacement of a histone. The following suggests that the histones eluted from preparative NaDodSO$_4$ gels are practically not contaminated with other proteins. Figure 6 shows the usual electrophoretic separation of acid-extracted material. After the removal of each histone band, further treatment, and elution of the protein, the recovered histones can be considered pure since when rerun on a second acetic acid–urea gel other protein(s) was (were) not found. For illustration, in Figure 6 are given the typical migration patterns of recovered H2A and H2B histones which in the first preparative NaDodSO$_4$ gel are separated closely. However, as far as the detection of the contamination was carried out on the basis of Coomassie staining but not after fluorography or autoradiography of labeled material, it is not possible to absolutely exclude traces of nonhistones being undetectable according to this method. Thus, it is reasonable

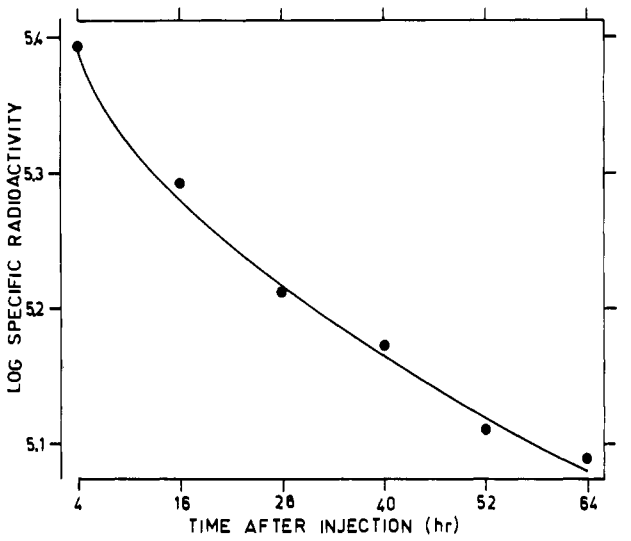

FIGURE 7: Changes of the specific radioactivity (dpm/mg) of mouse kidney nonhistone proteins with time after intraperitoneal administration of 150 μCi of [^{3}H]lysine.

Table II: Correlation between the Specific Radioactivity of Core Histones Isolated 28 h after Introduction of [^{3}H]Lysine and the Number of Lysine Residues

histone type	(a) sp radioact (dpm/mg)[a]	(b) no. of lysine residues[b]	a/b
H2B	15486	20	774
H2A	11802	14	843
H3	9325	13	717
H4	8355	11	759

to assume that if it exists, such a contamination should not change the specific radioactivity of the histones significantly. Although not directly, the striking difference in the overall view of the curves representing the changes of specific radioactivity of the histones and of the acid-insoluble nonhistones (compare with Figure 7) with time after introduction of [^{3}H]lysine also reinforces this conclusion. Finally, there is one more consideration that should be taken into account. The values of the specific radioactivity of the histones at 28 h after administration of the precursor (Figure 5), which prove to be close to the maximal values, correlate with the quantitative distribution of the lysine residues in each one of the core fractions (Table II), indicating that the labeled lysine used in our experiments has really been incorporated into the histone molecules. This fact suggests, in addition, that the cytoplasmic machinery for the synthesis of all core histones utilizes a common lysine precursor pool and that there should not be significant differences in the size of individual pools of histones nonintegrated into the chromatin core.

The experiments reported here obviously mark in both kidney and liver cells a continuous histone renewal proceeding independently of DNA replication. The main point to be discussed here is whether we face a true replacement or whether it accompanies nonreplicative repair processes in nontreated animals. As is seen in Table I, the proportion of the nuclei undergoing unsheduled DNA synthesis is even smaller than that of the replicating ones. These nuclei are lightly labeled (Figure 2) with an average of 4.6 grains/nucleus for kidney cells and 5.2 grains/nucleus for liver cells. For these and other tissues, such a low level of DNA lesions eligible for repair in nontreated animals has been reported by Price et al. (1971), Lieberman & Forbes (1973), and Epstein et al. (1971). In our opinion, this fact makes, the relation between the histone

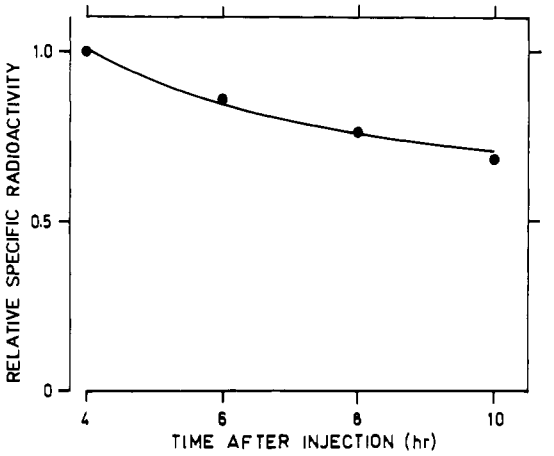

FIGURE 8: Plot of the relative specific radioactivity of recovered mouse bone marrow H2A + H2B histones vs. the time after intraperitoneal injection of 150 μCi of [^{3}H]lysine.

replacement and DNA repair hardly likely. The following line of evidence additionally corroborates such a conclusion. (1) It is known that part of the lesions induced by some agents in nonproliferating, differentiated cells are refractory to the excision repair systems (Goth & Rajewsky, 1974; Westra et al., 1976; Pegg, 1977). (2) In the case of chemical mutagenesis, there is a nonrandom distribution of the lesions within the chromatin structure. These ultimate carcinogens produce higher adduct concentration in linker DNA than in core DNA (Cech & Pardue, 1977; Jahn & Litman, 1979; Feldman et al., 1980; Kaneko & Gerutti, 1980), and after an appropriate posttreatment period, the relative concentration of the damaged core DNA is actually low. For example, in human fibroblasts treated with *N*-acetoxy-2-(acetylamino)fluorene, at the initial concentration of 20–40 adducts/10^6 deoxynucleotides, an average of 0.7–1.4% of the nucleosomes contain a damaged nucleotide only (Kaneko & Gerutti, 1980). (3) Although not yet confirmed, the results of Stein et al. (1976) indicate that the marked stimulation of DNA repair synthesis in human fibroblasts damaged with *N*-acetoxy-2-(acetylamino)fluorene is not accompanied by a stimulation of amino acid incorporation into the histones.

Nuclear Uptake of Newly Synthesized Histones. Figures 4 and 5 present the plot of the log of the specific radioactivity of the histones purified from kidney cells vs. the time after introduction of the precursor. Interestingly, the histones have reached maximum specific radioactivity between 16 and 28 h after the precursor has been administered. It is understandable that due to the extended time intervals chosen, our results are not able to mark the exact time of the maxima. Moreover, these results also found for the liver histones (data not shown) demonstrate a slow intranuclear uptake of the newly synthesized histone molecules which might be valid for all nonproliferating, terminally differentiated cells and thus specific for the histones synthesized in the absence of DNA replication. The following two experiments completely confirm this suggestion. Under the same conditions of labeling, extraction, and purification, the histones of continuously renewing mouse bone marrow cells have gained a maximum soon after intraperitoneal injection of the radioactive lysine (Figure 8). As is seen in Figure 9, the same was found in actively proliferating cultured cells. The retarded integration kinetics of the histones synthesized without DNA replication could be the consequence of specific changes in its translation within the cytoplasm, or intranuclear migration and association with chromatin, characterizing the differentiated state of the cells. The quantitative studies of Feldherr & Ogburn (1980) indicate

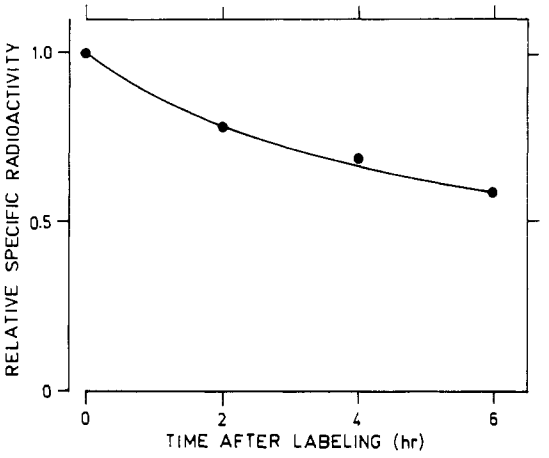

FIGURE 9: Plot of the relative specific radioactivity of recovered mouse erythroleukemia H2A + H2B histones vs. the time of chase. Actively proliferating erythroleukemia cells were labeled for 1 h with 1 μCi/mL [^{3}H]lysine; at zero time, the cells were washed and transferred to grow in a medium containing an excess of nonlabeled lysine. Similar curves were obtained for the specific radioactivity of H2A + H2B histones extracted from Namalva cells and from Chinese hamster fibroblasts (BII dii, FAF-28, clone 431) labeled as described for Friend cells.

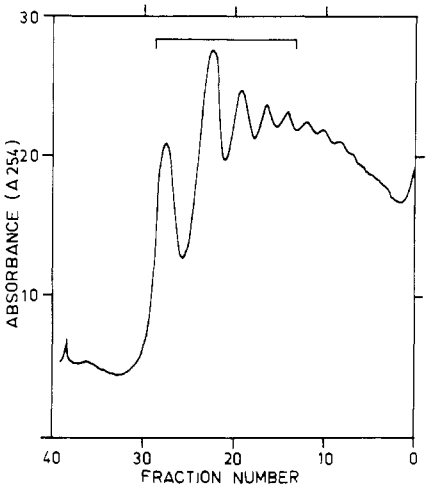

FIGURE 10: Sedimentation of short nucleosome oligomers in a 5–30% linear sucrose gradient. The fractions containing up to pentanucleosomes are indicated with the bar.

that specific binding and not the selection by the envelope is the main factor in maintaining the protein composition of the nucleus. In agreement with this, we favor the idea that the retarded integration into chromatin of nonreplicative histones is caused by a reduced amount of appropriate imperfect DNA–histone complexes available for integration.

Histones Undergoing Turnover Are an Integral Part of Intact Nucleosomes. Since the mass of the nuclear-soluble histones is negligible (Oliver et al., 1974; Jackson et al., 1976), the low specific radioactivity of the histones (compare with the specific radioactivity of the nonhistone proteins given in Figure 7) most probably indicates that at any time a relatively limited number of molecules are involved in replacement. In such a case, it is important to know whether these molecules are associated with structurally intact repeated units of chromatin. To see this, we isolated [^{3}H]lysine-labeled short nucleosome oligomers 16 h after injection of the precursor (Figure 10); the histones were extracted, fractionated, and purified as described. As is shown in Table III, their specific radioactivity is comparable to that of the core histones eluted from the total chromatin. This proves that the histones made under the conditions of nonreplicative synthesis participate in

Table III: Specific Radioactivity of Core Histones Purified from Short Nucleosome Oligomers Isolated 16 h after Intraperitoneal Administration of [^{3}H]Lysine

histone type	sp radioact (dpm/mg)
H2A	9005 (10455)[a]
H2B	12039 (14937)
H3	8840 (9012)
H4	6679 (7982)

[a] The data in parentheses are mean values of the specific radioactivity of the core histones from the total chromatin taken from Figure 5.

Table IV: Half-Lives of Major Mouse Kidney Histones

histone type	half-life (h)
H1A	60
H1°	41
H2A	135
H2B	90
H3	216
H4	361

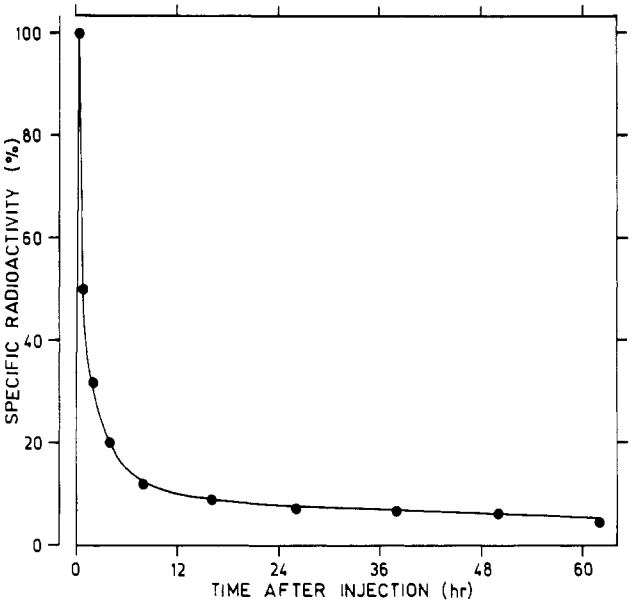

FIGURE 11: Changes of the specific radioactivity of the amino acid pool of mouse kidney cells after injection of 150 μCi of [^{3}H]lysine.

the organization of structurally complete nucleosomes. The assumption that the histone replacement is limited with respect to the number of molecules involved raises the question about the nature of the replacing nucleosomes. Our experiments do not clarify if the replacement proceeds continuously within a definite number of metabolically "active" nucleosomes or alternatively covers the entire nucleosomal pool in a random manner. In this direction, it would be of interest to know the metabolic behaviors of the modified histones, or histone variants. For the cycling cells, Wu & Bonner (1981) have already demonstrated the predominant involvement of certain variants in the basal histone synthesis not coupled to DNA replication.

Rate of Histone Turnover. For measurement of the turnover rate of each of the five histone types, their half-lives were determined. The values obtained are given in Table IV. As has been pointed out by Gan & Geffay (1967), such measurements are frequently minimal estimates of the degradation

rate due to the intensive reutilization of the labeled precursor. In our study, the Cl_3CCOOH-soluble radioactivity indicating the level of the radioactive amino acid within the intracellular pool of the kidney cells decreases to 12% 24 h after its administration but remains clearly detectable even 60 h later (Figure 11). For this reason, our calculations should also measure slightly longer turnover times. Relative to each other, the half-lives determined should be adequate, since as we have already noted, the intracellular pool of the lysine is common, or at least simmilar, for all core histones. The turnover rates of the liver histones do not principally differ from those calculated for the kidney histones, except that they were found to proceed 8–15% slower (data not shown).

Undoubtedly, the most intriguing finding in Table IV is the differences between turnover rates of individual histones. Such an uneven turnover has already been reported by Gurley et al. (1972), who, however, have computed different life spans. The differences do not sound unreasonable, since in the above-mentioned study the G1 phase induced in cultured Chinese hamster cells by the isoleucine-limiting method is not comparable to the G0 state of differentiated kidney and liver cells. The half-lives determined in both studies, however, are incomparably shorter than those of the brain and liver histones of mice found by Commerford et al. (1982). These authors computed a value of 117 days for the intracellular half-life of the liver histone and 223 days for the brain histone. Two points could be considered regarding this conspicuous discrepancy. First, it is possible that the net histone turnover may proceed with a striking divergence within the chromatin compartments, bearing differences in the degree and pattern of histone microheterogeneity. Further, since the actual labeling in our study is a pulse lasting no longer than a few hours (see Figure 11) while in the study of Commerford et al. (1982) the mice have been exposed to a constant level of tritiated water from conception till 8 months of age, it is clear why in either investigation the histones might have extremely different turnover rates. Second, in the same investigation, the high and unequal reutilization of tritium-labeled compounds to DNA and histones should present some problems in the exact calculation of the histone half-lives.

A number of conclusions follow from the uneven turnover of the histones. (1) The histone core does not degrade as a single unit, neither in the form of dimers nor in the form of tetramers, as could be supposed on the basis of reconstitution experiments (Kelley, 1973; D'Anna & Isenberg, 1974; Sperling & Bustin, 1975), cross-linking experiments (Van Lente et al., 1975; DeLange et al., 1979), or experiments on salt dissociation of the histones (Kornberg & Thomas, 1974). Similar to the "mosaic" degradation pattern of the protein complex of 40S monomer particles containing hnRNA (Ivanova et al., 1981), the replacement of each individual histone molecule follows its own route. This conclusion raises a number of questions about the mechanism of histone replacement and the conformational state of the nucleosomes undergoing histone replacement. There is no doubt that an appropriately changed conformation could provide better conditions for replacement. For this reason, a predominant involvement in this process of nucleosomes having some kind of histone modification or variant looks reasonable. (2) As far as a comparison between H1A and H1° could be indicative, the histones of the H1 group are also metabolically heterogeneous. Such a conclusion is consistent with the results recently reported by Pehrson & Cole (1982). (3) It is known that the different histone species possess a different degree of evolutionary conservatism in their primary structure [for a review, see Tsanev (1980)]. It appears from our results that the turnover rate of the five major histones is inversely correlated to the degree of this evolutionary conservatism, i.e., the higher the evolutionary conservatism, the lower the metabolic rate.

Acknowledgments

We are grateful to Dr. Konstantin Christov for microfluorometry.

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High-Pressure Nuclear Magnetic Resonance Studies of Hemoproteins. Pressure-Induced Structural Changes in the Heme Environments of Ferric Low-Spin Metmyoglobin Complexes†

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ABSTRACT: In order to gain an insight into nonbonded interactions in the heme microenvironments of hemoproteins, proton NMR spectra of the cyanide and methylamine complexes of metmyoglobin and its derivatives reconstituted with deutero- and *meso*-hemins in H_2O were studied under high pressures. The exchangeable NH proton of distal histidyl imidazole exhibits substantial pressure-induced shift while the proximal histidyl NH proton shows no pressure effect for the cyanide complexes. The heme peripheral proton signals, especially 5- and 8-methyl and vinyl $C_\alpha H$ resonances, were also affected by pressure. These observations are interpreted as arising from pressure-induced structural changes in the heme crevice in which the pressure effects are localized to the distal side rather than the proximal side and from possible changes in the van der Waals contacts at the heme periphery with nearby amino acid residues.

The heme group in hemoprotein is buried in the heme crevice made up by the three-dimensional arrangements of the polypeptide chain by covalent or coordinative bonds and by a multitude of weak bonds, i.e., hydrogen bonds, ionic bonds, and van der Waals interactions. The structure of polypeptide chain and the heme group in hemoprotein molecules are closely related to their functions. The importance of the heme–apoprotein interactions is underscored by their proposed focal role in modulating the reactivity of the iron center. Of particular interest are the roles of the heme proximal ligand and the heme distal amino acid residues at the heme proximal, distal, and peripheral sides in modulating the heme electronic structures and ligand exchange phenomena. These primary

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0006-2960/83/0422-4102$01.50/0 © 1983 American Chemical Society