CHEMISTRY AND METABOLISM OF MACROMOLECULES:

Metabolism of Histones of Brain and Liver

R. Sakari Piha, Michel Cuénod and Heinrich Waelsch

J. Biol. Chem. 1966, 241:2397-2404.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 241, No. 10, Issue of May 25, pp. 2397-2404, 1966
Printed in U.S.A.

Metabolism of Histones of Brain and Liver*

(Received for publication, September 17, 1965)

R. SAKARI PIHA,‡ MICHEL CUÉNOD,§ AND HEINRICH WAELSCH

From the New York Psychiatric Institute and the College of Physicians and Surgeons, Columbia University, New York, New York 10032

SUMMARY

Turnover of whole histones of brain and liver nuclei was studied after administration of ^{14}C-lysine to adult mice. The radioactivity of histones and other nuclear and cytoplasmic proteins was determined at intervals from 2 hours to 245 days. Two fractions of cerebral histones with half-lifetimes of 54 and 104 days and three fractions of hepatic histones with half-lifetimes of 18, 56, and 93 days were found. A larger number of fractions with gradually increasing turnover rates are not excluded.

The histones of brain and liver turn over at a lower rate than any of the nuclear and cytoplasmic protein fractions measured in the respective organs.

In order to label histones with a very low rate of turnover, ^{14}C-lysine was administered to pregnant mice and the turnover of histones was estimated in their offspring after reaching maturity. In the brain only a slow histone fraction with a half-lifetime of 117 days and in the liver two fractions with half-lifetimes of 58 and 105 days were observed.

Over a period from 2 to 8½ months in the brain and from 5 to 8½ months in the liver the decrease in the specific activity of histones was very small, corresponding to a replacement rate of about 0.6% per day. The turnover rates of histones may reflect division and replacement of various cellular species. The data suggest a metabolic stability of histones which corresponds to that of deoxyribonucleic acid.

The heterogeneity of organ proteins expresses itself in a wide spectrum of turnover rates when measured with the aid of isotopic amino acids (3). In the brain some protein fractions show a high rate of turnover such as the rapidly synthesized proteins localized in the microsomes while others such as the proteolipids (4) have a half-lifetime of many months. At present no data are available concerning the turnover of cerebral histones, the principal protein moiety of the deoxyribonucleoproteins.

This information would be of particular interest in relation to the turnover of deoxyribonucleic acid since it is generally accepted that DNA is synthesized only in the process of cell division. A second question of importance in view of the possible role of histones as regulators of genetic activity is whether individual histone components are replaced at similar or different rates. We chose as an experimental system the brain and the liver of the mouse. In the adult brain, cell division is a rare occurrence, being restricted to occasional divisions in the glial and ependymal cell populations. This is indicated by the metabolic stability of cerebral DNA which retains a labeled purine moiety without loss of radioactivity for as long as 12 months (5). In the normal adult liver cell replacement is considerably higher.

In order to compare the half-lifetimes of histones in both organs with the data given in literature for DNA, long term experiments were carried out with adult mice. Since cerebral histones with a very low rate of turnover may not be labeled significantly by pulse labeling with an isotopic amino acid in the adult animal, in some experiments ^{14}C-lysine was administered to pregnant mice and the turnover of histones estimated in the offspring after they had reached maturity. For comparative purposes the rates of turnover of other nuclear and cytoplasmic proteins in the respective organs were studied.

EXPERIMENTAL PROCEDURE

Adult Animal Experiments—Male or female white mice 3 to 3½ months old at the time of injection of the ^{14}C-L-lysine (125 mC per mmole, Schwarz) were used, the male mice weighing between 28 and 35 g and the female mice between 24 and 31 g. Animals of the same sex were used for the same experiment. In one experiment, 6.7 μC of ^{14}C-lysine were administered intraperitoneally to female mice in one dose (Table I, Experiment 1). In another experiment (Table I, Experiment 2), male mice received subcutaneously 10 μC of ^{14}C-lysine in two equal portions, 72 hours apart. In a third experiment (Table I, Experiment 3) the female mice received 22.5 μC of ^{14}C-lysine divided into three equal portions and injected subcutaneously at 24-hour intervals. The experimental periods of Experiments 1, 2, and 3 were 45, 31, and 245 days, respectively. Observations of the specific activity of proteins were made at varying times after the initial

* This work was supported in part by grants from the National Institute of Neurological Diseases and Blindness, United States Public Health Service (NB 00557), and from the Supreme Council, 33rd Degree Scottish Rite Masons of the Northern Jurisdiction, United States of America, and by a contract between the Office of Naval Research and the Research Foundation for Mental Hygiene, Inc. (New York State Psychiatric Institute Branch). Preliminary reports have appeared (1, 2).

‡ United States Public Health Service International Fellow, on leave of absence from the Department of Physiology, University of Helsinki, Helsinki, Finland. Present address, Department of Biochemistry, University of Turku, Turku, Finland.

§ Present address, Hirnforschung Institut der Universität Zürich, Zürich, Switzerland.

injection of the isotopic amino acid (see Table I). The value obtained at each time interval represents the average of five mice injected and killed simultaneously.

Experiments with Labeling in Utero—Female mice about 3 months old were mated. The beginning of gestation was estimated from the appearance of the vaginal plug. In the first experiment (Table II, Experiment 4) five pregnant mice were treated by subcutaneous injection with 5 μC of ^{14}C-lysine per animal on the 12th day of gestation. In the second experiment (Table II, Experiment 5) eight pregnant mice received, on 3 alternate days, 7.5-μC portions of ^{14}C-lysine, the first injection being given on the 8th day. In the third experiment (Table II, Experiment 6) 7.5-μC portions of ^{14}C-lysine were administered on 5 alternate days, the first injection being given on the 5th day of gestation. The offspring were killed in groups of five to eight at intervals between the ages of 2 and 8 months (see Table II), the individual animals being taken from different litters of the same age. The time of delivery was estimated to within 12 hours.

Isolation of Nuclei—Brain and liver nuclei were isolated according to a modified method (Fig. 1) of Sporn, Wanko, and Dingman (6). All operations were carried out at 4°. Five to eight mice were decapitated and brains and livers were rapidly removed and weighed. The average weights of these organs were 0.46 and 1.8 g, respectively. The organs were rinsed with ice-cold 0.9% NaCl solution and minced with scissors. The tissues were homogenized in an all-glass homogenizer with 7 volumes of 0.32 M sucrose solution containing 0.001 M $MgCl_2$, 0.0004 M KH_2PO_4, and 0.0004 M K_2HPO_4, pH 6.7 to 6.8. The homogenates were centrifuged in 40-ml conical centrifuge tubes at 820 × *g* for 10 min in a horizontal rotor (International refrigerated centrifuge).

After decantation of the supernatant, the crude nuclear sediment was washed three times by homogenization in the above solution and centrifugation of the suspension at 820 × *g*, 580 × *g*, and 580 × *g*, for 10 min each. After the last centrifugation, the crude nuclear sediment was homogenized with the 0.32 M sucrose solution (see above), the final volume being 4.5 ml. About 28.5 ml of a hypertonic sucrose solution (2.39 M sucrose, 0.001 M $MgCl_2$, 0.0035 M K_2HPO_4, pH 6.7 to 6.8) were added to give a final sucrose concentration of 2.05 to 2.10 M. After vigorous mixing the suspension was centrifuged at 90,000 × *g* (maximum) for 2 hours in the swinging bucket rotor (SW 25.1, Spinco ultracentrifuge, model L). A small amount of a white nuclear pellet was obtained from the brain and a considerable amount of a pink nuclear pellet from the liver. The nuclei were checked for purity by phase contrast microscopy. Only a small amount of contaminating non-nuclear material was present.

TABLE I

Biological half-lifetimes of histones labeled in adult

In Experiment 1 the mice were killed 2, 5, 12, and 24 hours and 4, 8, 21, 31, and 45 days after the administration of ^{14}C-lysine. In Experiment 2 the mice were killed 11, 20, and 31 days and in Experiment 3, 69, 113, 155, and 245 days after the first dose of ^{14}C-lysine.

Conditions	Half-lifetimes calculated between days			
	1 and 45	69 and 110	110 and 150	150 and 245
Experiment 1, 6.7 μC of ^{14}C-lysine injected per adult mouse				
Brain	56			
Liver	18			
Experiment 2, 2 × 5 μC of ^{14}C-lysine injected per adult mouse				
Brain	52			
Liver	19			
Experiment 3, 3 × 7.5 μC of ^{14}C-lysine injected per adult mouse				
Brain		104	104	104
Liver		58	58	93

TABLE II

Biological half-lifetimes of histones labeled in embryo

In Experiment 4 the offspring were killed 71, 104, and 138 days after the administration of ^{14}C-lysine into pregnant mice. In Experiment 5 the offspring were killed 69, 90, 111, 134, 153, 195, and 257 days and in Experiment 6, 88, 108, and 150 days after the first dose of ^{14}C-lysine.

Conditions	Half-lifetimes calculated between days		
	69 and 110	110 and 150	150 and 257
Experiment 4, 5 μC of ^{14}C-lysine injected per pregnant mouse			
Brain	45	115	
Liver	58	58	
Experiment 5, 3 × 7.5 μC of ^{14}C-lysine injected per pregnant mouse			
Brain	117	117	117
Liver	58	58	105
Experiment 6, 5 × 7.5 μC of ^{14}C-lysine injected per pregnant mouse			
Brain	52	Very long	
Liver	55	55	

Separation of Cytoplasmic Fractions—The combined supernatants from the first two centrifugations at 820 × *g* were centrifuged at 20,000 × *g* for 15 min in the Servall refrigerated centrifuge (SS-34 rotor) in order to separate the mitochondrial fraction. The pellet was homogenized in the 0.32 M sucrose solution and the suspension was centrifuged at 13,000 × *g* for 15 min. The supernatant, containing microsomes, was centrifuged at 144,000 × *g* (maximum) for 60 min (Spinco, No. 40 rotor). The microsomal fractions of brain and liver were washed by homogenization with the 0.32 M sucrose solution, followed by centrifugation at 144,000 × *g* (maximum) for 60 min.

For the preparation of ribosomes, 50 to 75% of the total amount of microsomes was treated by homogenization with sodium deoxycholate, the final concentration being 0.5% (7). After standing for 30 min at 2–4° the suspension was centrifuged at 144,000 × *g* (maximum) for 60 min and a ribosomal pellet was obtained.

Preparation of Proteins

Histones—The nuclear pellets were washed three times by homogenization with 0.14 M sodium chloride-0.01 M trisodium citrate solution and twice with 70% ethanol (*cf.* Reference 8) (Fig. 1). The suspensions were centrifuged at 1000 × *g* for 10 min. The sediment was extracted three times by stirring with 0.25 N hydrochloric acid (18 hours with 3 and 5 ml, 3 hours with

2 and 3 ml, and 1 hour with 1 and 2 ml for brain and liver, respectively). The supernatant solutions containing the histones were separated by centrifugation at 35,000 × *g* for 20 min after each extraction. The combined extracts were freeze-dried, and the residue was taken up in a small volume of 0.02 N hydrochloric acid. The histones were precipitated with trichloracetic acid, the final concentration being 20%. The resulting precipitate formed overnight at 4° was washed three times with ethanol-ether (1:2), and three times with ether, before being dried in a vacuum to constant weight.

From the amino acid analyses of the isolated histones, assuming 6 mole % of amide nitrogen (9), the theoretical nitrogen content of the samples was calculated (19.9%) and compared with the value found by Kjeldahl analysis (average, 15.4%). On the basis of this calculation 100 mg of prepared histone, dried to constant weight in a vacuum, would contain 78% of histone protein, the remainder being trichloracetic acid, anions, ash, and moisture. The yield of histones from 100 g of brain was about 0.04 g and from 100 g of liver about 0.16 g. These figures represent minimal values since only a partial recovery of nuclei is obtained. On disk electrophoresis (see below) the various histone preparations gave similar patterns.

Nuclear Proteins Other than Histones—A protein fraction referred to as "NaCl-soluble nuclear protein" was precipitated with trichloracetic acid from the combined sodium chloride-citrate and ethanol washings of the nuclei (Fig. 1). The solution was brought to dryness in a vacuum at 35° and the residue was taken up in a small volume of water and precipitated with trichloracetic acid, the final concentration being 20%. The nuclear material left after the extraction of the histones with dilute acid contains a protein fraction referred to as "HCl-insoluble nuclear proteins" (Fig. 1). The NaCl-soluble proteins were washed four times with 20% trichloracetic acid and the HCl-insoluble proteins four times with 5% trichloracetic acid and processed further according to Siekevitz (10).

Cytoplasmic Proteins—Ribosomes, microsomes, and mitochondria were treated with 5% trichloracetic acid at room temperature and the suspensions were centrifuged. The soluble proteins of the cytoplasm were precipitated by adding 50% trichloracetic acid to a final concentration of 7%. The proteins of the particles and the soluble proteins were prepared for counting by the procedure of Siekevitz (10).

Measurement of Radioactivity of Proteins—After drying the proteins to constant weight in a vacuum, about 1 mg of brain histones, 1 to 2 mg of brain HCl-insoluble nuclear protein, 0.2 to 0.5 mg of brain NaCl-soluble nuclear protein, 0.5 mg of brain ribosomal protein, and about 5 mg of other brain proteins and of each of the liver proteins were transferred to steel planchets for counting. The proteins were dissolved in 1 ml of formic acid (sp. gr. 1.2), and the solution was evaporated to dryness. Radioactivity was determined in a gas flow low background counter (Nuclear-Chicago, model C-110A). The values were corrected for self-absorption. All specific activities are expressed as counts per min per mg of protein. Since animals of different weight received the same amount of radioactive lysine, the specific activity of the proteins was corrected accordingly. A similar correction was applied for growth of the liver during the experimental period, whereas the values for brain were not corrected, owing to the insignificant growth of this organ.

Electrophoretic Analysis of Histones—Since the method of preparation of nuclei and of histones differed from that used by Neidle and Waelsch (11), it seemed of interest to compare the patterns obtained by disk electrophoresis. This was carried out on polyacrylamide gels (11–13).

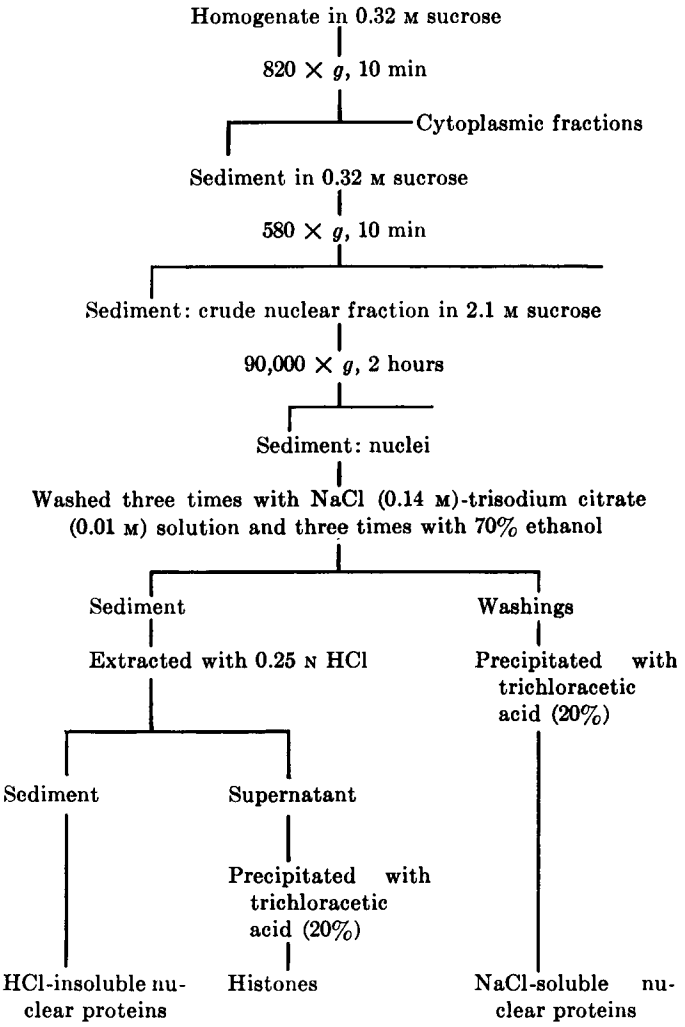

FIG. 1. Scheme of isolation of histones and other nuclear proteins from brain and liver.

Amino Acid Analysis of Histones—Quantitative analysis of the amino acids of histone hydrolysates was performed with the aid of an automatic amino acid analyzer (AutoAnalyzer, Technicon). About 1 mg of brain and liver histones was hydrolyzed in 2 ml of 6 N HCl for 20 hours at 110° in sealed tubes. The hydrolysates were evaporated to dryness in a vacuum at 35°, and HCl was removed by repeated addition and removal of water. Finally the dry material was dissolved in 1 ml of 0.1 N HCl, and aliquots containing 0.5 mg of histone hydrolysate were taken for analysis.

Estimation of Turnover Rate of Proteins—The biological half-lives of brain and liver proteins at different time intervals were determined from the specific activities of the proteins plotted against time on semilogarithmic graph paper. The half-lifetime ($t_{\frac{1}{2}}$) of a protein at a particular point on the decay curve is defined as the hypothetical time required for the specific activity of the protein to decay to half, assuming that decay continues at a rate determined by the slope of the decay curve at that point. The percentage of the protein pool replaced per day was calculated from the formula $100 \ln (2)/t_{\frac{1}{2}}$.

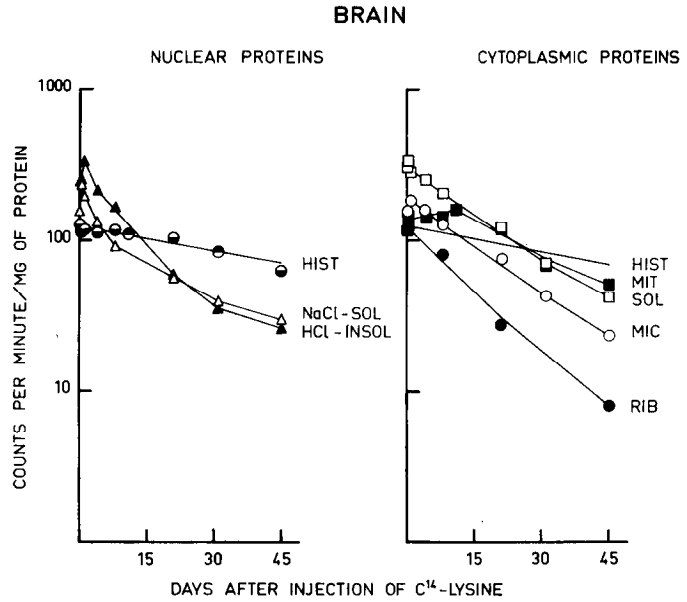

FIG. 2. Specific activity of nuclear and cytoplasmic proteins of the brain after injection of ^{14}C-lysine to adult mice. Histones: Experiment 1, ◒; and Experiment 2, ◓. Nuclear proteins: NaCl-soluble, △; and HCl-insoluble, ▲. Soluble proteins, □; ribosomal, ●; microsomal, ○; and mitochondrial, ■.

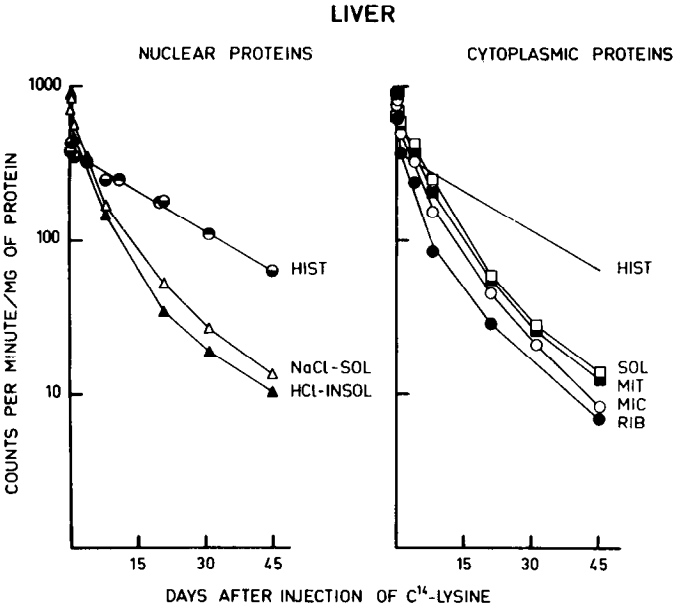

FIG. 3. Specific activity of nuclear and cytoplasmic proteins of the liver after injection of ^{14}C-lysine to adult mice. Histones: Experiment 1, ◒; and Experiment 2, ◓. Nuclear proteins: NaCl-soluble, △; and HCl-insoluble, ▲. Soluble proteins, □; ribosomal, ●; microsomal, ○; and mitochondrial, ■.

RESULTS

In the first group of experiments carried out with adult mice and lasting for as long as 45 days, histone decay in brain and liver was found to follow first order kinetics (Figs. 2 and 3). For the histones of brain $t_{\frac{1}{2}}$ of 56 days (B_1)[1] and for those of liver $t_{\frac{1}{2}}$ of 18 days (L_1)[2] were calculated (Table I, Experiment 1). These values correspond to a replacement rate of 1.24% in brain and 3.86% in liver per day.[3] In another experiment, $t_{\frac{1}{2}}$ values of 52 and 19 days were calculated for brain and liver histones, respectively, from the slopes of the decay curves from 11 to 31 days after ^{14}C-lysine injection (Table I, Experiment 2).

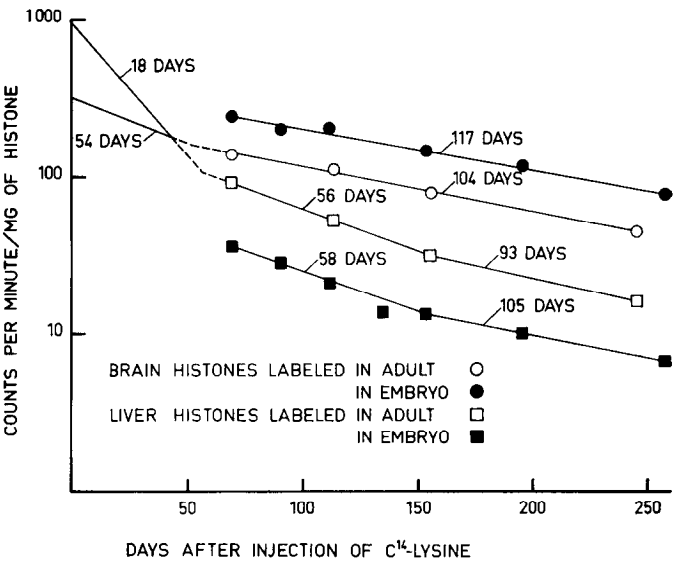

FIG. 4. Turnover of brain and liver histones labeled in adult and embryo. Specific activities of histones are those obtained between 69 and 257 days after injection of ^{14}C-lysine in three equal portions at 24-hour intervals to adult mice (Experiment 3) and pregnant mothers (Experiment 5). The initial slopes for the brain and liver histones labeled in adults were obtained from the 45-day experiment (Experiment 1, *cf.* Figs. 2 and 3) and brought up to the corresponding level by multiplying the values by 2.6.

When the experimental period was extended beyond 45 days with adult mice, histone fractions with slower rates of turnover than B_1 and L_1 were found. In the interval between 49 and 245 days (Table I, Experiment 3) decay of specific activities of brain histones showed an approximately constant exponential rate corresponding to a mean $t_{\frac{1}{2}}$ of 104 days (B_2).[1] In the liver two mean $t_{\frac{1}{2}}$ figures of 58 days (L_2)[2] and 93 days (L_3)[2] could be derived from the specific activity curve of histones (Fig. 4). Histones with $t_{\frac{1}{2}}$ longer than those of B_2 and L_3 were not found within the experimental period of 8½ months.

In another set of experiments the proteins were labeled in the embryo by injecting ^{14}C-lysine into the pregnant mothers; the administration of the labeled amino acid was started at a period of gestation when a large part of the cell division was still to come. In such experiments all histone fractions, particularly those with a very slow turnover, may be expected to be labeled significantly. After the offspring had reached maturity the specific activity of their brain and liver histones was determined between 69 and 257 days after the administration of ^{14}C-lysine to the pregnant mothers (Fig. 4). During this period specific activities of cerebral histones decayed at a rate corresponding to $t_{\frac{1}{2}}$ of 117 days (B_2) (Table II, Experiment 5; Fig. 4). This is equivalent to a replacement rate of 0.60% per day. In two other experiments $t_{\frac{1}{2}}$ values derived from the specific activity curves between 69 and

[1] Histone fractions of the brain with apparent turnover rates corresponding to $t_{\frac{1}{2}}$ of 52 to 56 and 104 to 117 days are referred to as B_1 and B_2.

[2] Histone fractions of the liver with apparent turnover rates corresponding to $t_{\frac{1}{2}}$ of 18 to 19, 55 to 58, and 93 to 105 days are referred to as L_1, L_2, and L_3, respectively.

[3] The results reported in our preliminary note (1) referred only to the data obtained in experiments of 45-day duration.

110 days, drawn between two points only, were 45 days (Table II, Experiment 4) and 52 days (Table II, Experiment 6). In the succeeding experimental period from 110 to 150 days, in the former experiment $t_{\frac{1}{2}}$ of 115 days was calculated, while in the latter experiment no decrease in the specific activity of cerebral histones was found.

Histones from livers of animals labeled in the embryo decreased in specific activity at a rate corresponding to a mean $t_{\frac{1}{2}}$ of 55 to 58 days (L_2) (Table III, Experiments 4 to 6) in the period between 69 and 150 days after injection. In the succeeding period between 150 and 257 days a mean $t_{\frac{1}{2}}$ of 105 days (L_3) was observed. These $t_{\frac{1}{2}}$ values correspond to replacement rates of 1.2 and 0.65% per day.

The histones of brain and liver measured during the 45-day period had a rate of turnover lower than any of the nuclear and cytoplasmic protein fractions measured in the respective organs (Figs. 2 and 3, Tables III and IV). Nuclear non-histone and cytoplasmic proteins did not decay according to first order kinetics at any time during this period, indicating the great heterogeneity of these protein fractions (Figs. 2 and 3, Tables III and IV). In contrast, histone decay was linear during this period and showed evidence of heterogeneity only at longer time intervals.

The HCl-insoluble nuclear fractions of both brain and liver, in agreement with previous reports (14–17), have half-lives much shorter than those of the histones (Tables III and IV, Figs. 2 and 3). This fraction consists in part of non-histone components of chromatin. Histones isolated by acid extraction may contain small amounts of such acidic and neutral proteins. The first order decay of histone radioactivity found during the 45-day experimental period, however, argues against the presence in the histone preparations of significant contamination with proteins of shorter half-lives than the bulk of the histones.

The heterogeneity of turnover rates found with proteins other than histones in the present experiments is in full accord (Tables V and VI) with the results obtained from previous decay experiments (18) as well as with the previous data obtained from experi-

TABLE III

Biological half-lifetimes of proteins in particular fractions of mouse brain

Protein fraction	Half-lifetimes calculated at different time intervals between days					
	0 and 1	1 and 4	4 and 8	8 and 21	21 and 31	31 and 45
Nuclear proteins						
Histones	56.0	56.0	56.0	56.0	56.0	56.0
NaCl-soluble	2.0	5.1	7.6	19.2	19.2	33.5
HCl-insoluble		4.8	9.1	9.1	13.7	32.4
Cytoplasmic proteins						
Ribosomal	0.5	10.7	10.7	10.7	12.5	12.5
Microsomal		13.2	13.2	14.6	14.6	16.3
Soluble	2.9	14.1	14.1	15.0	15.0	18.9
Mitochondrial				18.6	18.6	24.4

TABLE IV

Biological half-lifetimes of proteins in particular fractions of mouse liver

Protein fraction	Half-lifetimes calculated at different time intervals between days					
	0 and 1	1 and 4	4 and 8	8 and 21	21 and 31	31 and 45
Nuclear proteins						
Histones	18.0	18.0	18.0	18.0	18.0	18.0
NaCl-soluble	1.2	4.0	4.0	7.8	10.3	13.5
HCl-insoluble	0.9	3.9	3.9	5.9	11.8	15.8
Cytoplasmic proteins						
Ribosomal	1.0	3.5	3.5	7.7	11.8	11.8
Microsomal	0.9	4.3	4.3	6.4	9.3	10.5
Soluble	2.7	5.6	5.6	5.8	9.3	13.9
Mitochondrial	1.2	5.5	5.5	6.7	9.0	13.4

TABLE V

Turnover rates of total brain proteins measured from radioactivity decay curve of proteins and from ascending part of incorporation of ^{14}C-amino acids

Half-lifetimes obtained from the present decay experiments		Incorporation experiment				Decay experiment, ^{14}C-algal protein hydrolysate (18)	
		^{14}C-Lysine (3)[a]		^{14}C-Leucine (19)			
$t_{\frac{1}{2}}$	Between days	$t_{\frac{1}{2}}$	Experiment time	$t_{\frac{1}{2}}$	Experiment time	$t_{\frac{1}{2}}$[b]	Between days
days		*days*	*min*	*days*	*min*	*days*	
2.9[c]	0–1	2.8	2			2.6	0–1
		3.5	5	3.8	3	4.5	1–2
		6.2	20			6.1	2–3
		6.9	30	8.1	5		
12.6	1–8	10.4	45	11.0	10		
15.0	8–31	15.2	60	15.0	20		
18.0	31–45			20.0	30	19.4	3–6
				24.0	45	25.3	6–12

[a] Numbers in parentheses give reference of source of results.
[b] Mean half-lifetimes of proteins of different parts of the brain.
[c] Half-lifetimes of soluble protein fraction; other values in this column were obtained from the whole cytoplasmic protein fraction.

TABLE VI

Turnover rates of total liver proteins measured from radioactivity decay curve of proteins and from ascending part of incorporation of ^{14}C-amino acid

Half-lifetimes obtained from the present decay experiments		Incorporation experiment				Decay experiment, ^{14}C-algal protein hydrolysate (18)	
		^{14}C-Lysine (3)[a]		^{14}C-Leucine (19)			
$t_{\frac{1}{2}}$	Between days	$t_{\frac{1}{2}}$	Experiment time	$t_{\frac{1}{2}}$	Experiment time	$t_{\frac{1}{2}}$	Between days
days		*days*	*min*	*days*	*min*	*days*	
0.9[b]	0–1	0.9	2	0.9	5		
1.6	0–1	1.3	5	1.3	10	1.5	0–1
		2.6	10	2.4	20		
				3.9	30	4.0	1–3
5.7	1–4			5.7	45	5.5	3–6
5.9	4–8					5.9	6–12
7.5	8–12						
12.0	21–45						

[a] Numbers in parentheses give reference of source of results.
[b] Half-lifetime of microsomal proteins; other values in this column were obtained from total liver proteins.

ments of short duration in which the turnover of proteins of brain, liver, and other organs was calculated from the uptake of ^{14}C-lysine (3) and ^{14}C-leucine (19).

All non-histone nuclear and cytoplasmic proteins studied, with the exception of the brain mitochondrial fraction, showed a rapid loss of radioactivity. The specific activity of the proteins of this fraction increased slowly up to the 8th day of the experiment, followed by a steady decrease, corresponding to a half-lifetime of 18.6 days (Fig. 2). This observation may be related to the finding that proteins of nerve endings which are included in the crude mitochondrial fraction from brain (20) increase in specific activity during a 5½-day experimental period after administration of ^{14}C-leucine to mice (21).

DISCUSSION

The experiments reported here were designed to compare histone turnover in the brain, an organ in which cell turnover is minimal, with that in liver, an organ with a considerably higher rate of cell replacement. In the first series of experiments in which histone decay was followed for as long as 45 days after a single injection of ^{14}C-lysine into adult mice, the decrease in histone specific activity in both liver and brain was found to follow first order kinetics. All other cellular proteins studied yielded more complex decay curves, undoubtedly resulting from the greater heterogeneity of these proteins. The histones were found to have the slowest turnover rate of all the fractions studied, corresponding to average half-lives of 54 and 18 days, respectively, for brain and liver. Since the first points obtained in the decay curves were 2 and 5 hours following lysine injection, these data do not rule out the possibility of very rapidly turning over histone fractions. The report that histone components are uniformly labeled a short time after a single injection of amino acid (14), however, argues against the presence of a histone fraction with a turnover rate very much faster than that of the bulk of the histone. The exponential decay curves obtained for histones also suggest that during this 45-day period the various histone species were turning over at similar rates. In preliminary experiments[4] we have isolated lysine-rich, intermediate, and arginine-rich subfractions at various times during 30 days after an initial ^{14}C-lysine injection. All the fractions were labeled to a similar extent and decayed at first order rates comparable to that of whole histone.[5]

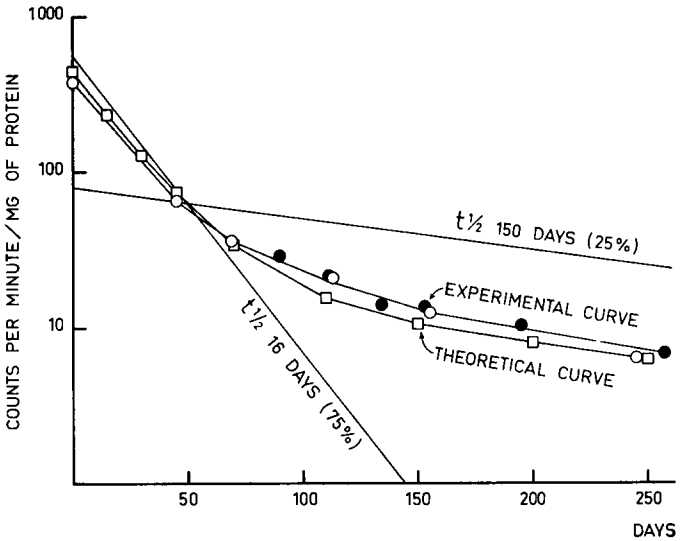

FIG. 5. The course of the theoretical decay process based on two fractions with half-lifetimes of 16 and 150 days, the relative amounts being 75 and 25%, respectively. The experimental curve was prepared from the experimental data available (*cf.* Fig. 4). *Open circles*, ○, correspond to specific activities of the liver histones labeled in adult and brought to the level of the values of the histones labeled in the embryo (*filled circles*, ●) by multiplying by 0.4. Calculated values for the theoretical curve are denoted by *open squares*, □.

The low rates of histone replacement combined with similar rates of turnover of the various histone subfractions suggests the possibility that histones may be replaced only during cell division. The finding that brain histones had a longer $t_{\frac{1}{2}}$ than those of liver (54 days compared to 18 days) is consistent with this hypothesis in view of the greater stability of the cell population in brain. The values found for histone half-lives, however, are considerably shorter than estimates of cell turnover in these organs (5, 24–26). It should be emphasized, however, that a single injection of isotope preferentially labels the most rapidly metabolizing cellular components and it is the turnover of these fractions that is observed in the subsequent decay curves. If histones are labeled only with the formation of new cells and if the label persists for the life of the cell, the half-lives observed would represent the most rapidly dividing cell populations and not average cell half-lives.

If histones form a stable DNA complex only during cell division, histones with considerably longer half-lives should be observable than were apparent in the 45-day experiments. Two types of experiments were therefore performed; in one, larger doses of isotopes were given to adult animals in order to follow the decay curves for a longer period of time; and in the other advantage was taken of the higher initial specific activities that could be obtained when the histones were labeled *in utero*. In the latter case since cells are rapidly dividing at the time of injection of the ^{14}C-amino acid, cells of long half-life should be appreciably labeled. In both types of experiment histones with longer half-lives than B_1 (54 days) and L_1 (18 days) were found. The longest half-lives observed were 117 days for brain histone and 105 days for liver histone. Thus for both brain and liver at least one relatively stable histone component and one with more rapid turnover has been demonstrated. Whether the intermediate values represent fractions with intermediate half-lives or represent the transition region in a two-component system cannot be decided from these data. A model for two components as shown in Fig. 5 can yield intermediate half-lives similar to those obtained for liver. An analogous calculation could be done with the brain histone data. While two components appear to be the minimal number consistent with these results, a larger number of fractions with intermediate turnover rates obviously cannot be excluded.

It appears unlikely that the histone inhomogeneity that appears when the experimental period is extended beyond 45 days can be due to the presence of histone subfractions with differing turnover rates. While no direct measurement of the distribution of label among histone fractions has been carried out at these long

[4] R. S. Piha, A. Neidle, and H. Waelsch, unpublished results.

[5] In a recent report by Chalkley and Maurer (22) it is claimed that in nondividing cells of rat liver histone turnover is restricted to the arginine-rich fraction. This conclusion is in contradiction to the argument presented here as well as to the results of Laurence and Butler (14) and of Hnilica and Busch (23).

TABLE VII

Relationship between turnover rates of histones and DNA

Experiments carried out with mice in References 5, 25, 26, 27, and 31–34. Half-lifetimes calculated from the data given in References 5, 24, 28, 30, and 32–35. Decay experiments (5, 29, 30–34, 37, 38). Autoradiography experiments (25–27, 34).

Organ	Fraction of nucleoprotein	Half-lifetimes							
		Fast fractions, B_1 and L_1	References	Intermediate fraction L_2	References	Slow fractions, B_2 and L_3	References	Stable fraction	References
		days		*days*		*days*		*days*	
Brain	Histones	52–56				104–117			
	DNA					115	26, 27	800	26, 27
						116	28	Very long	5
Liver	Histones	18–19		55–58		93–105			
	DNA	14	29, 30	56	24	89	32	487–5250	38
		16	31	58	35	90	34	Very long	5, 25, 33, 34, 39
		17	32	61	24	150	37		
		21	27, 33	69	28	164	32		
		25	29, 34	70	36	258–318	5		

intervals after isotope injection, a stable histone fraction necessary to explain these data would have been observed in the experiments discussed above carried out in a 30-day interval after initial ^{14}C-lysine injection.[4]

It is generally agreed that DNA is synthesized only during periods characterized by cell division. Since the histones are associated with DNA in nucleoprotein, the question arises whether their rate of turnover has any relationship to that of the respective DNA. In Table VII data from the literature on the turnover of DNA in brain and liver of mouse and rat are contrasted with those found by us for the histones in the two organs. Some of the results on DNA turnover were obtained by measurement of the decay of the radioactivity in a manner similar to our experimental design, others by radioautographic techniques. No selection of the DNA data has been attempted. It should be pointed out that in decay experiments the accuracy of $t_{\frac{1}{2}}$ longer than 100 days is obviously subject to considerable error because a small experimental error in specific activity within the period of very slow decay would cause a large change in calculated $t_{\frac{1}{2}}$ values. The $t_{\frac{1}{2}}$ found for liver DNA may be divided into four groups, three of which roughly correspond to those found by us for liver histones. In brain, DNA with a $t_{\frac{1}{2}}$ corresponding to B_2 was found. A DNA corresponding to B_1 has not, up to now, been discovered in brain, but the results obtained by radioautography (40, 41) make the presence of such a DNA fraction probable. The comparison of the data suggests that in the nucleoprotein, DNA and histones have very similar rates of turnover and that the metabolic stability of histones corresponds to that of the DNA. On the basis of somewhat different arguments than those discussed above, Laurence and Butler (14) have also very recently concluded that histone may be replaced concurrently with DNA.

For the histones, as well as for the DNA, it is obvious that

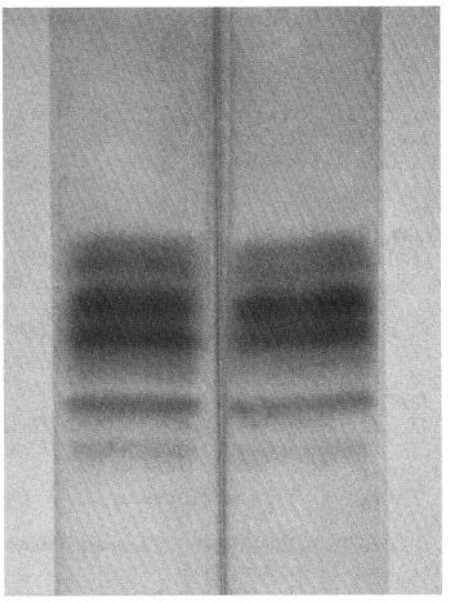

FIG. 6. Electrophoretic patterns of the whole histones of brain and liver. *Left*, brain; *right*, liver.

TABLE VIII

Amino acid composition of whole mouse histones

Amino acid	Brain	Liver
	moles/100 moles all amino acids	
Aspartic acid	4.7	5.1
Glutamic acid	8.3	8.8
Glycine	10.8	10.8
Alanine	12.1	11.9
Valine	5.8	6.0
Leucine	8.1	8.1
Isoleucine	3.9	3.9
Phenylalanine	1.8	1.9
Tyrosine	2.2	2.4
Serine	5.4	5.2
Threonine	5.3	5.4
Proline	5.8	4.5
Methionine	0.7	0.9
Arginine	9.1	9.3
Histidine	1.9	1.9
Lysine	14.0	13.6
Lysine:arginine	1.54	1.46

different turnover rates may be due to the turnover of different cellular species, such as neurons and glia in the brain or parenchymatous and connective tissue cells in the liver. The rate of turnover of L_1 would reflect a cell population characterized by rapid cell division (for instance, reticuloendothelial cells and about 10% of parenchymal cells (42)) and that of L_2 and L_3 would reflect cell populations with progressively longer life spans. Although cell division is only a rare occurrence in the brain, the division of the glial and ependymal cells (40, 41) may account for the half-life of 54 days for brain histones.

The slow and very similar turnover found over a period from 2 to 8 months in the brain (0.6% per day) and from 5 to 8 months in the liver (0.65% per day) could be ascribed to cell death and replacement, even in the most stable cell populations. However, an independent intracellular turnover of a certain fraction of histone present in the nuclei (for instance, free histone which is not combined with DNA) cannot be excluded.

In the adult because of the smaller number of cell divisions the presence of stable compounds will not be as apparent as in animals labeled in the embryo. In some of our experiments the adult mice and the pregnant mothers received the same amount of radioactivity. The specific activity of the cerebral histones of the offspring was higher than that found in the histones of the animals labeled as adults, while in liver the reverse ratio was observed (Fig. 4). This observation may be explained by the fact that a considerably larger part of cell division in liver takes place during a period when the free ^{14}C-lysine has already been diluted out, whereas the major part of cell division in brain occurs at an earlier period.

In confirmation of previous results (11) the patterns on gel electrophoresis of histones of brain and liver were identical with each other (Fig. 6); in addition they were identical with the patterns of brain, liver, and kidney histones of the mouse, reported earlier (11), although the methods of preparation were different from each other. To this may be added the fact that no significant differences were found in the amino acid composition of the whole histones from the two organs (Table VIII). Therefore, it seems that, as with DNA, the over-all composition of histones is not organ-specific, at least for brain, liver, and kidney, but that their turnover is determined by the biological activity of the cells of the particular organ.

Whereas the type of experiments carried out on normal animals under physiological conditions does not permit conclusions as to the function of the histones, the similarity of the electrophoretic patterns and of the composition of brain and liver histones, combined with the apparent close coupling of their turnover to that of DNA, have to be taken into consideration in any hypothesis regarding the regulation of DNA activity by histones in the process of transcription (43).

Acknowledgment—We are highly indebted to Dr. Amos Neidle for his valuable help during this work and in the preparation of the manuscript.

REFERENCES

1. Piha, R. S., and Waelsch, H., *Federation Proc.*, **23**, 267 (1964).
2. Piha, R. S., Cuénod, M., and Waelsch, H., in H. Peeters (Editor), *Protides of the biological fluids*, Elsevier Publishing Company, Amsterdam, 1965, p. 145.
3. Lajtha, A., Furst, S., Gerstein, A., and Waelsch, H., *J. Neurochem.*, **1**, 289 (1957).
4. Furst, S., Lajtha, A., and Waelsch, H., *J. Neurochem.*, **2**, 216 (1958).
5. Bennett, L. L., Jr., Simpson, L., and Skipper, H. E., *Biochim. Biophys. Acta*, **42**, 237 (1960).
6. Sporn, M. B., Wanko, T., and Dingman, W., *J. Cell Biol.*, **15**, 109 (1962).
7. Acs, G., Neidle, A., and Waelsch, H., *Biochim. Biophys. Acta*, **50**, 403 (1961).
8. Hnilica, L., Johns, E. W., and Butler, J. A. V., *Biochem. J.*, **82**, 123 (1962).
9. Phillips, D. M. P., *Progr. Biophys. Chem.*, **12**, 213 (1962).
10. Siekevitz, P., *J. Biol. Chem.*, **195**, 549 (1952).
11. Neidle, A., and Waelsch, H., *Science*, **145**, 1059 (1964).
12. Ornstein, L., and Davis, B. J., *Disc electrophoresis*, Canal Industrial Company, Bethesda, Maryland.
13. Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281 (1962).
14. Laurence, D. J. R., and Butler, J. A. V., *Biochem. J.*, **96**, 53 (1965).
15. Daly, M. M., Allfrey, V. G., and Mirsky, A. E., *J. Gen. Physiol.*, **36**, 173 (1952).
16. Steele, W. J., and Busch, H., *Cancer Res.*, **23**, 1153 (1962).
17. Wang, T.-Y., *Biochim. Biophys. Acta*, **68**, 52 (1963).
18. Piha, R. S., Bergström, R. M., Bergström, L., Uusitalo, A. J., and Oja, S. S., *Ann. Med. Exptl. Biol. Fenniae (Helsinki)*, **41**, 485 (1963).
19. Lajtha, A., *J. Neurochem.*, **3**, 358 (1959).
20. Gray, E. G., and Whittaker, V. P., *J. Anat.*, **96**, 79 (1962).
21. Barondes, S. H., *Science*, **146**, 779 (1964).
22. Chalkley, G. R., and Maurer, H. R., *Proc. Natl. Acad. Sci. U. S.*, **54**, 498 (1965).
23. Hnilica, L. S., and Busch, H., *J. Biol, Chem.*, **238**, 918 (1963).
24. Stevens, C. E., Daoust, R., and Leblond, C. P., *J. Biol. Chem.*, **202**, 177 (1953).
25. McDonald, R. A., *Arch. Internal Med.*, **107**, 335 (1961).
26. Pelc, S. R., *Exptl. Cell Res.*, **29**, 194 (1963).
27. Pelc, S. R., *J. Cell Biol.*, **22**, 21 (1964).
28. Hevesy, G., and Ottesen, J., *Acta Physiol. Scand.*, **5**, 237 (1943).
29. Bendich, A., Russell, P. J., Jr., and Brown, G. B., *J. Biol. Chem.*, **203**, 305 (1953).
30. Gerber, G., Gerber, G., and Altman, K. I., *J. Biol. Chem.*, **235**, 1433 (1960).
31. Bennett, E. L., and Krueckel, B. J., *Biochim. Biophys. Acta*, **17**, 503 (1955).
32. Garder, K. H., and Devik, F., *Intern. J. Radiation Biol.*, **6**, 157 (1963).
33. Jardetzky, C. D., Barnum, C. P., and Vermund, H., *J. Biol. Chem.*, **222**, 421 (1956).
34. Devik, F., and Halvorsen, K., *Nature*, **197**, 148 (1963).
35. Hammarsten, E., and Hevesy, G., *Acta Physiol. Scand.*, **11**, 335 (1946).
36. Swick, R. W., Koch, A. L., and Tahara, D. M., *Federation Proc.*, **14**, 149 (1955).
37. Swick, R. W., Koch, A. L., and Handa, D. T., *Arch. Biochem. Biophys.*, **63**, 226 (1956).
38. Fresco, J. R., and Bendich, A., *J. Biol. Chem.*, **235**, 1124 (1960).
39. Daoust, R., Leblond, C. P., Nadler, N. J., and Enesco M., *J. Biol. Chem.*, **221**, 727 (1956).
40. Koenig, H., *J. Biophys. Biochem. Cytol.*, **4**, 785 (1958).
41. Messier, B., Leblond, C. P., and Swart, I., *Exptl. Cell Res.*, **14**, 224 (1958).
42. Post, J., Huang, C.-Y., and Hoffman, J., *J. Cell Biol.*, **18**, 1 (1963).
43. Bonner, J., and Ts'o, P. (Editors), *The nucleohistones*, Holden-Day, Inc., San Francisco, 1964.