

Histone-Acetylating Enzyme of Brain

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1. Acetylation of histones by an enzyme system derived from rat brain and liver (histone acetylase) was studied by using [$1-^{14}\text{C}$]acetyl-CoA as the acetyl group donor. 2. The activity of this enzyme was largely confined to the nucleus. 3. Histone-acetylating activity of cerebral nuclei purified by centrifugation through 1.9M-sucrose was not altered by the presence of the cytoplasmic fraction. 4. Cerebral nuclei from adult rats exhibited greater histone-acetylating activity than did the corresponding preparation from newborn animals. 5. Nuclear acetylating activity was higher in brain than in liver of adult rats but not in newborn animals. 6. The partially purified enzyme from cerebral nuclei, prepared by ammonium sulphate fractionation of an acetone-dried powder, specifically catalysed histone acetylation. 7. Polylysine, protamine, serum albumin and γ -globulin were not enzymically acetylated by this preparation. 8. Soluble acetylating preparations from both brain and liver nuclei were more active towards arginine-rich F3 and slightly lysine-rich F2a and F2b histone fractions than towards the lysine-rich F1 fraction. 9. Enzymic acetylation of chromatin-bound proteins was much less extensive than that of free histones. 10. The high histone acetylase activity in mature brain may reflect the importance of this process in the genetic control of cerebral function.

The synthesis of RNA and protein in the mature brain appears to proceed at rates comparable with those observed in other active tissues of the adult animal (Zomzely, Roberts & Rapaport, 1964; Bondy, 1966; Jacob, Stevenin, Jund, Judes & Mandel, 1966; Roberts & Zomzely, 1966). Evidence has been presented that these processes are unusually sensitive to changes in the environment (Roberts, Zomzely & Bondy, 1970). Since the adult brain possesses a relatively stable DNA population (Adrian & Walker, 1962; Adams, 1966), alterations in genetic expression in this organ must be dependent on mechanisms that proceed at a rapid rate and are highly responsive to regulatory influences.

Histones and other nuclear proteins are thought to repress the genome by interacting with chromosomal DNA (Bonner *et al.* 1968). Gene expression may involve, in part, modification of these histones and resultant weakening of their association with DNA. The presence of acetylated lysine groups has been demonstrated in histones derived from various plant and animal sources (Allfrey, 1968; DeLange, Fambrough, Smith & Bonner, 1969). The degree of histone acetylation may be related to the physiological state of the tissue (Pogo, Pogo, Allfrey & Mirsky, 1968). For example, selective

acetylation of certain bound histones, by decreasing their basicity, may favour their dissociation from DNA (Gershey, Vidali & Allfrey, 1968; Libby, 1968). The present investigations were initiated in an attempt to assess the significance of histone acetylation in the regulation of RNA and protein synthesis in the brain. This paper describes the preparation and some characteristics of a cerebral enzyme system (histone-acetyl-CoA acetyltransferase) that is confined to the nuclear fraction of rat brain and acetylates histones selectively. This enzyme is highly active in the mature brain and may reflect the dynamic state of the cerebral genome in this organ.

EXPERIMENTAL

Preparation of cytoplasm and purified nuclei. Rats of an inbred Sprague-Dawley strain were used. Newborn animals were 1-16 h old. Adult animals were maintained after weaning on Purina laboratory chow *ad libitum* until they were 6 weeks old. Newborn rats were killed by decapitation; adult animals were killed by exsanguination from the abdominal aorta under light anaesthesia with sodium pentobarbital (Nembutal). Brains and livers were rapidly removed and placed in cold 0.32 M-sucrose. All subsequent procedures were carried out at 0-4°C. Tissues were homogenized by hand in 12 vol. of cold 0.32 M-sucrose

with the use of a Teflon pestle and a glass homogenizing tube and the homogenate was centrifuged at 750g for 10 min. The resulting supernatant constituted the cytoplasmic fraction. The pellet was vigorously shaken with 3.16 vol. of a medium composed of 2.4 M-sucrose, 3 mM-CaCl₂ and 50 mM-tris-HCl buffer, pH 7.2; the final sucrose concentration was approx. 1.9M. This preparation was then centrifuged for 30 min at 35 000g in a Sorvall RC-2 centrifuge. A white pellet was obtained that appeared to consist entirely of morphologically intact nuclei when examined by phase-contrast microscopy. The nuclear pellet was resuspended in 0.9% NaCl, and samples of the suspension were taken for measurement of protein by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin (Sigma Chemical Co., St Louis, Mo., U.S.A.) as a standard. DNA was assayed by the diphenylamine method of Burton (1956), with calf thymus DNA (Calbiochem, Los Angeles, Calif., U.S.A.) as a standard.

Preparation of soluble acetylating enzyme from nuclei. The acetylating enzyme was prepared by the method of Gallwitz (1968). The nuclear pellet obtained from 50 g of rat brain or 25 g of rat liver was suspended in 7 ml of water. This preparation was then added dropwise with continuous stirring to 70 ml of acetone kept at -30°C. The resulting suspension was passed through Whatman no. 1 filter paper on a Buchner funnel and the precipitate was washed three times with 50 ml of acetone at -30°C and dried in a desiccator at 0°C. The dry powder obtained was then homogenized in 13 ml of 0.1 M-tris-HCl buffer, pH 8.2, and centrifuged at 2000g for 10 min. The resulting supernatant was treated with neutral saturated (NH₄)₂SO₄. The fraction that was precipitated between 35% and 60% saturation with (NH₄)₂SO₄ was dialysed against two successive 500 ml portions of a solution containing 68 mM-KCl, 1 mM-β-mercaptoethanol and 20 mM-NaHCO₃ at pH 8.0 (Chou & Lipmann, 1952). This dialysed preparation contained the soluble acetylating enzyme. Samples were taken for assay of protein content. The remainder was frozen at -40°C in 1 ml batches.

Assay of acetylating enzyme. The acetylating capacities of the enzyme preparations were determined by incubation with various protein substrates in the presence of 25 nCi of [1-¹⁴C]acetyl-CoA (61 mCi/mmol; New England Nuclear Corp., Boston, Mass., U.S.A.) as described by Gallwitz (1968). All solutions were made up in 80 mM-tris-HCl buffer, pH 7.0. Assays for the soluble acetylating enzyme also included 1 mM-β-mercaptoethanol in the reaction mixture. The total reaction volume was 0.25 ml. Non-enzymic acetylation was assayed with enzyme preparations denatured by heating at 65°C for 10 min. The difference in incorporation of [¹⁴C]acetate in the presence of the heat-denatured and native enzyme preparations was taken as a measure of true enzymic acetylating activity.

After incubation, the reaction was stopped by the addition of 3 ml of 15% (w/v) trichloroacetic acid. The precipitate obtained was collected by filtration through a Millipore filter (25 mm diam., 0.8 μm pore size), and washed twice with 15 ml of cold 15% trichloroacetic acid and twice with 15 ml of cold chloroform-ethanol (1:1, v/v). The filter containing the air-dried precipitate was then placed in 5 ml of a scintillator solution containing 0.5% of 5-(4-biphenyl)-2-phenyl-1-oxa-3,4-diazole and 0.01% of

1,4-bis-(5-phenyloxazol-2-yl)benzene (Packard Instruments Inc., LaGrange, Ill., U.S.A.). Radioactivity was measured in the Packard Tri-Carb spectrometer at an efficiency of 84%. All assays were performed in triplicate.

Acetylation substrates. Cerebral and hepatic chromatin were prepared as described by Bondy & Roberts (1969). Calf thymus histone was obtained from Calbiochem. Purified histone fractions derived from calf thymus were obtained from the Worthington Corp., Freehold, N.J., U.S.A. The histone nomenclature used is that of Johns, Phillips, Simson & Butler (1960). Polylysine, protamine, and human γ-globulin were obtained from Mann Research Laboratories, New York, N.Y., U.S.A.

RNA synthesis on cerebral chromatin. The influence of the soluble acetylating enzyme on template capacity of cerebral chromatin from the adult rat was measured. Each reaction mixture contained (in 0.25 ml): 40 mM-tris-HCl buffer, pH 8.0, 4 mM-MgCl₂, 1 mM-MnCl₂, 12 mM-β-mercaptoethanol, 0.4 mM-ATP, 0.4 mM-GTP, 0.4 mM-CTP, 0.4 mM-[³H]UTP (12.5 mCi/mmol; Schwarz BioResearch, Orangeburg, N.Y., U.S.A.), 1 mM-acetyl-CoA and 88 μg of DNA as chromatin. The acetylating enzyme (22 μg) was added with or without 15 units of RNA polymerase (*Escherichia coli* K12; Miles Laboratories Inc., Elkhart, Ind., U.S.A.); 1 unit of RNA polymerase corresponded to the incorporation of 1 nmol of CMP in 1 h under the incubation conditions described here. Incubation was carried out for 40 min at 37°C. The reaction was stopped by addition of 0.25 ml of m-K₂HPO₄, followed by cold 5% (w/v) trichloroacetic acid. The resulting precipitate was collected by centrifugation at 3000g for 10 min and washed three times with 5% trichloroacetic acid. The final pellet was dissolved at 60°C in 0.5 ml of NCS solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.). This solution was added to 20 ml of the scintillator solution described above. Radioactivity was measured in the Packard spectrometer at an efficiency of 48% with a background of 14 c.p.m. All experiments were repeated at least three times.

RESULTS

Properties of the acetylating system in purified nuclei. With calf thymus histone as the substrate, acetylase activities of purified cerebral nuclei were similar at pH 7.0, 7.5 and 8.0. Since the extent of acetylation with heat-denatured enzyme was minimal at pH 7.0, all subsequent incubation experiments were carried out at this pH.

Enzymic acetylation by cerebral nuclear preparations continued for at least 25 min, but at a gradually declining rate. An initial lag phase was not observed. In contrast, acetylation in the presence of heated nuclei reached a lower maximal value in less than 5 min and remained constant thereafter. The standard incubation time of 18 min was chosen, since this interval permitted measurement of a high proportion of true enzymic activity in spite of considerable acetylation in the presence of the heated nuclei (Table 1). Protein acetylation in the presence of heat-denatured enzyme was non-specific and therefore not due to contamination

Table 1. *Acetylation properties of purified brain nuclei from adult rats*

Protein substrates (100 μg) were incubated at 37°C with cerebral nuclei (equivalent to 100 μg of protein) and 25 nCi of [^{14}C]acetyl-CoA (61 mCi/mmol) in 0.25 ml of 80 M-tris-HCl buffer, pH 7.0. The standard incubation time of 18 min was employed.

Nuclei	Reaction mixture	Substrate	Radioactivity incorporated into protein (c.p.m.)		
			Native enzyme	Heated enzyme	Difference
Fresh	Standard	—	528	258	270
Fresh	Standard	Histone	1131	311	820
Fresh	+ 1 mM-MgCl ₂	Histone	931	269	662
Fresh	+ 0.1 M-(NH ₄) ₂ SO ₄	Histone	344	240	104
Fresh	Standard	Bovine serum albumin	610	351	259
Stored (0°C for 1 week)	Standard	Histone	835	281	554

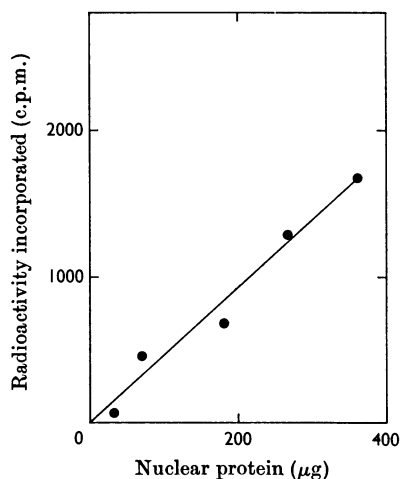


Fig. 1. Enzymic acetylation of histone in the presence of various amounts of fresh brain nuclei from adult rats. Calf thymus histone (100 μg) was incubated with purified brain nuclei and 25 nCi of [^{14}C]acetyl-CoA (61 mCi/mmol) in 80 mM-tris-HCl buffer, pH 7.0. The final volume was 0.25 ml. Incubation was allowed to proceed at 37°C for 18 min. Incorporation of [^{14}C]acetate into protein was measured in the presence of both native and heat-denatured nuclei. True enzymic acetylation was calculated as the difference between these two incorporation values.

of the substrates with acetylases (see also Patel & Crockett, 1969).

The amount of acetylase added as fresh brain nuclei was rate-limiting up to 350 μg of nuclear protein/0.25 ml reaction mixture (Fig. 1). Nuclear suspensions were always prepared freshly before each incubation experiment, since storage at 0°C resulted in decreased enzymic activity (Table 1); aggregation and loss of nuclear structure occurred at lower temperatures.

Enzymic acetylation of calf thymus histone by cerebral nuclei from adult rats was inhibited by 0.1 M-ammonium sulphate (Table 1). The presence of Mg^{2+} had little effect. Enzymic acetylation of endogenous nuclear proteins could be demonstrated in the absence of added substrate. Addition of bovine serum albumin did not significantly increase this basal extent of incorporation of [^{14}C]acetate into protein. These results suggested that the acetylating enzyme in rat brain nuclei was specific for histones. Additional support for this conclusion was obtained with the solubilized acetylase preparation (see below).

Acetylating capacities of purified nuclei prepared from brain and liver of newborn and adult rats were compared (Table 2). Nuclei isolated from brain or liver of newborn animals exhibited similar enzyme activities. In contrast, enzyme activity was consistently higher in brain nuclei from the adult rat than in the corresponding liver preparation. Acetylation capacity of nuclear preparations from rat brain increased during development. These differences were not significantly altered when the incorporation results were expressed on a DNA basis, since the DNA/protein ratios for all nuclear preparations were very similar (Table 2). The latter observation also suggested that the degree of nuclear purity was comparable for all preparations.

Morphological localization of the histone acetylase.

Acetylating enzyme activity was also investigated in brain and liver postnuclear supernatants. Brain cytoplasm from adult rats exhibited very low acetylase activity towards various protein substrates (Table 3). Moreover, this activity was non-specific, since polylysine was acetylated more readily than histones. A relatively low capacity for auto-acetylation was also noted in brain cytoplasm. In contrast, liver cytoplasmic preparations were active in the acetylation of endogenous proteins, but revealed no additional acetylating

Table 2. *Acetylating capacity of brain and liver nuclei from adult and newborn rats*

Acetylase activity was measured under the conditions described for Table 1. Incubation was carried out at 37°C for 18 min with 100 µg of histone and nuclei equivalent to 100 µg of protein.

Source of nuclei	Protein/DNA ratio (w/w)	Radioactivity incorporated into protein (c.p.m.)		
		Native enzyme	Heated enzyme	Difference
Newborn brain	4.18	1023	390	633
Newborn liver	4.29	856	238	618
Adult brain	4.46	1227	370	857
Adult liver	4.33	998	533	465

Table 3. *Acetylating capacity of brain and liver cytoplasm from adult rats*

Acetylase activity was measured under the conditions described for Table 1. Incubation was carried out at 37°C for 18 min with cytoplasmic fractions equivalent to 100 µg of protein.

Substrate	Radioactivity incorporated into protein (c.p.m.)			Radioactivity over value without added substrate (c.p.m.)
	Native enzyme	Heated enzyme	Difference	
Brain				
—	195	87	108	—
Arginine-rich histone F3	364	170	194	86
Slightly lysine-rich histone F2b	291	199	92	0
Bovine serum albumin	170	117	53	0
Polylysine	734	416	318	210
Liver				
—	1022	128	894	—
Arginine-rich histone F3	1055	141	914	20
Slightly lysine-rich histone F2b	1025	216	809	0
Bovine serum albumin	960	123	837	0
Polylysine	952	500	452	0

activity in the presence of added polypeptides. The high endogenous activity in liver may reflect the presence of non-specific acetylases that are active in detoxification. These findings suggest that the specific histone-acetylating enzymes may be confined to the nucleus in both brain and liver.

Evidence has been presented that cytoplasmic mechanisms may intervene in the drastic curtailment of cell division in the mature brain (Gurdon, 1967; Gurdon & Woodland, 1968; Jacobson, 1968). This could be due to the appearance during development of cytoplasmic factors that inhibit nuclear acetylation of histones. However, no inhibition was observed when brain or liver nuclei from adult rats were incubated with either brain or liver cytoplasm from the same animals (Table 4).

Specificity of the solubilized acetylating enzyme from nuclei. The soluble acetylating enzyme, prepared by ammonium sulphate fractionation of an acetone-dried powder of nuclei, was stable on storage at -40°C but rapidly lost activity at 0°C. Enzyme preparations were therefore stored in small portions to avoid repeated freezing and thawing.

Contamination with DNA or histone protein was very low (<5%).

During incubation, the soluble enzyme preparation from adult rat brain acetylated histones for at least 18 min, but at a progressively declining rate (Fig. 2). Substrate concentration was saturating above 100 µg of histone/0.25 ml of incubation medium (Fig. 3). The specificity of this soluble enzyme towards a variety of polypeptide substrates was examined (Table 5). Calf thymus histone was acetylated to a much greater extent than any other substance tested. Other basic proteins (such as protamine), other lysine-containing proteins (such as bovine serum albumin and γ -globulin) and polylysine were acetylated enzymically to only a slight extent. The soluble enzyme preparation provided a poorer substrate for endogenous acetylation than the nuclear suspension, presumably because of the relative absence of cerebral histones.

Acetylase activity and substrate specificity of the brain and liver soluble enzyme preparations were very similar (Table 6). Arginine-rich histone F3 and the slightly lysine-rich F2 histones were better

Table 4. *Acetylation of histones by nuclear and cytoplasmic fractions from liver and brain of adult rats*

Acetylase was measured under conditions similar to those described for Table 1. Incubation was at 37°C for 18 min with 250 μg of histone and nuclear and cytoplasmic fractions equivalent to 100 μg of protein.

	Radioactivity incorporated into protein (c.p.m.)		
	Native enzyme	Heated enzyme	Difference
Liver nuclei	1576	368	1208
Brain nuclei	1830	321	1509
Liver cytoplasm	897	317	580
Brain cytoplasm	561	301	260
Liver nuclei+ liver cytoplasm	2337	347	1990
Liver nuclei+ brain cytoplasm	2163	318	1845
Brain nuclei+ liver cytoplasm	2796	303	2493
Brain nuclei+ brain cytoplasm	2313	295	2018

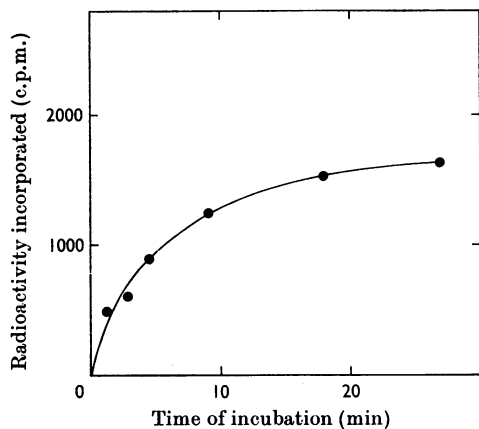


Fig. 2. Kinetics of enzymic acetylation of calf thymus histone by a soluble acetylating preparation from brain nuclei of the adult rat. Incubation conditions were similar to those described for Table 1. The soluble enzyme preparation (100 μg of protein) and 100 μg of histone were incubated together at 37°C for various time-intervals.

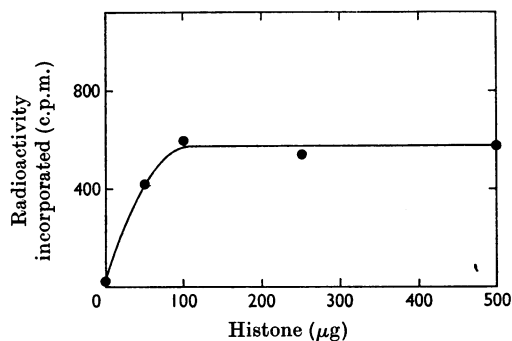


Fig. 3. Influence of substrate concentration on enzymic acetylation of calf thymus histone by soluble histone acetylase preparations from brain nuclei of the adult rat. Incubation conditions were similar to those described for Table 1. The soluble enzyme preparation (40 μg of protein) was incubated at 37°C for 18 min with various amounts of histone.

substrates than was the lysine-rich histone F1. These specificities are consistent with results reported for liver and uterus by Gallwitz (1968) and Libby (1968) respectively. However, the enzyme studied by Libby (1968) was largely localized in the uterus postmicrosomal fraction.

Measurements were also made of the capacity of histones present in the form of chromatin in rat brain and liver to act as substrates for a soluble acetylase preparation (Table 7). Acetylation of chromatin protein was low. This finding suggested that binding of histones to DNA partially protects these proteins against subsequent acetylation.

RNA synthesis on acetylated chromatin. Experiments were performed to test the possibility that enzymic acetylation of chromatin proteins may increase the amount of DNA available as a template for RNA synthesis (Table 8). The ability of cerebral chromatin to support RNA synthesis in the presence of bacterial RNA polymerase was increased by about 25% on addition of the native brain acetylating system. RNA polymerase activity in the acetylase preparation accounted for less than one-half of this increase. The difference was significant at the 2% level. Accentuation of RNA synthesis on acetylated chromatin may be due to decreased basicity of the histones in this complex and a consequent loosening of their attachment to DNA.

Table 5. *Acetylation of various proteins and polypeptides by a soluble enzyme preparation from brain nuclei of adult rats*

Acetylase activity was measured under the conditions similar to those described for Table 1. The soluble enzyme preparation (102 μ g of protein) was incubated at 37°C for 15 min with 500 μ g of various acceptor proteins.

Acceptor	% of lysine in acceptor	Radioactivity incorporated into polypeptide (c.p.m.)		
		Native enzyme	Heated enzyme	Difference
None	—	111	95	16
Calf thymus histone	15-22	2043	316	1727
Polylysine	100	470	386	84
Protamine	0	436	300	136
Bovine serum albumin	13	163	122	41
Human γ -globulin	8	116	61	55

Table 6. *Acetylation of histone fractions by soluble acetylating preparations from brain and liver nuclei of adult rats*

Acetylase activity was measured under conditions similar to those described for Table 1. Various histone fractions (100 μ g) were incubated at 37°C for 18 min with the soluble acetylating enzyme (40 μ g of protein).

Substrate	Radioactivity incorporated into substrate (c.p.m.)		
	Native enzyme	Heated enzyme	Difference
Brain			
—	77	69	8
Mixed histones	645	190	455
Arginine-rich histone F3	782	216	566
Lysine-rich histone F1	329	145	184
Slightly lysine-rich histone F2a	780	160	620
Slightly lysine-rich histone F2b	621	171	450
Liver			
—	103	114	—
Mixed histones	681	203	478
Arginine-rich histone F3	1259	480	779
Lysine-rich histone F1	439	128	311
Slightly lysine-rich histone F2b	893	312	581

Table 7. *Acetylation of histones in chromatin from brain and liver of adult rats*

Acetylase activity was measured under conditions similar to those described for Table 1. Soluble enzyme fractions from liver nuclei (50 μ g) were incubated at 37°C for 18 min with chromatin or free histone (48 μ g of protein).

Substrate	Radioactivity incorporated into protein (c.p.m.)		
	Native enzyme	Heated enzyme	Difference
Mixed histone	673	140	533
Liver chromatin	218	146	72
Cerebellum chromatin	286	177	109

DISCUSSION

These investigations have revealed that a highly active histone-acetylating system is concentrated in the nucleus of adult rat brain cells. This enzymic activity was highly specific for histones; other

polypeptides and proteins were acetylated only to a minor extent. In contrast, protein-acetylating activity in brain cytoplasm was very low and highly non-specific. Only one histone acetylase appeared to be present in brain nuclei. Thus no change in substrate specificity occurred during solubilization

Table 8. *Influence of acetylase on RNA synthesis on chromatin*

The reaction mixture contained 40 mM-tris-HCl buffer, pH 8.0, 4 mM-MgCl₂, 1 mM-MnCl₂, 12 mM-β-mercaptoethanol, 0.4 mM concentrations of ATP, GTP, CTP and UTP (the CTP was ³H-labelled to 12.5 mCi/mmol), 1 mM-acetyl-CoA and 88 μg of DNA as cerebral chromatin. Where indicated soluble cerebral acetylase (22 μg) and/or bacterial RNA polymerase (15 units) were also added. The final volume was 0.25 ml. Incubation was carried out for 40 min at 37°C.

RNA polymerase	Native enzyme	Heated enzyme	Radioactivity incorporated into protein (c.p.m.)	
			Total	Due to acetylase
+	+	-	2502 ± 81	569
+	-	+	1933 ± 28	
-	+	-	600 ± 33	260
-	-	+	340 ± 10	

of the enzyme. These findings suggested that histones of cerebral nuclear chromatin may be the physiological substrates for cerebral acetylase. A similar situation may hold for other tissues in view of the demonstration that endogenous nuclear histones were selectively acetylated by enzymes present in isolated liver nuclei (Gallwitz & Sekeris, 1969).

The turnover rate of cerebral histones in the adult rat has been shown to be extremely low (Piha, Cuénod & Waelsch, 1966). This phenomenon may indicate that the turnover of nuclear histones is linked to the replacement of DNA (Byvoet, 1966), which occurs at a very low rate in the mature brain (Adams, 1966). Metabolic stability of chromatin-bound histones was also suggested by the observation that these substances were very poor substrates with cerebral acetylase preparations, whereas free histones were very rapidly acetylated. However, in view of the high acetylase activity of brain nuclei the brain may have the potential for histone acetyl groups to turn over faster than the whole histone molecule (see also Pogo, Allfrey & Mirsky, 1966; Byvoet, 1968). Since the sites of histone acetylation *in vivo* appear to be restricted to specific lysine residues (DeLange *et al.* 1969), the lower extent of acetylation of chromatin-bound proteins compared with free histones may indicate that these particular sites are partially blocked by interaction with DNA.

Available evidence suggests that histones fall into two diverse functional classes, based on differences in chemical structure (Sadgopal & Bonner, 1969; Spelsberg, Tankersley & Hnilica, 1969). The lysine-rich histones seem to be concerned primarily with cell division and the regulation of DNA synthesis (Ord & Stocken, 1967; Stevely & Stocken, 1968; Dick & Johns, 1969), whereas the arginine-rich histones may play a major role in the regulation of RNA synthesis (Hindley, 1963; Sluysers, 1966; Pogo *et al.* 1968; Sunaga & Koide, 1968). The arginine-rich histones are more active metabolically

than the lysine-rich group (Busch, Steele, Hnilica, Taylor & Mavioglu, 1963) and may turn over even in non-replicating cells (Chalkley & Maurer, 1965). In the present investigations solubilized cerebral acetylase exhibited considerably more activity towards the arginine-rich histone F3 than towards the lysine-rich histone F1. Total histone acetylase activity in brain nuclei increased with maturation and significantly exceeded the activity of liver nuclei. These findings may reflect a high rate of production of mRNA in brain (Bondy & Roberts, 1968), as well as the genetic flexibility of this organ under various physiological conditions (Hydén, 1967). Cerebral chromatin is relatively high in non-histone protein compared with hepatic chromatin (Bondy & Roberts, 1969). The suggestion has been made that this observation may indicate the presence of an active de-repressed genome (Frenster, Allfrey & Mirsky, 1963; Arbuzova, Gryaznova, Morozova & Salganik, 1968; Teng & Hamilton, 1969). Although certain non-histone cerebral nuclear proteins have been shown to be tissue-specific (Dravid & Burdman, 1968), the possible occurrence of brain-specific histones has not yet been resolved (Neidle & Waelsch, 1964; Tomasi & Kornguth, 1968; Martenson, Deibler & Kies, 1969).

The present investigations provide direct evidence for the concept that acetylation of histones within the chromatin complex may result in de-repression of the DNA genome (see also Allfrey, 1968; Mukherjee & Cohen, 1969). Thus exposure of cerebral chromatin to solubilized acetylase preparations from brain augmented its capacity to act as a template for RNA synthesis. This phenomenon was probably due to decrease in the basicity of certain bound histones and the resultant weakening of their interaction with DNA. In this connexion an elevated rate of acetylation of cerebral histones has been reported in the neurological disease scrapie (Caspary & Sewell, 1968), which involves glial proliferation (Kimberlin & Anger, 1969) and enzymic changes in the brain (Mould, 1969). This

observation is consistent with the conclusion that the cerebral genome may be qualitatively and quantitatively regulated by selective acetylation of nuclear histones. Other modifications of cerebral histones may also be involved in the control of genetic expression in the mature brain, e.g. binding to acidic proteins (Hydén & McEwen, 1966) or phosphorylation (Langan, 1968). By these various modifications, the output of information from the cerebral genome in the form of mRNA and rRNA may be regulated, resulting in changed patterns of protein synthesis in response to various physiological demands.

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