LITERATURE CITED

1. É. Ya. Baumanis, I. É. Kalninya, T. A. Moskvitina, et al., Biokhimiya, 43, 1496 (1978).
2. L. G. Vasil'evykh, R. G. Glushkov, V. Z. Gorkin, et al., Khim.-farm. Zh., No. 7, 20 (1979).
3. V. Z. Gorkin, Amine Oxidases and Their Importance in Medicine [in Russian], Moscow (1981).
4. M. D. Mashkovskii, V. Z. Gorkin, I. V. Verevkina, et al., Byull. Éksp. Biol. Med., No. 2, 169 (1981).
5. T. P. Blackburn, G. A. Foster, D. T. Greenwood, et al., Eur. J. Pharmacol., 52, 357 (1978).
6. A. Coppen and V. A. Rama Rao, Psychopharmacology, 63, 125 (1979).
7. A. L. Green and M. A. S. Hait, J. Pharm. Pharmacol., 30, 262 (1978).
8. J. A. Roth and C. N. Gillis, Mol. Pharmacol., 11, 28 (1975).
9. R. F. Squires, Adv. Biochem. Pharmacol., 5, 355 (1972).
10. R. J. Ziance, K. Moxley, M. Mullis, et al., Arch. Int. Pharmacodyn., 228, 30 (1977).

SELECTIVE LOSS OF HISTONE H1 — A NEW CHROMATIN RESPONSE TO ADDED TOTAL HISTONE IN A MEDIUM OF PHYSIOLOGICAL IONIC STRENGTH

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Analysis of the chemical composition of chromatin in most eukaryote cells gives a histone/DNA ratio close to 1 [6]. However, there is some evidence that this ratio in the nuclei of some cells may reach 3.3 [5], or even 2000 [9]. Moreover, in all proliferating cells a local rise in the histone/DNA ratio can be expected, if only temporarily, in the case of addition of extra histone octamers to individual nucleosomes in the S phase [14].

To analyze the consequences of such situations, when the cell chromatin comes into contact with an extra quantity of histones, the effect of total histone of calf thymocyte chromatin on chromatin isolated from these same cells in a medium of physiological ionic strength was studied. It was shown previously [14] that under these conditions chromatin can bind a limited number of extra histones. A new type of response of chromatin to added histone was discovered, namely selective loss of its own histone H1.

EXPERIMENTAL METHOD

Chromatin was obtained [15] by washing tissue homogenate in a medium consisting of 0.025 M EDTA–NO_2 + 0.075 M NaCl (pH 8.0). After five washings the chromatin suspension was dispersed in a solution of physiological ionic strength consisting of 0.15 M NaCl + 0.7 mM Na-phosphate buffer (pH 7.0) + 1 mM PMSF. The total histone of the chromatin was extracted with 0.4 N HCl from the same chromatin preparation obtained previously (30 min at 0°C), after which the extract was dialyzed against 0.9 N CH_3COOH and lyophilized.

Preparations of chromatin (C_{DNA} = 25 μg/ml) were mixed with equal volumes of solutions of total histone of different concentrations in an appropriate solvent. The mixtures were incubated for 48 h at 4°C, the stand with the tubes being slowly rotated to prevent the suspension from being thrown down. The mixtures were then centrifuged for 30 min on an L2-65B centrifuge (Beckman, USA; 40.3 rotor) at 40,000 rpm. The residues and walls of the tubes were washed with distilled water. The residues were then dispersed in water by means of an ultrasonic disintegrator and used for electrophoretic analysis of the protein composition of the nucleoprotein complexes formed in mixtures of chromatin and total histone. Electrophoresis was carried out by Laemmli's method [7]. Densitometry of the polyacrylamide gels, stained with Coomassie blue R-250, was carried out on a spectrophotometer (Gilford, England) at 580 nm.

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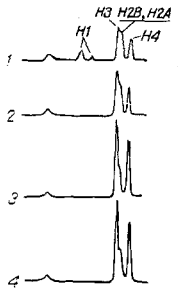

Fig. 1. Electrophoretic analysis of proteins in residues of chromatin–histone mixtures. Added histone/DNA chromatin ratios: 0 (initial chromatin), 1, 20, and 200 correspond to traces 1, 2, 3, and 4 respectively.

EXPERIMENTAL RESULTS

Densitograms of the gels after electrophoresis of residues from mixtures of chromatin and total histone are shown in Fig. 1. Equal volumes of samples were applied to each column of gel. Since all DNA of the chromatin suspension was in the residue after centrifugation, samples applied to different columns of gels were equalized for DNA content although their protein content could differ.

Clearly when the ratio of added total histone to chromatin DNA was 1 no histone H1 was found in the chromatin. In the writers' opinion this phenomenon is the result of competition between histones for binding sites on DNA. Loss of histone H1 is accompanied by an increase in the content of all four histones of the nucleosome nucleus in the chromatin (H3, H4, H2A, H2B). However, in the presence of a large excess of total histone added to the chromatin, the increase in the protein content in the chromatin takes place only on account of histones H3 and H4 (compare the first densitogram with the third and fourth, characterizing complexes formed in the presence of a 20- and 200-fold excess of histones over chromatin DNA). It was shown previously that in medium of physiological ionic strength and in the presence of a 20-fold excess of total histone over DNA, only histones H3 and H4 bind with it [2]. Competitive relations between histones and chromatin are thus the same as those with pure DNA.

Over the whole range of histone/DNA chromatin ratios from 20 to 200 the quantity of histones H3 and H4 bound with chromatin remains unchanged, as is clear from comparison of the bottom two densitograms in Fig. 1. It is interesting to note that in the presence of these large excesses of total histone relative to chromatin DNA, the content of histones H2A and H2B in the chromatin does not decrease (compare the top densitogram with the bottom two). The writers reported previously [3] that histones H2A and H2B disappear practically completely from chromatin obtained in medium of low ionic strength (0.7 mM Na-phosphate buffer, pH 7.0) in the presence of a 40-fold excess of added histone. The difference revealed in responses of chromatin to added total histone may be the result of structural differences between the preparations used.

There is evidence that in medium of low ionic strength the nucleosome nuclei of chromatin become unpacked [8]. In the light of these data it can be postualted that the presence of nucleosome nuclei in chromatin prevents exchange of the histones present in them with histones added to the chromatin. Unpacking of nucleosome nuclei is the condition which permits histone exchange and, as a result, replacement of histones H3 and H4, possessing high affinity for DNA, by histones H2A and H2B. This histone replacement in medium of low ionic strength takes place, incidentally, despite a decrease in differences between the competitive powers of the histones to bind with DNA when the ionic strength of the medium is lowered, which the writers found previously [1, 10].

The absence of even partial competitive displacement of histones H2A and H2B from chromatin in medium of physiological ionic strength in the presence of excesses of total histone five times greater than those which displace H2A and H2B almost completely from chromatin in medium of low ionic strength (provided that the difference in the affinity of histones for DNA is greater in physiological medium than in water), leads to the conclusion this histones of nucleosome nuclei are more firmly bound with DNA in medium of physiological than of low ionic

strength. This is reflected in the fact that if the ionic strength of the medium falls below the physiological level stable relations between histones of nucleosome nuclei and DNA are replaced by dynamic relations, i.e., exchange of intrinsic histones of the chromatin with free histones becomes possible.

According to the usual notion of purely electrostatic interaction between histones and DNA in medium of lower ionic strength, histones should be more firmly bound with DNA (see [11]). This conclusion can be taken as valid only for histone H1, for in a medium of low ionic strength a greater excess of total histone is required to displace H1 from chromatin than in physiological saline. Even if the added histone/DNA chromatin ratio is 2, histone H1 can still be found in the chromatin in a medium of low ionic strength [3], whereas at a physiological ionic strength H1 is displaced from the chromatin when the quantity of total histone added is equivalent by weight to the DNA content in the chromatin (Fig. 1).

This weakening of the ability of histone H1 resident in chromatin to be exchanged with free histones when the ionic strength of the medium falls below physiological is in agreement with recent work [4] showing that under these same circumstances the ability of histone H1 to be redistributed among oligonucleosomes obtained by digestion of the chromatin with DNase is weakened.

During the transition from medium of low ionic strength to physiological, the dynamic character of relations between DNA and histone H1 is thus intensified, but it is no longer manifested with histone of the nucleosome nuclei. This conclusion is in agreement with views on the purely electrostatic interactions of histone H1 and DNA in chromatin, but it requires the participation of certain other interactions in determining the strength of the bonds between DNA and histones H3, H4, H2A, and H2B. Views of some workers on the primary role of hydrophobic DNA–histone interactions in the relations of these histones with DNA (see [10]) were disproved by the present writers' experiments in which polyphosphate was used as a model of DNA deprived of its hydrophobic functional groups [1, 10]. Strengthening of bonds of histones of the nucleosome nuclei with DNA on an increase in ionic strength of the medium may perhaps take place through hydrophobic interhistone interactions which, in accordance with the mechanism described previously [1, 10], can increase the strength of ionic binding of DNA with histone.

The data on stabilization of relations between histones of nucleosome nuclei and DNA during the transition from medium of low to physiological ionic strength, obtained in the present experiments, may be useful when explaining the location of histones on DNA, for the dynamic character of these interrelations in medium of low ionic strength and, in particular, oscillation of histones between DNA strands, as found by Shick et al. [13] is a factor which makes this problem more difficult.

Exclusion of histone H1 from chromatin on the addition of total histone equivalent in weight to the quantity already present, in medium of physiological ionic strength, can be understood to mean that binding of one additional octamer with the nucleosomes removes one H1 molecule from it. This is an interesting fact, for during the S phase a process such as this in the cell may be one of the stages essential for replication and transcription of chromatin unpacking. The basis for this suggestion is the extensive evidence for the participation of histone H1 in the organization of supranucleosomal levels of chromatin packing [8] and data on reversible binding of histone octamers with nucleosome nuclei [14].

The response of chromatin to excess histones synthesized in cells treated with hydroxyurea, which inhibits DNA synthesis, has recently been analyzed [12]. The authors cited concluded that these excess histones are exchanged with the chromatin's own histones. The possibility cannot be ruled out that in such cells the nucleosome nuclei have been subjected to unpacking on account either of the direct or indirect action of hydroxyurea or of certain intracellular factors, enabling exchange not only of histone H1 to take place, but also of histones of nucleosome nuclei with excess free histones. The further development of research into the response of cell chromatin to excess histone may prove to be a promising trend in the study of the work of the genetic apparatus of eukaryote cells both under normal conditions and when DNA synthesis is inhibited, which may arise, in particular, under the influence of various pharmaceutical agents.

LITERATURE CITED

1. V. D. Paponov, Biokhimiya, 45, 1539 (1980).

2. V. D. Paponov, P. S. Gromov, and V. V. Rupasov, Byull. Éksp. Biol. Med., No. 8, 163 (1980).
3. V. D. Paponov, P. S. Gromov, and D. M. Spitkovskii, Byull. Éksp. Biol. Med., No. 6, 672 (1981).
4. F. Caron and J. O. Thomas, J. Mol. Biol., 146, 513 (1981).
5. F. Grellet, M. Delseney, and Y. Guitton, Nature, 267, 724 (1977).
6. R. D. Kornberg, Sciences, 184, 865 (1974).
7. U. K. Laemmli, Nature, 227, 680 (1970).
8. J. D. McGhee and G. Felsenfeld, Ann. Rev. Biochem., 49, 1115 (1980).
9. A. D. Mills, R. A. Laskey, P. Black, et al., J. Mol. Biol., 139, 561 (1980).
10. V. D. Paponov, P. S. Gromov, N. A. Sokolov, et al., Eur. J. Biochem., 107, 113 (1980).
11. I. Polacow, L. Cabasso, and H. J. Li, Biochemistry (Washington), 15, 4559 (1976).
12. G. Russev, L. Vassilev, and R. Tsanev, Nature, 285, 584 (1980).
13. V. V. Shick, A. V. Belyavsky, S. G. Bavykin, et al., J. Mol. Biol., 139, 491 (1980).
14. A. Stein, J. Mol. Biol., 130, 103 (1979).
15. G. Zubay and P. Doty, J. Mol. Biol., 1, 1 (1959).

INCREASE IN SYNAPTOSOMAL GLUTAMATE DECARBOXYLASE ACTIVITY UNDER THE INFLUENCE TETANUS TOXIN

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Tetanus toxin (TT) disturbs synaptic secretion of GABA [11]. Investigation of the action of TT on isolated nerve endings (synaptosomes) revealed a decrease in the transmembrane K^+ gradient [6, 3] and membrane depolarization [16], and disturbance of the release and uptake of mediators [2, 4, 6, 10]. The decrease in synaptic secretion of GABA may be explained by inhibition of glutamate decarboxylase (GDC) (EC 4.1.1.15), which synthesizes GABA. Meanwhile the membrane-mediated increase in GABA synthesis may be an indication of depolarization of the nerve ending membrane. The study of GDC activity in synaptosomes is therefore interesting as a means of elucidating the target and mechanism of action of TT.

Accordingly in the investigation described below the effect of TT was studied directly on GDC and also on the glutamate-decarboxylase activity of nerve endings during poisoning with TT *in vivo* and *in vitro*.

EXPERIMENTAL METHOD

TT purified by gel filtration [8] was used. To obtain local tetanus, 0.2 MLD (for rats) TT in 0.5 ml physiological saline was injected into the gastrocnemius muscles of both hind limbs of albino rats weighing 180-220 g. The lumbar enlargement of the spinal cord was removed 48 h later from seven to 10 rats treated with TT and the same number of healthy animals. Spinal synaptosomes were isolated and GDC activity determined in the experimental and control groups parallel in each experiment. Spinal cord tissue was homogenized in 10 volumes 0.32 M sucrose in a glass homogenizer with Teflon pestle. The suspension was centrifuged on the TsLR-1 centrifuge (1000g, 10 min, at 2°C) and the residue was resuspended and centrifuged under the same conditions. The two supernatants were pooled and centrifuged at 15,000g for

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