6. O. Desiderato, J. R. Mackinnon, and H. Hisson, Physiol. Behav., 87, 208 (1976).
7. S. S. Goldman and P. W. Albers, J. Biol. Chem., 248, 867 (1973).
8. G. A. Langer, J. Mol. Cell. Cardiol., 12, 231 (1980).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
10. W. Rathbun and V. Betlach, Anal. Biochem., 28, 439 (1969).
11. H. Reuter and H. Scholz, Pflüg. Arch. Ges. Physiol., 300, 86 (1968).

DNA METABOLISM IN RAT BRAIN DURING CONDITIONING

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During conditioning, RNA synthesis on a DNA template is known to be activated in the neurons and glial cells [9] and this is an essential stage in the mechanism of conditioned reflex formation [5, 4]. This may be accompanied by structural changes in the DNA itself. Changes in the level of DNA methylation [2, 14] and amplification and reparative DNA synthesis [7, 10, 15] are changes of this type. It has been shown for bacterial systems that a fall in the methylation level of DNA methylated by monofunctional agents takes place through excision of 5-methylcytosine, followed by repair of the single-stranded DNA breaks thus formed [13]. It has recently been suggested that a decrease in the 5-methylcytosine content in the DNA of animals, notably a return to normal after conditioning, can also take place through excision repair [1].

Since reparative DNA synthesis when replicative synthesis is suppressed during conditioning has not previously been studied, it was decided to undertake a parallel investigation of the 5-methylcytosine content and the level of reparative DNA synthesis during the formation of a conditioned active avoidance reflex in rats.

EXPERIMENTAL METHOD

Male Wistar rats weighing 190-210 g were used. Hydroxyurea was injected intraperitoneally into the animals 2 h before sacrifice in a dose of 50 mg/100 g body weight, sufficient to inhibit replicative DNA synthesis by 98% [12]. An intraperitoneal injection of [^{3}H]thymidine (specific radioactivity 55 Ci/mmole, USSR origin) in a dose of 100 μSi/100 g body weight also was given 10 min later. The conditioned active avoidance reflex was formed in the animals. For this purpose the animal was placed in a chamber through the floor of which a current of 4 mA was passed. The rats could avoid electric shocks by mounting on a platform placed in the center of the chamber. This conditioned reflex of active avoidance of nociceptive stimulation was formed in all animals in a time which varied from a few seconds to 5 min from the beginning of stimulation. Animals trained to avoid nociceptive stimulation remained on the platform in the chamber for up to 30 min, after which they were taken from the chamber and kept under ordinary conditions. The rats were decapitated between 6 min and 8 h after the beginning of the experiment. All subsequent procedures were carried out at 4°C. The brain was removed, the gray matter of the cerebral hemispheres was detached, and the cell nuclei isolated [6]. DNA was then isolated from the nuclei [11], its content [8] and nucleotide composition [3] determined, and the radioactivity of the DNA measured in residues on membrane filters in toluene scintillator.

EXPERIMENTAL RESULTS

As Fig. 1 shows, immediately after the beginning of conditioning, the 5-methylcytosine content in DNA from the cortical nuclei fell sharply below normal, but only 15 min later it increased, and after 30 min it reached a maximum, namely, 35% above normal. Activation of

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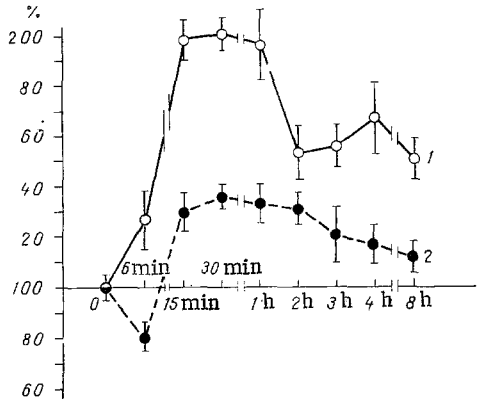

Fig. 1. Changes in 5-methylcytosine content in cerebral cortical DNA of rats during formation of conditioned active avoidance reflex and corresponding reparative DNA synthesis. Abcissa, time from beginning of experiment; ordinate, 1) level of reparative synthesis, 2) 5-methylcytosine content (in percent of control). In control: level of reparative synthesis and 5-methylcytosine content, amounting to 70 cpm/μg DNA and 1.07%, respectively, taken as 100%. Each point represents mean of three independent preparations of cell nuclei. Each preparation was obtained from 3-5 animals. For all DNA preparations, Chargaff's rule was observed; content of guanine + cytosine for all preparations did not differ significantly and was between 42.9 and 43.6 moles %.

reparative DNA synthesis under these circumstances had two phases. The first, which was more marked, followed primary demethylation of DNA, whereas the second, which was less well defined, corresponded to a slow decrease in the level of DNA methylation during the period when conditioned reflex formation was complete. The two phases of reparative DNA synthesis are thus evidently closely linked with the demethylation process, evidence in support of excision repair of regions containing 5-methylcytosine.

It can be postulated on the basis of these findings that reversible DNA methylation, involving excision of 5-methylcytosine, participates in expression of the genome in cells of higher organisms, and, in particular, the genome of cerebral cortical cells, and it can thus take part in temporary connection formation, one of the stages in the formation of systemic structural traces.

LITERATURE CITED

1. V. V. Ashapkin, G. A. Romanov, N. A. Tushmalova, et al., Nauchn. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 11, 30 (1981).
2. B. F. Vanyushin, N. A. Tushmalova, L. V. Gus'kova, et al., Mol. Biol., 11, 181 (1977).
3. V. K. Vasil'ev, Nauchn. Dokl. Vyssh. Shkoly, Biol. Nauki, No. 9, 118 (1971).
4. R. I. Kruglikov, in: Textbook of Physiology. Physiology of Higher Nervous Activity [in Russian], Moscow (1971), p. 34.
5. F. Z. Meerson, Plastic Provision for Functions of the Body [in Russian], Moscow (1967).
6. J. Austoker, D. Cox, and A. P. Marthias, Biochem. J., 129, 1139 (1972).
7. A. Bregnard, C. C. Kuenzle, and F. Ruch, Exp. Cell Res., 107, 151 (1977).
8. K. Burton, Methods Enzymol., 12-B, 163 (1968).
9. H. Hyden and P. W. Lange, Naturwissenschaften, 53, 64 (1966).
10. C. C. Kuenzle and A. Bregnard, Exp. Cell Res., 113, 151 (1978).
11. J. Marmur, J. Mol. Biol., 3, 208 (1961).
12. M. F. Rajewsky, Exp. Cell Res., 60, 269 (1970).
13. H. Reiter, R. B. Straus, M. Robbins, et al., J. Bact., 93, 1056 (1967).
14. B. F. Vanyushin, L. E. Nemirovsky, V. V. Klimenko, et al., Gerontologia, 19, 138 (1973).
15. R. J. Williams, Adv. Radiat. Biol., 6, 161 (1976).