respiration rate v on the time interval in the case of constant Mt area S=const, whereas the dependence on Mt area under constant time t = const is expressed weakly. The combined correlation gives a more accurate description of the dependence of Mt activity on both factors at once and leads to a number of conclusions.

Thus, 1) the Mt respiration rate can be described by a linear correlation with the time period and Mt area; 2) negative coefficients in the equations indicate that the respiration rate drops both with an increase of the time and with augmentation of the Mt area; 3) the equations obtained permit an estimatation of the state of the Mt population according to Mt sizes and the time period and show that the larger the Mt is, the shorter life it has; 4) it may be assumed that the rate of Mt mortality will depend on the dose of radiation, and this leads to changes of the equation coefficients.

Probably, further investigations will prove or refine our conclusions. This calls for appropriate studies using the proposed scheme of correlation analysis. In our view, such an approach makes the evaluation of morphofunctional relationships in medical and biological assays more objective.

We are indebted to Dr. N.A.Yurina for her help in the discussion of some aspects of this investigation.

REFERENCES

1. G. G. Avtandilov, V. V. Sirotkin, and E. V. Kozyreva, *Byull. Eksp. Biol.*, **92**, № 8, 96-97 (1981).
2. A. I. Karasev, *Probability Theory and Mathematical Statistics* [in Russian], Moscow (1979).
3. V. M. Mityushin, V. V. Sirotkin, and E. V. Kozyreva, *Ninth Conference on Electron Microscopy*, Moscow (1973), pp. 389-390.
4. V. V. Sirotkin, in: *Statistical Properties of Microstructures* [in Russian], Moscow (1978), pp. 192-193.
5. V. V. Sirotkin, *Med. Ref. Zh.* **XXII**, № 12, (1985).
6. V. V. Sirotkin, *Method of Estimating the Morphofunctional of Mitochondria State, Pat. № 14887200 SSSR* (1989).
7. V. V. Sirotkin and N. D. Volodin, *Byull. Eksp. Biol.*, **110**, № 7, 100-103 (1990).
8. V. V. Sirotkin, A. Yu. Grishin, V. A. Smirnov, *et al.*, *Byull. Eksp. Biol.*, **115**, № 2, 215-218 (1993).

Electron-Microscopical Study of Cardiomyocyte Chromatin in Epinephrine-Treated Dogs

R. I. Klyasheva

UDC 576.3.315.42

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 3, pp. 305–307, March, 1993
Original article submitted April 13, 1992

Key Words: *cardiomyocytes; chromatin rearrangement; epinephrine; nucleoplasmic chromatin; perimembrane chromatin*

It is well known that one of the key pathogenic mechanisms switched on by extreme conditions is endogenous epinephrine, the boosted synthesis and release of which into the blood leads to a hypertoxic effect on the cardiomyocytes (CM), resulting in a significant drop of the level of DNA methylcytosine (5-MC). Biological methods [4,5] previously used by us are associated with the study of DNA methylation by cytosine.

In the present work we studied the chromatin ultrastructure and, in particular, the area of dense

Department of Pharmacology, I. N. Ul'yanov State University, Chuvash. (Presented by A. D. Ado, Member of the Russian Academy of Medical Sciences)

0007–4888/93/0003–0336$12.50

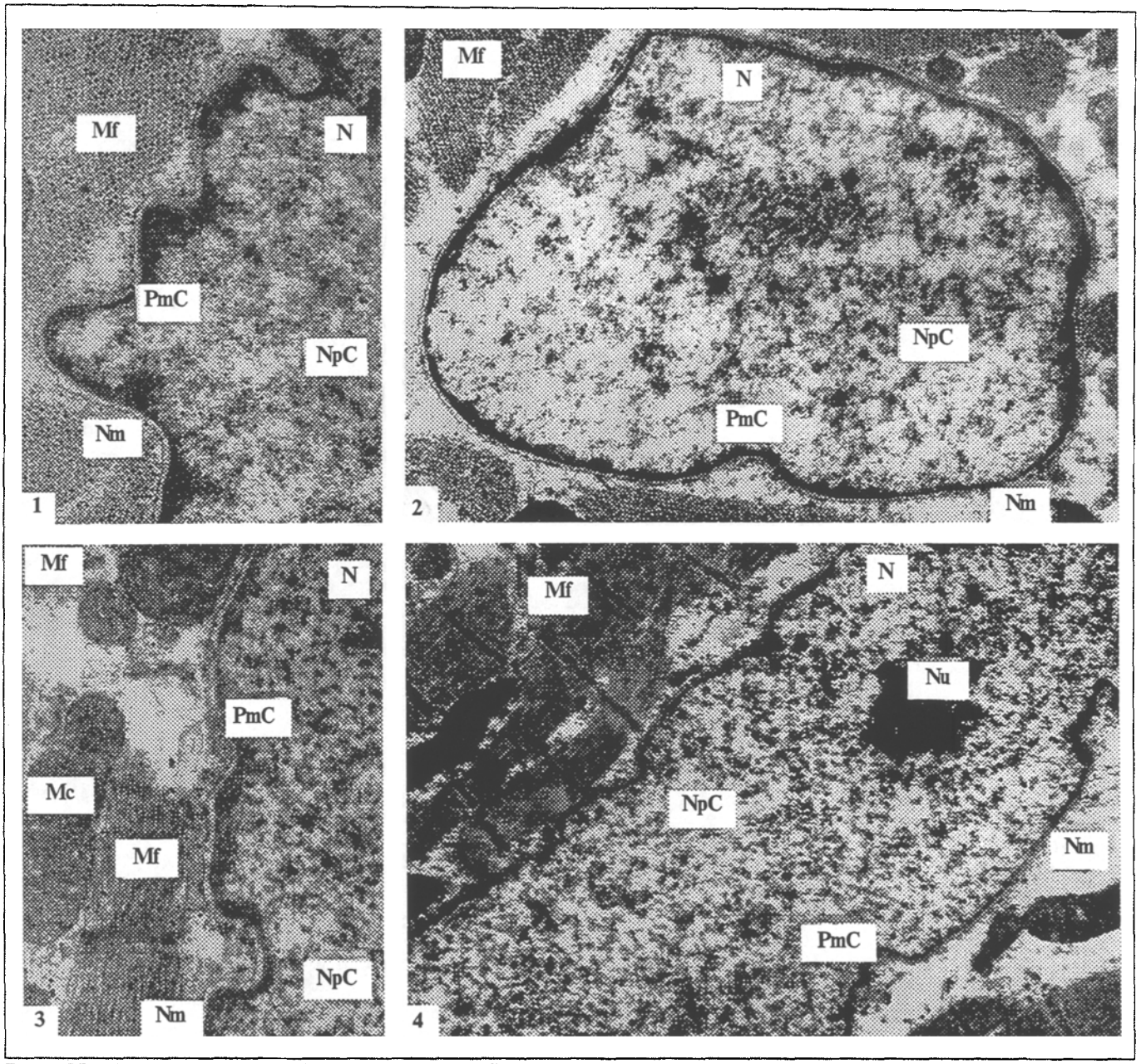

Fig. 1. Ultrathin section of dog cardiomyocyte nuclei. *1, 2*) control groups; *3, 4*) treated groups. At left: fragments of nuclei (×16,000), at right: nuclei (×7500). N: nucleus; Nu: nucleolus; NM: nuclear membrane: NpC: nucleoplasmic chromatin; PmC: perimembrane chromatin; Mf: myofibrils; Mc: mitochondria.

perimembrane chromatin (PmC) in dog CM nuclei under epinephrine treatment using the electron microscopic technique.

MATERIALS AMD METHODS

The experiments were carried out on 10 male mongrel dogs weighing 10-14 kg (3-5 years old). The experimental dogs received epinephrine i.v. 100 μg/kg during 5 days. Directly before injection the dose of epinephrine was dissolved in 10 ml saline.

The intravenous infusion lasted 5 min. The control animals were injected with saline. On the fifth day the animals were sacrified with sodium pentobarbital (1g in 50 ml sterile water). The heart and liver were removed immediately. Pieces of tissue 1 mm^3 were fixed in 2.5 % glutaraldehyde solution (Serva, Germany), on Na-cacodylate buffer (pH 7.4) during 4 hours. Postfixation was performed with 2% osmium tetroxide using the same buffer for 1 hour. The tissue were then dehydratated in ascending grades of alcohol and acetone according to the following scheme: alcohol: 30°, 50°, 70°, 80°, 96° - 10 min × 2, 100° - 15 min × 2; acetone 100%- 15 min × 2.

After dehydration the tissue specimens were embedded in an Epon-812, DDSA, MNA, and DMP-

TABLE 1. Areas (conventional units) of NpC and PmC in Cardiomyocyte Nuclei of Dogs under Epinephrine Treatment

Group	Area			Ratio of Pmc to nucleus area	PmC per cent of nucleus area
	nuclei $x \pm x_s$	NpC $y \pm y_s$	PmC $z \pm z_s$		
Control	0.6862±0.0001	0.6130±0.0002	0.0734±0.0001	0.1069	10.69 $p<0.001$
Treated	0.5328±0.0001	0.5054±0.0001	0.0274±0.0002	0.0514	5.14

30 mixture. Polymerization was performed at temperatures of 37°, 45°, 60°C during 3 days. The specimens were sectioned on an LKB ultramicrotome. Serial ultrathin (70nm) sections were double-stained with uranyl acetate (2% aqueous solution) and lead citrate for 90 and 10 min, respectively, at 37°C. The grids were examined in a JEM-7 electron microscope. The areas of dense PmC in the CM nuclei were studied in the following manner. Tracing paper was placed over the nuclear image obtained at electron microscopic magnification ×10,000 and at photographic magnification ×3.5. The zones of nucleoplasmic chromatin and dence PmC were traced, then cut out, and weighed on an analytical balance to within 1 mg and expressed in conventional units [1,7]. The data were processed statistically by Student's *t* test.

RESULTS

Electron-microscopic study of CM nuclei of control animals showed that the dense PmC was intimately connected with the internal layer of karyolemma and had a friable, scalloped margin (Fig. 1, *1, 2*). The CM of treated animals CM had a thin, even layer of dense PmC (Fig. 1, *3, 4*). The results of the electron-microscopic study are presented in Table 1.

As may be seen from the table, the ratio of the area of dense PmC to the nuclear area in the control animals is twice that in the treated animals.

It is well known that epinephrine does not penetrate into target cells [2,6], but launches a chain biochemical reactions connected with cAMP and cGMP production, followed by phosphorylation of chromosomal proteins [2,3,6]. In addition, the epinephrine signal lowers the level of DNA 5-MC (by some mechanism that is not understood) [4]. Phosphorylation of chromosomal proteins and methylation of DNA cytosine bases are connected with changes in chromatin state, and thus with variations in gene expression [7].

From the results obtained it may be stated that under epinephrine injection dog cardiomyocyte nuclei undergo a decrease the area of dense PmC, apparently as a result of chromatin reorganization.

We are indebted to Dr. D. S. Gordon for her help in the research.

REFERENCES

1. G. G. Avtandilov, *Medical Morphometry: A Manual* [in Russian], Moscow (1990), pp. 275-280 .
2. B. Alberts, D. Bray, J. Lewis, *et al.*,(Eds.) *Molecular Biology of the Cell*, Garland (1983).
3. T. T. Berezov and B. F. Korovkin, in: *Biological Chemistry: A Textbook* [in Russian], ed. S.S.Debov, 2nd adition, Moscow (1990), pp. 189-191.
4. R. I. Klyasheva and Zh. Kh. Vasil'ev, *Nauchn. Dokl. Vyssh. Shkol., Biol. Nauki.*, № 10, 31-33 (1984).
5. R. I.Klyasheva, Zh. Kh. Vasil'ev, and Z. I. Ivanova, *Nauchn. Dokl. Vyssh. Shkol., Biol. Nauki.*, № 12, 32-34 (1987).
6. A. Lehninger, *Biochemistry: The Molecular Bases of AM Structure and Function*, 2nd ed., Worth (1975).
7. V. M. Manteifel', E. B. Romanenko, D.P. Babadzhanyan, *et al.*, *Molek. Biol.*, **22**, № 4, 1087-1095 (1988).