cytoplasm of the enterocytes, and swelling and desquamation of individual microvilli [1, 4, 6, 7]. No such changes could be found in germfree rats with a chronic vibrio carrier state. Intensification of the functional activity of the enterocytes (hypertrophy of the lamellar complex, an increase in the number of lysosomes in the cytoplasm) probably took place because of absorption of some soluble metabolic products of the vibrios. Projection of the base of the enterocytes through the basement membrane must evidently be regarded as potentiation of epitheliomesenchymal interaction [9].

The pattern of activation of immunocompetent cells in response to administration of *cholera vibrios* described above, in the absence of any pathological changes in the mucous membrane of the small intestine, must be regarded as evidence of the basically healthy state of germfree rats with a chronic vibrio carrier state.

LITERATURE CITED

1. A. P. Avtsyn, V. A. Shakhlamov, and O. F. Sageeva, Arkh. Patol., No. 3, 41 (1973).
2. O. V. Baroyan, O. V. Chakhava, I. N. Gailonskaya, et al., Byull. Eksp. Biol. Med., No. 5, 561 (1976).
3. E. M. Gorskaya, O. V. Chakhava, and N. M. Shustrova, Arkh. Patol., No. 2, 51 (1978).
4. Yu. E. Polotskii, E. M. Dragunskaya, E. S. Snigirevskaya, et al., Arkh. Patol., No. 2, 10 (1977).
5. N. B. Shalygina, T. S. Efremova, F. A. Tumanov, et al., Arkh. Patol., No. 12, 55 (1973).
6. N. B. Shalygina, Arkh. Patol., No. 11, 16 (1974).
7. H. Asakura, N. Tsuchiya, Y. Watanabe, et al., Gut, 15, 531 (1974).
8. E. J. Gangarosa, W. R. Beisel, C. Benyjati, et al., Am. J. Trop. Med. Hyg., 9, 125 (1960).
9. M. Mathan, J. Hermes, and J. S. Trier, J. Cell Biol., 52, 577 (1972).

INTENSITY OF RNA SYNTHESIS AND DNA CONTENT IN THE NEONATAL RAT MYOCARDIUM DURING ADAPTATION TO HIGH ALTITUDE HYPOXIA

V. A. Kononova

UDC 612.172.015.36:547.963.32]:[612.648:612.275.1.017.2

KEY WORDS: myocardium; hypoxia; RNA synthesis; DNA; light autoradiography.

In the genesis of heart muscle differentiation of myocytes is not accompanied immediately by their quitting the mitotic cycle. According to data obtained by various workers, proliferative activity of rat cardiomyocytes is considered to continue for a long period after birth [2, 4, 7, 9]. Meanwhile doubts have been expressed even about the data of light microscopy and autoradiography on DNA synthesis and mitotic activity of cardiomyocytes during early postnatal development [10, 12]. Some particularly heated discussions have taken place on the question of the behavior of DNA in muscle cell nuclei during hyperfunction and hypertrophy of the myocardium both in adults and during the period of its early ontogeny [1, 6].

It was accordingly decided to use the method of light autoradiography to study RNA synthesis and DNA content in nuclei of muscle and nonmuscle cells of the neonatal rat heart during gradual adaptation to high-altitude hypoxia, throwing a measurable functional load on the myocardium, and also during a single exposure to high-altitude hypoxia under different conditions.

Kirghiz Research Institute of Obstetrics and Pediatrics, Frunze.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yanninov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 5, pp. 100-102, May, 1982. Original article submitted December 22, 1981.

0007-4888/82/9305-0670$07.50

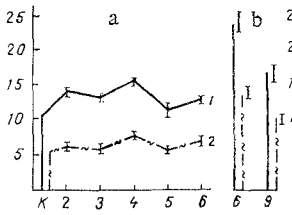

Fig. 1

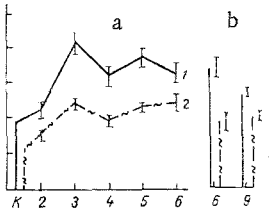

Fig. 2

Fig. 1. Time course of RNA synthesis in neonatal rat heart during gradual adaptation (a) and after a single exposure (b) to high-altitude hypoxia. Abscissa, altitude (in thousands of meters); ordinate, number of grains of silver above nuclei of muscle (1) and connective-tissue (2) cells. K) Control.

Fig. 2. Time course of DNA content in neonatal rat heart during gradual adaptation (a) and after a single exposure (b) to high-altitude hypoxia. Remainder of legend as to Fig. 1.

EXPERIMENTAL METHOD

Training in a pressure chamber under an atmospheric pressure corresponding to altitudes of 2000, 3000, 4000, 5000, and 6000 m successively above sea level, for 6 h daily, was given to 48 newborn rats aged 1-2 days. The animals were divided into three groups: 1) rats receiving an intraperitoneal injection of labeled RNA precursor [5-^{3}H]uridine in a dose of 30 μCi (specific activity 22.5 Ci/mmole) 1 h after every ascent to a high altitude, and sacrificed 6 h later, 2) rats receiving an intraperitoneal injection of the DNA precursor [^{3}H]-thymidine in a dose of 1 μCi (specific activity 22 Ci/mmole) after each ascent to a high altitude, and killed 1 h later, 3) rats taken up in a pressure chamber to an altitude of 6000 and 9000 m for 6 h once only. These animals were given [5-^{3}H]uridine and [^{3}H]thymidine in the same doses as the rats of the first two groups.

The control group consisted of eight rats of the same age as the experimental animals, which received [5-^{3}H]uridine and [^{3}H]thymidine without training in the pressure chamber.

After sacrifice of the animals the heart was fixed in Carnoy's fluid and embedded in paraffin wax.

Sections were coated with type M photographic emulsion and exposed for 32-56 days. The sections were stained with hematoxylin and eosin. The intensity of RNA and DNA synthesis was judged by the number of grains of silver above the nuclei of the muscle and stromal cells. Visual counting of the grains was carried out in 100 muscle and nonmuscle cells of each rat. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of experiments to study RNA synthesis in the muscle and nonmuscle cells of the rat heart are given in Fig. 1. They show a statistically significant increase in the number of muscle nuclei with increased ability to synthesize RNA after the first few days of gradual adaptation to high altitudes. The increase in the number of grains above nuclei of cardiac myocytes took place in cycles; the number of grains of silver at each stage of the experiments remained higher than in the control animals.

The total quantity of [5-^{3}H]uridine taken up by the muscle nuclei increased toward the 5th day of training in the pressure chamber by 1.6 times: 1750 grains of silver were counted above 100 muscle nuclei compared with 1050 grains in the control ($P < 0.001$). The number of grains of silver above stromal cell nuclei in the control animals was less than the number above cardiomyocyte nuclei (5.2 ± 0.44 and 10.5 ± 0.28 respectively). During gradual adaptation to a high altitude the intensity of incorporation of [5-^{3}H]uridine into the nuclei of

the stromal cells increased gradually. By the 4th day of training the number of grains above the stromal nuclei was increased by 2.3 times ($P < 0.001$).

A single ascent to an altitude of 6000 m was accompanied by the most intensive incorporation of [5-^{3}H]uridine into nuclei of both muscle and nonmuscle cells.

During exposure to high-altitude hypoxia at an altitude of 9000 m the number of grains of silver above the cardiomyocyte nuclei and in the stroma decreased significantly. This observation is a further reminder of the heterogeneity of the myocardium and the structural and functional heterogeneity of its cells. A similar pattern was found in a study of adaptive changes in the DNA content in the neonatal rat heart, the results of which are illustrated in Fig. 2. The following conclusion can be drawn from analysis of these data. In the early postnatal period in rats under the influence of high-altitude hypoxia the intensity of incorporation of [^{3}H]thymidine into nuclei of muscle and nonmuscle cells of the heart rises compared with that in control animals. The increase in the number of grains of silver above nuclei of interstitial cells takes place more rapidly than that above cardiomyocyte nuclei as the duration of gradual adaptation to a high altitude increases. For instance, on the 5th day of training in the pressure chamber at an altitude of 6000 m the number of grains of silver above the muscle cell nuclei had increased by 66.6%, but in the interstitial cells by 101.7%; after a single exposure of the animals to an altitude of 9000 m the increase was 40.6 and 101.3% respectively ($P < 0.001$).

These observations suggest that the level of RNA synthesis and the DNA content may be indicators of the relationship between destructive and regenerative processes in the cell during compensatory hyperfunction of the heart during exposure to pressure chamber hypoxia under different conditions. The emergency stage of gradual adaptation to high-altitude hypoxia during early postnatal development of rats, given the natural proliferative activity of the cardiomyocytes, is characterized by intensification of nucleic acid synthesis in the muscle and interstitial cells of the heart.

More rapid incorporation of protein precursors into cardiac myocytes of adult and newborn animals was observed separately in response to an increased functional load and in certain pathological states [5, 8, 11].

The reduced RNA synthesis and DNA content in the heart of animals at a high altitude (9000 m) probably reflect a deficiency of high-energy compounds arising as a result of an excessively increased functional strain on the cardiomyocytes, followed by the development of dystrophic changes [3, 6]. These dystrophic changes are a unique triggering mechanism facilitating the development of compensatory and adaptive reactions of the myocardium, determined by intracellular processes of regeneration. High-altitude hypoxia, a nonspecific stimulus for the myocardium, causes activation of RNA synthesis and an increase in the DNA content in the nuclear populations of muscle and stromal cells. The results suggest that in early postnatal development of heart muscle regulatory links exist between muscle and connective-tissue cells, through which the level of contractile function of the muscle cells can determine the intensity of protein synthesis and, possibly, of cell proliferation. These links can play a role in the provision of an adequate substrate for the activity of the heart during its hyperfunction under conditions of high-altitude hypoxia.

LITERATURE CITED

1. L. N. Belov and M. E. Kogan, Tsitologiya, No. 6, 722 (1973).
2. V. N. Galankin, Arkh. Patol., No. 2, 37 (1975).
3. V. N, Galankin, A. A. Pal'tsyn, and A. K. Badikova, Byull. Eksp. Biol. Med., No. 6, 751 (1977).
4. M. E. Kogan, L. N. Belov, and T. A. Leont'ev, Arkh. Patol., No. 1, 77 (1976).
5. P. P. Rumyantsev, Arkh. Anat., No. 7, 15 (1973).
6. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Electron-microscopic Autoradiography of the Cell [in Russian], Moscow (1980), p. 140.
7. V. F. Sidorova and G. B. Bol'shkova, Byull. Eksp. Biol. Med., No. 4, 475 (1980).
8. P. Anversa, L. Vitali-Mazza, and A. Gandolfi, Lab. Invest., 33, 125 (1975).
9. F. J. Manasek, in: Developmental Regulation. Aspects of Cell Differentiation, New York (1973), pp. 193-217
10. J. S. Polinger, Exp. Cell Res., 76, 253 (1973).
11. G. Unge, Acta Pathol. Microbiol. Scand., Sec. A, 81, 806 (1973).
12. R. Zak, Am. J. Cardiol., 31, 211 (1973).