THE NEW ARMENIAN
MEDICAL JOURNAL
Vol. 2 (2008), N 3, 83 - 102
www.ysmu.am

ROLE OF PUTRESCINE IN PROCESSES OF INCREASED HEART CONTRACTILE ACTIVITY FORMATION

(Experimental Research: First Communication)

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Abstract

The influence of putrescine low concentrations on contractile activity of myocardium in large and small laboratory animals and possible mechanisms of its direct and/or mediated influence on morphofunctional state of cardiomyocytes was studied. Single intravascular injection of 10^{-8} *mg/ml* putrescine to mice was accompanied by elevation of functional indices of heart contractile functions, which were expressed as the increase of heart mechanical contractions amplitude, increase of the apical potential in electrocardiogram. No signs of rapid or reduced heart rate were observed.

Echo-tests /ultra-sound/ in young puberal male pigs revealed that already in a minute after the injection of putrescine at the same concentrations as administered to experimental mice, the contractile ability of myocardium increased. This latter was expressed in increase of emission fraction of the left ventricle of heart. The most positive inotropic effect of putrescine was observed on the fourth minute after the injection of putrescine. The indicated parameters were normalized, i.e. reached the control values on the tenth minute of the experiment. Structural alterations characterising the high contractile ability of myocardium of the left ventricle in experimental mice were revealed by phase-contrast microscopy. With the help of enzyme immunoassay, it was established that on the 20th minute post injection of putrescine the content of adrenalin was considerably increased in heart, adrenals, and blood serum. The established fact of activation of synthetic processes occurring in the medulla of adrenal glands was confirmed by means of fluorescent microscopic research that allowed to register structural changes in endocrynocytes testifying to their hyperfunction.

On the background of an increase of intracellular ionised calcium content, the decrease of adrenergic nerve fibrils number with low content of noradrenaline was observed in myocardium of experimental animals. Due to the research performed, the conclusion might be drawn suggesting that the positive inotropic effect to heart under the conditions of injection of putrescine to experimental animals is mostly conditioned by processes of activation of catecholamine synthesis in the adrenals followed by their enhanced inflow into blood and heart.

In relatively later period of observation (8 hours post the injection of putrescine), the marked increase in the content of insulin-like growth factor (IGF-1) was observed in heart of experimental mice, thus allowing to conclude on its modulator influence on processes proceeding in myocardium, which are "concerned" in realization of the contractile function. The administration of rather low concentrations of putrescine to experimental animals was not accompanied by emergence of heart dystrophic disorders, signs of myocardium remodeling, activation of nitrogen oxide (NO) and cytokines.

Keywords: polyamines, putrescine, cardiomodulatory effect, catecholamines, cytokines, nitrogen oxide, growth hormones.

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Introduction

Cardiomodulatory effects of such polyamines as putrescine, spermine, and spermidine make the subject of comprehensive investigation during the last twenty years. The aspects relevant to the mentioned direction of research are rather intensively developed and aimed at elucidation of the probable mechanisms, underlying synthesis and inhibition of polyamines in myocardium.

In overwhelming majority of works shifts of the content of polyamines and, first of all, that of putrescine, are connected to activity of ornithine decarboxylase (ODC) and spermidine-spermine aminotransferase (SSAT) in myocardium, to their influence on β-adrenergic receptors, influence on cardiomyocytes of cytokines and other immunomodulating factors [*Flamigini F. et al., 1986; Tipnis U.R., Skiera C., 1989; Hasegawa S. et al., 1997; Cepero M. et al., 1998; Cubria J.C. et al., 1998; Mackintosh C.A. et al., 2000; Tantini B. et al., 2001; Zhao et al., 2007*].

Carrying out experimental research many authors adhere to concrete methodological principle based on studying cardioprotective (modulating) effects of polyamines by means of modeling various pathological processes in myocardium: ischemia, "myocardial infarction", injection of agonists and antagonists to β-adrenergic receptors, use of transgenic animals with hypertensive syndrome and high ODC content in heart [*Ruskoaho H., Raunio H., 1987; Mezl V.A. et al., 1988; Solani G. et al., 1991; Tagliavini S. et al., 1991; Shimizu M. et al., 1992; Tipnis U.R. et al., 1994; Nakano M. et al., 1995; Ibrahim J. et al., 1996; Cepero M., et al., 1998; Lopatin A.N., et al., 2000; Tipnis U.R. et al., 2000; Panama B.K, Lopatin A.N., 2006; Tantini B. et al., 2006; Flamigini F. et al., 2007; Stanic I. et al., 2008*].

It should be specially mentioned that rather high concentrations of putrescine were used in almost all studies (exceeding those determined in blood serum and myocardium of intact rats and mice, - grater by orders of magnitude) [*Tagliavini S. et al., 1991; Ohta H. et al., 1993; Morrison R.F., Seidel E.R., 1995a; Til H.P. et al., 1997; Stefanelli C. et al., 2000*]. Therefore, in this case the authors studied dose-dependent pharmacological effects of putrescine, which in no case would allow to extrapolate the obtained results from the point of view of interpreting the cardiomodulatory role of putrescine under conditions of cardiovascular system normal functioning. In our opinion, many biological effects of putrescine should be directly and/or are indirectly conditioned by its low concentrations, which are practically identical to concentrations of many endogenous active factors of hormone- and mediatory spectrum of action, both in blood serum, and in studied target-organs.

Therefore, to our mind, it seems more promising, to perform research aimed at biological effects of putrescine in myocardium with the use of its precisely low concentrations that are rather close to those determined in blood serum and myocardium of mammals.

The influence of exogenous putrescine on morphofunctional state of myocardium in test animals is studied in this research in order to define contractile activity of the latter, taking into consideration precise endogenously active factors, directly and/or indirectly concerned in processes of myocardium remodeling and its contractile functions.

Materials and methods

The research was performed in 110 female mice (40-50 *g*) and 12 male pigs aged 1 year (110-120 *kg*) In all series and test groups, with the observance of antiseptic rules, the same single dose of putrescine was intravascularly injected to experimental animals: 10^{-8} *mg/ml*. Putrescine was injected to jugular vein of mice and to aural vein of pigs.

Cardio-ultrasound research in pigs was performed at the Ultrasonic educational centre of YSMU, on Ultramerk-9 (USA) device with M-B and Doppler region studies before and after the injection of putrescine. The control group involved 6 pigs and 20 mice with only single intravenous administration of saline of the same volume as that used to dissolve putrescine (0.1 *ml* for mice; 2 *ml* for pigs). Using Echo-tests, the following indices of functional condition of the left ventricle were evaluated:

1. Final diastolic size;
2. Final-Systolic size;
3. Ejection fraction;.
4. Doppler colasters of diastolic function

Photoelectric registration of mechanical contractions of heart was performed in mice under nembutal narcosis before and after the injection of putrescine.

The apical potential of heart deduced off thorax was simultaneously registered. Echo-examination and registration of muscular contractions were done under nembutal narcosis (single intraperitoneal injection of 30 *mg/kg* nembutal).

While carrying out morphological and immune-fluorescent research, mice under study were withdrawn from the experiment in 20 minutes, 4 and 8 hours after injection of putrescine, with observance of all generally accepted ethical standards. After the sacrificce the slices from the left ventricle were fixed in Karnua liquid and after corresponding processing were fixed in paraffin. Slices were stained with hematoxylin-eozine. In another series of the experiment freshly frozen cryostate-derived tissue sections of the left ventricle were examined with the help of phase-contrast microscope in order to establish contractile potentialities of microfibriles

In two other series of the experiment, the topical features of cathecholamines and ionized calcium distribution were defined in fresh-frozen sections prepared from the left ventricle and adrenal glands.

For determination of cathecholamines (well-known Falck-Hillarp method) glasses were fixed on the support made of organic glass and placed in a glass cylindrical chamber with the volume of 1 litre. Fifteen grams of paraformaldehyde powder preliminary kept for 7 days in an extract containing 40% sulfuric acid was filled to the bottom of the chamber. The chamber was closed by a cover and kept in the thermostat at 80°C for an hour. Paraformaldehyde obtained from "Sigma" Co. was used in the experiments. Upon determination of the ionized calcium the method was used for its indication by means of a chlortetracyclin probe [*Vladimirov Yu., Dobretsov G., 1980*].

Preparations were examined with the help of luminescent microscope (BOECO, Germany), with reproduction of the obtained image on a digital camera (CANON, Japan).

Carrying out the immune-enzyme research in order to determine adrenalin, IGF-1, such cytokines as interleikines IL-1, IL-2, IL-6, interferon-γ and nitrogen oxide (NO), slices taken from the left ventricle of heart of mice were dried on filter paper, weighed on torsion balance, then treated with the help of glass-teflon homogenizer in cooling conditions (at 4°C). The homogenate was diluted in the physiological saline at 6°C and then centrifuged at 15000 *g* for 15 minutes in cold setting

The content of adrenalin and IGF-1 in blood serum and supernatants was defined by means of commercial kits (DRG-International Inc., USA-Germany). Adrenaline and IGF-1 levels were expressed as *ng/ml*. The content of prolactin was determined with the help of appropriate kits (SYNTRON Bioresearch Inc., USA-Germany), while NO - by kits of Assay Design Inc. (USA). The content of prolactin was expressed in *ng/ml*, and the NO content was expressed as μmole/*L*. Cytokines were determined with the help of anti-murine commercial kits for IL-1, IL-2, IL-6 and INF-γ, (DRG International Inc., USA-Germany) and the content was expressed in *pg/ml*. The choice of precise terms for determination of cytokines (4 and 8 hours of observation) was done, taking into consideration kinetics and increase of their transcripts to mRNA and to appearance of cytokines in cultural medium of stimulated lymphocytes [*Simbirtsev A. 1998 a;b*].

The calculation of immune-enzyme assay results was done using Stat Fax 3200 automatic analyzer (AWARENESS Technology Inc., USA) in compliance with the requirements of the instruction on methods of determination available for each kit. Construction of the calibrating curve and determination of concentration of samples was done by means of Statistical software SPSS.

In order to calculate the average values and root-mean-square deviation of errors of ELISA, the Compare Means mode for Independent

Samples t-test (Student's test) was used, as well as the One-Way ANOVA that considered Student' criteria, so that to allow comparison of values in Control and test groups.

The study on the influence of putrescine on contractile activity of myocardium in rats under conditions of an acute experiment *in situ* and mechanical ventilation of lungs demonstrated that its injection at the dose level of 10^{-8} *μg/ml* was accompanied by the increase of heart contractile function indices during the precise period of observation (Table 1).

Whereas on the first, fifth and tenth minutes after injection of putrescine the indices of heart contractions amplitude were within the range of control values, on the twentieth minute of the experiment the given indicator exceeded the control level 1.7 times. Similar high level was also observed on the 40th minute of the experiment. In the subsequent intervals (from 60 to 120 minutes), the heart contraction amplitude indices practically did not differ from those in rats of control group.

It is especially worth to mention that the increase of cardiac mechanical contractions amplitude registered by us in precise time intervals rather accurately correlate with the dynamics of apical potential of the electrocardiogram, which exceeded the control level 2.9 and 1.9 times on the 20th and 40th minutes, accordingly (Table 1).

During the period of the experiment, no signs of frequency increase or decrease in cardiac contractions were observed. Thus, indices, char-

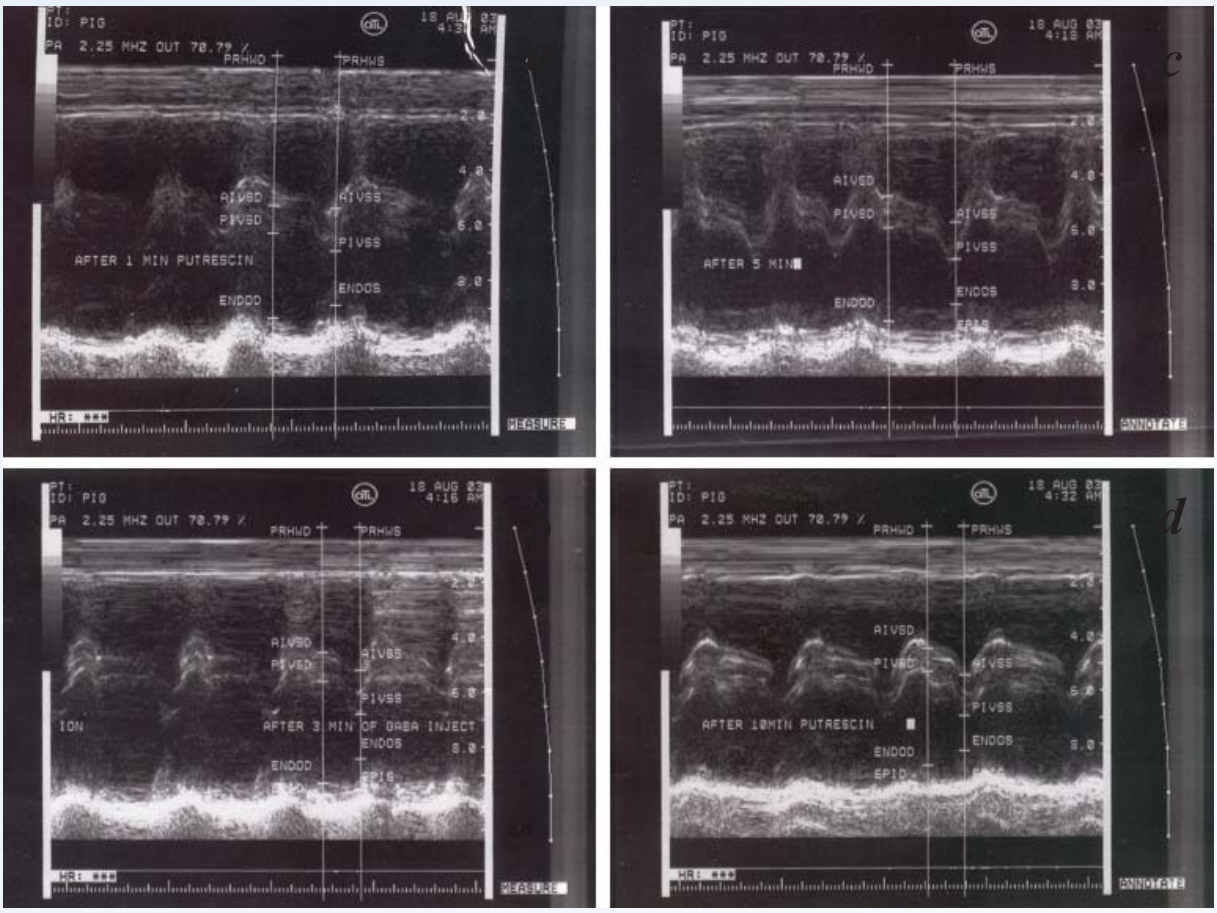
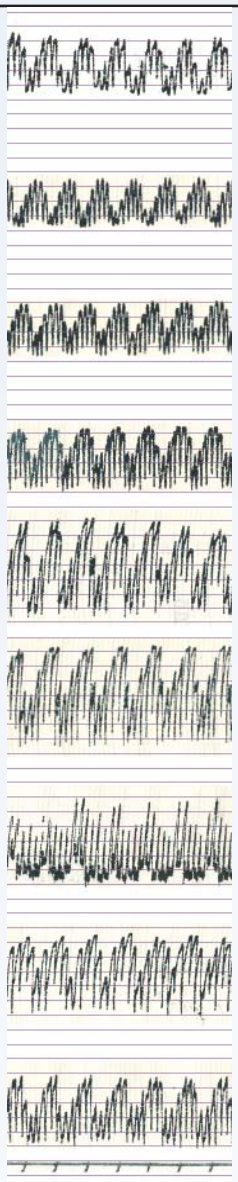


Figure 1. *M- mode echocardiographic studies after 1min (a) , 3 min (b) , 5 min (c) and 10 min (d) of putrescine injection with measurements of left ventricular structural parameters.*

Table 1.

Influence of putrescine on heart contractile activity in rats under conditions of mechanical lung ventilation

Time interval after injection of putrescine (in minutes)	Amplitude cardiac contraction (in mm)	Frequency of cardiac contractions (in bpm)	Apical electrocardiogram (in mechanical ventilation)	Mechanogramm
Control group	9,5±1,3	184,2±9,6	17,3±1,4	
1	8,1±1,0 P>0,5	167,1±10,5 P>0,5	25,2±3,6 0,1>P>0,05	
5	8,3±1,1 P>0,5	173,0±7,4 P>0,5	20,4±2,2 P=0,25	
10	9,8±1,4 P>0,5	182,4±6,3 P>0,5	19,3±2,4 P=0,25	
20	16,3±1,7 0,01>P>0,002	165,6±6,0 0,25> P>0,1	49,6±5,8 P<0,001	
40	15,8±1,6 0,01>P>0,002	182,9±7,4 P>0,5	32,5±6,3 0,05>P>0,02	
60	13,3±1,3 0,1>P>0,05	171,0±9,1 P>0,5	24,4±3,5 0,1>P>0,05	
90	13,2±1,5 0,1>P>0,05	163,7±7,0 P=0,1	21,4±2,7 0,25>P>0,1	
140	13,0±1,6 0,25>P>0,1	170,4±4,5 0,25>P>0,1	20,7±2,1 0,25>P>0,1	

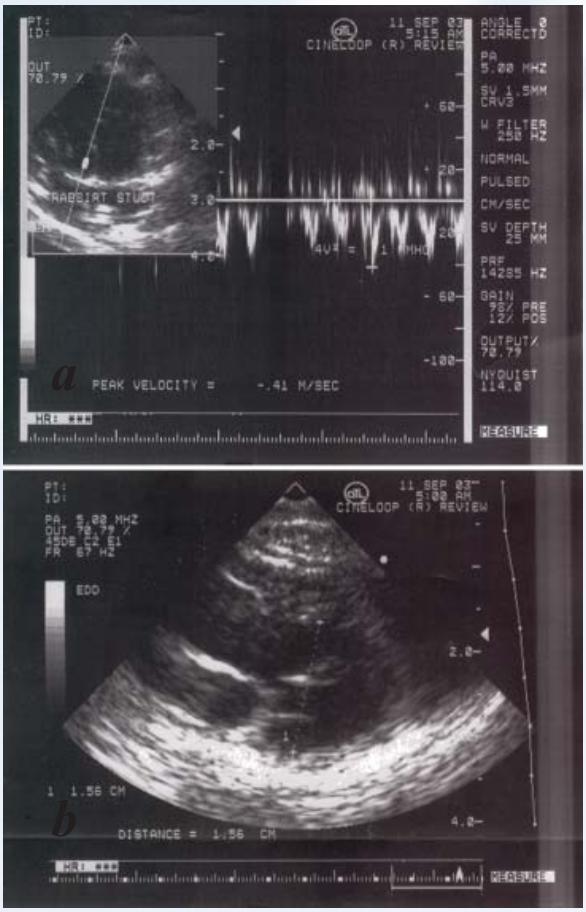


Figure 2.
a/ *Doppler echocardiographic study after 10 min of putrescine injection*
b/ *B-mode study with measurement of end-diastolic diameter of left ventricle after 10 min of putrescine injection.*

acterizing the frequency of cardiac contractions in different time intervals (from 1 to 120 minutes of registration on mechanogramm), practically never differed from those in control animals.

As demonstrated by the results of Echo-studies in pigs in one minute after intravascular injection of putrescine, the contractile ability of the myocardium increased, which was expressed in an increase of ejection fraction of the left ventricle from 65% to 70%. The maximal positive inotropic effect of putrescine was observed on the fourth minute after its injection; this being also expressed in an increase of ejection function to 76%. Mentioned indices were normalized, i.e. reached the control values in 10 minutes (Figure 1, 2).

Thus, based on the results of mechanogramm of cardiac contractions in small laboratory animals it is possible to draw the conclusion, according to which rather low concentrations of putrescine caused positive inotropic effect on heart, without changes of cardiac contractions frequency. On the 20th and 40th minutes of experiment, the positive inotropic effect was registered on the background of chronotropic indicator, which was within the range of control values during all time intervals. A positive inotropic effect was also registered by us in large laboratory animals (male pigs) using Echo-tests.

The research findings of laboratory-based analysis were compared with the results of phase-contrast microscopy, which allowed to reveal the morphological substrate characteristic for the high contractile ability of myocardium of the left ventricle, with coverage of a considerable number of longitudinally oriented cardiomyocytes (sarcomeres). Thus, on the 20th and 40th minutes after the injection of putrescine to mice, the method of phase-contrast microscopy allowed to reveal that, to significant extent, the cross-section striation of cardiomyocytes was disturbed; this latter being expressed in disturbance of regulated orientation of M and Z disks. Alongside the muscular fibers, a noticeable reduction of the distance between M and Z striae of myofibrils occurred with extreme increase of anisotropia in the interspaces and with enhancement of anisotropia on their periphery (Figure 3).

Thus, in our opinion, dynamic and morphological indices of contractile activity of the heart under the influence of putrescine demonstrate a typical rhythmic dependence on internal sympathic effects realized in the frames of sympatoadrenal system.

Therefore, as the subsequent stage of our research, the immune-enzyme and fluorescent-microscopical investigations were performed in order to determine adrenaline content in blood serum, heart, and adrenal glands.

The research results were compared with the results of fluorescent microscopy analysis aimed

Table 2.

Shifts of adrenaline content in heart, adrenal glands and blood plasma of the experimental animals after injection of putrescine

Study Groups	Object of study		
	blood plasma	heart	adrenal glands
Control	3.9±0.3	1.4±0.8	9.7±1.5
Experiment: Group I (20 minuts after putrescine injection)	9.5±0.6 P<0.001	5.9±0.9 0.01>P>0.001	19.1±2.4 0.01>P>0.002
Experiment: Group II (4 hours after putrescine injection)	6.26±0.9 0.25>P>0.01	3.35±0,8 0.25>P>0.01	12.3±201 0.5>P>0.25
Experiment: Group III (8 hours after putrescine injection)	5.08±0.7 0.25>P>0.1	2.9±0.7 0.25>P>0.1	13.0±1.1 0.1>P>0.05

to study topical features of distribution of ionising calcium in myocardium which, as known, plays a key role in the "contraction-relaxation" cycle of myocardiofibrils.

The results of the enzyme immunoassay for adrenaline content in blood plasma are presented in Table 2.

As obvious from the table, approximately identical dynamics of shifts of adrenaline content was observed in heart and adrenal glands of experimental animals in all study groups. High indices of adrenaline were registered only in 20 minutes after injection of putrescine. Thus, adrenaline levels in blood plasma, heart and adrenal glands exceeded the control level 2, 4, 4.2 and 1.9 times, accordingly. In relatively later intervals of observation (in 4 and 8 hours after injection of putrescine), the content of adrenaline reached the initial (control) levels in all studied biological objects. A similar pattern was traced in blood plasma of experimental animals as well.

To our mind, the study on cardiomodulatory effects on myocardium should be performed alongside with the obligatory consideration of structural alterations, shifts in adrenergic nerve fibrils of myocardium atrium and ventricles, as their functional state in many respects influences the cardiac performance, in particular, the contractile activity of myocardium. Thus, it is considered to be established [*Pshennikova M.G., 1980*] that adrenergic effects on heart are realized due to

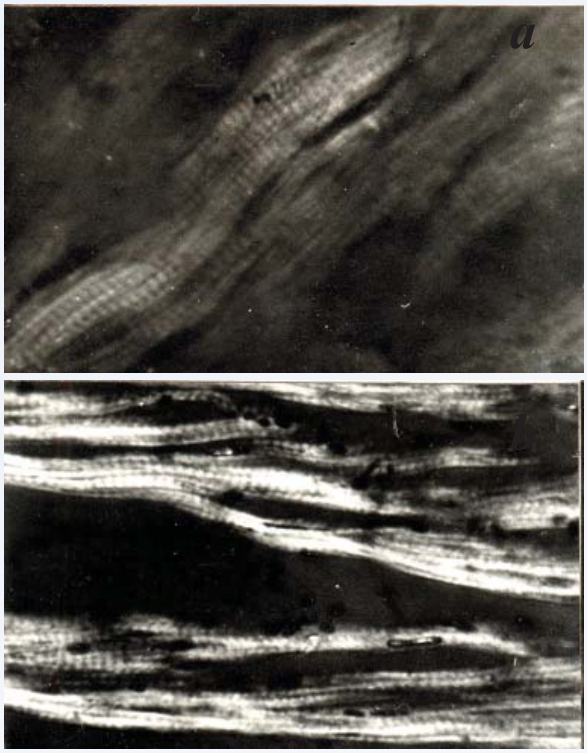


Figure. 3 *Structural shifts in cardiomyocytes of the left ventricle of experimental animals, in 20 minutes after injection of putrescine. Phase-contrast microscopy. (10 x 10).*
a/ *Control group. Regulated orientation of M and Z disks throughout interfacing cardiomyocytes with a uniform moderate cross-section anisotropic luminescence.*
b/ *Experimental group. Constriction of interdisk area with anisotropic intensifying in the central and peripheral areas of M and Z disk location.*

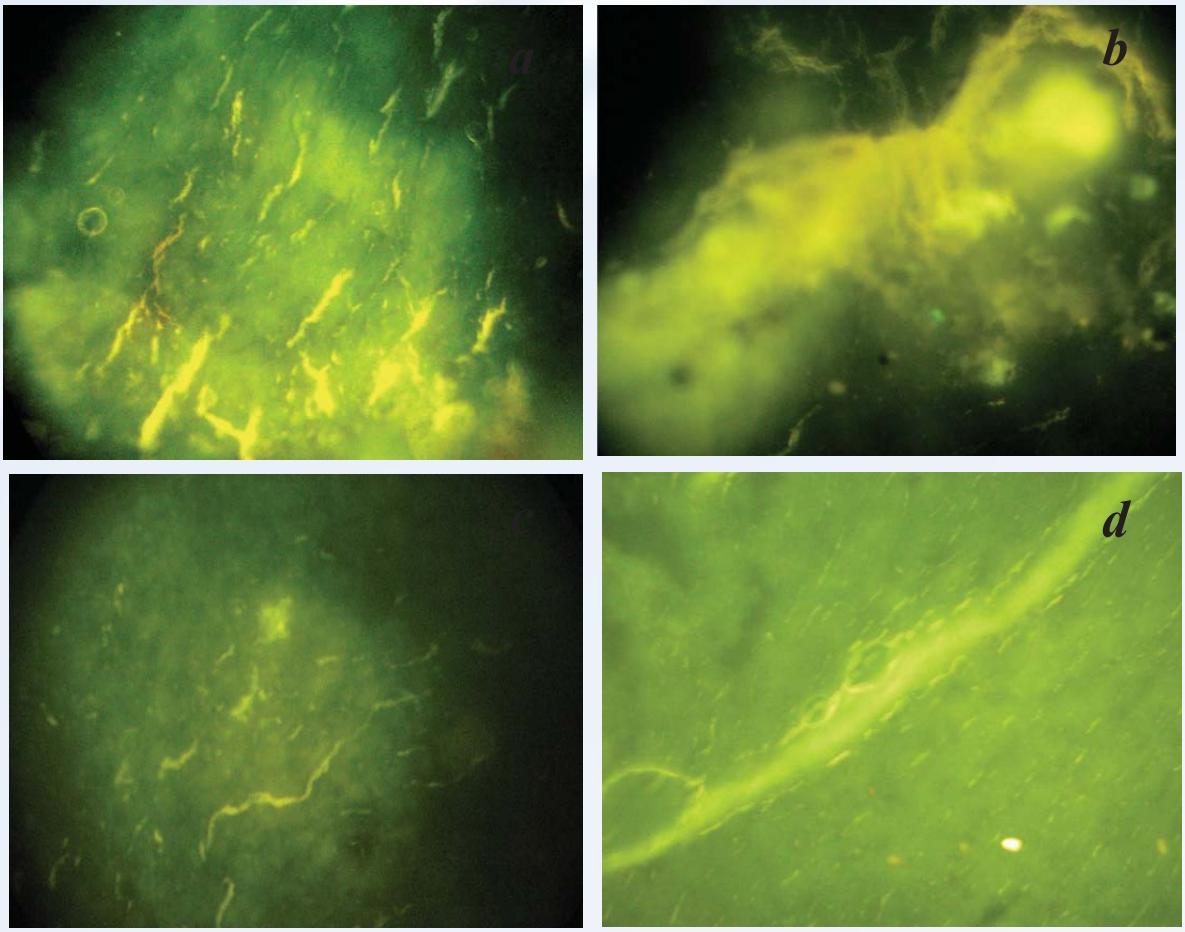


Figure. 4 *Topical features of distribution of catecholamines in myocardium of experimental animals in 20 minutes after injection of putrescine. Luminescent microscopy (Falck-Hillarp) (40 x 10).*
a, b / *Control group. Neuroplexes and fibers with intensive fluorescence of catecholamine are traced in myocardium of the left auricle and ventricle;*
c, d / *Experimental group. Appreciable dropping of specific fluorescence in adrenergic nerve fibrils and plexuses.*

catecholamines of sympatic-adrenal system. Noradrenalin is responsible for the basic role in adrenergic realization of heart. The constance of homeostasis of catecholamines in myocardium is ensured by their synthesis, deposit, release and reciprocal capture by adrenergic plexuses with which the atria, especially the right atrium are rich. All these processes altogether define the optimum concentrations of the mediator influencing the effector cells - cardiomyocytes [*Manukhin B.N., 1968*].

Therefore, as a separate series of the experiment we studied topical features of localization of adrenergic nerve structures in myocardium of experimental mice in the conditions of putrescine injection

As demonstrated by results of fluorescent microscopy analysis, the state was identical in myocardium of both atria and ventricles of control group mice. Thus, in control animals nerve fibrils were characterized by relatively dense orientation, especially in the areas localized in immediate proximity of microvessels of muscular cells. Adrenergic fibers differed by the expressed luminescence that had diffuse homogeneous or granular character. The highest density of adrenergic plexuses and fibers was found in both atria, that is conditioned by topical features of localiza-

a

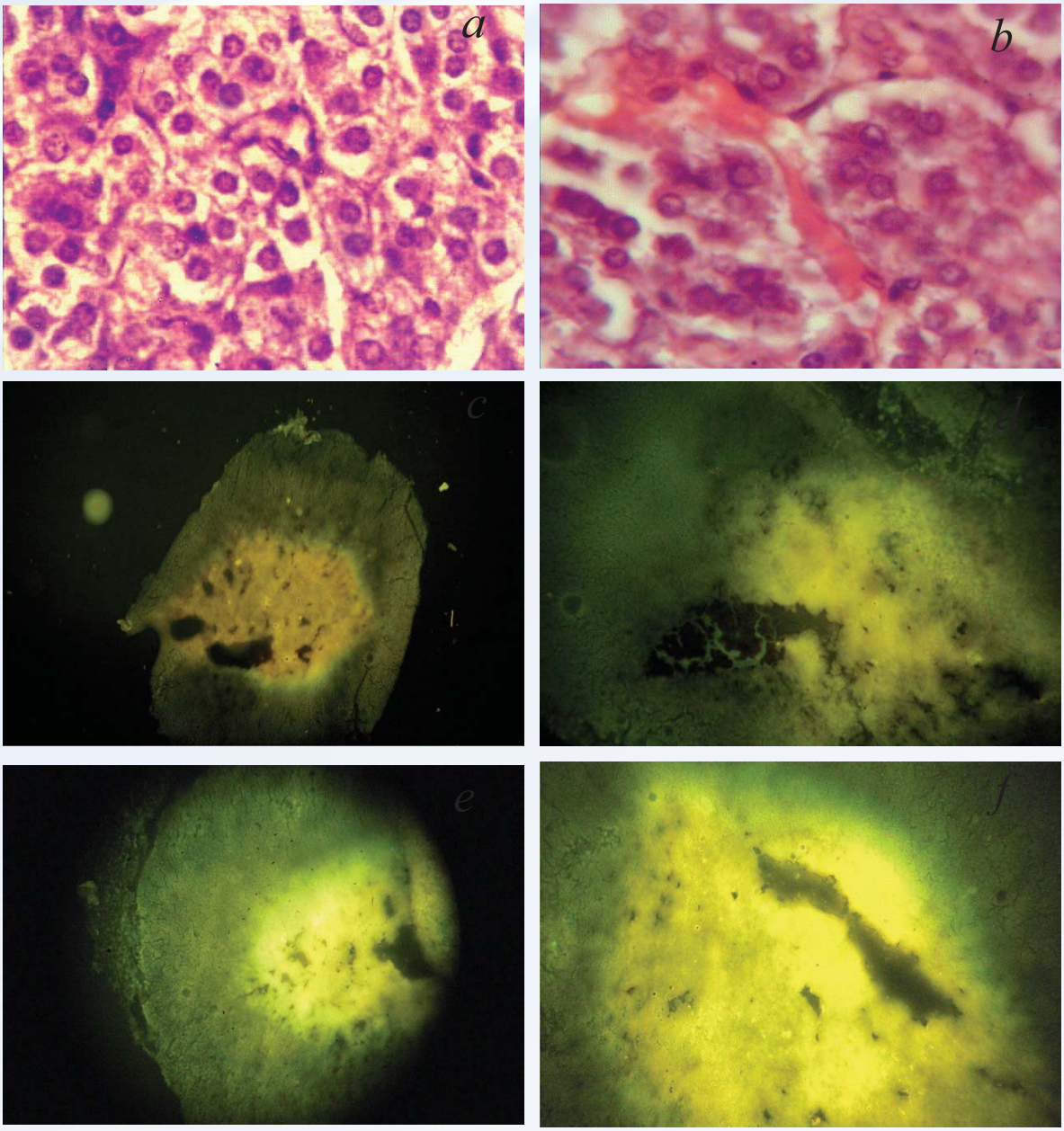


Figure. 5 *Topical features of allocation of the ionized calcium in myocardium of the left ventricle and in adrenal gland of animals, in 20 minutes after injection of putrescine. Luminescent microscopy, with application of chlortetracyclin probe (a and c :10 x10; b, d, e, f: 40 x 10).*
a, b / *Control group. Focal moderate luminescence of calcium - chlortetracyclin complex throughout the separate cardiomyocytes;*
c, d / *Experimental group. Intensive specific luminescence throughout adjacent groups of cardiomyocytes.*
e / *Control group. Moderate specific fluorescence in cytoplasm of separate groups of secretory cells of the cerebral matter of adrenals is found.*
f / *Experimental group. The content of secretory cells characterized by intensive specific luminescence of calcium-chlortetratcyclin complex of the cerebral matter is increased considerably.*

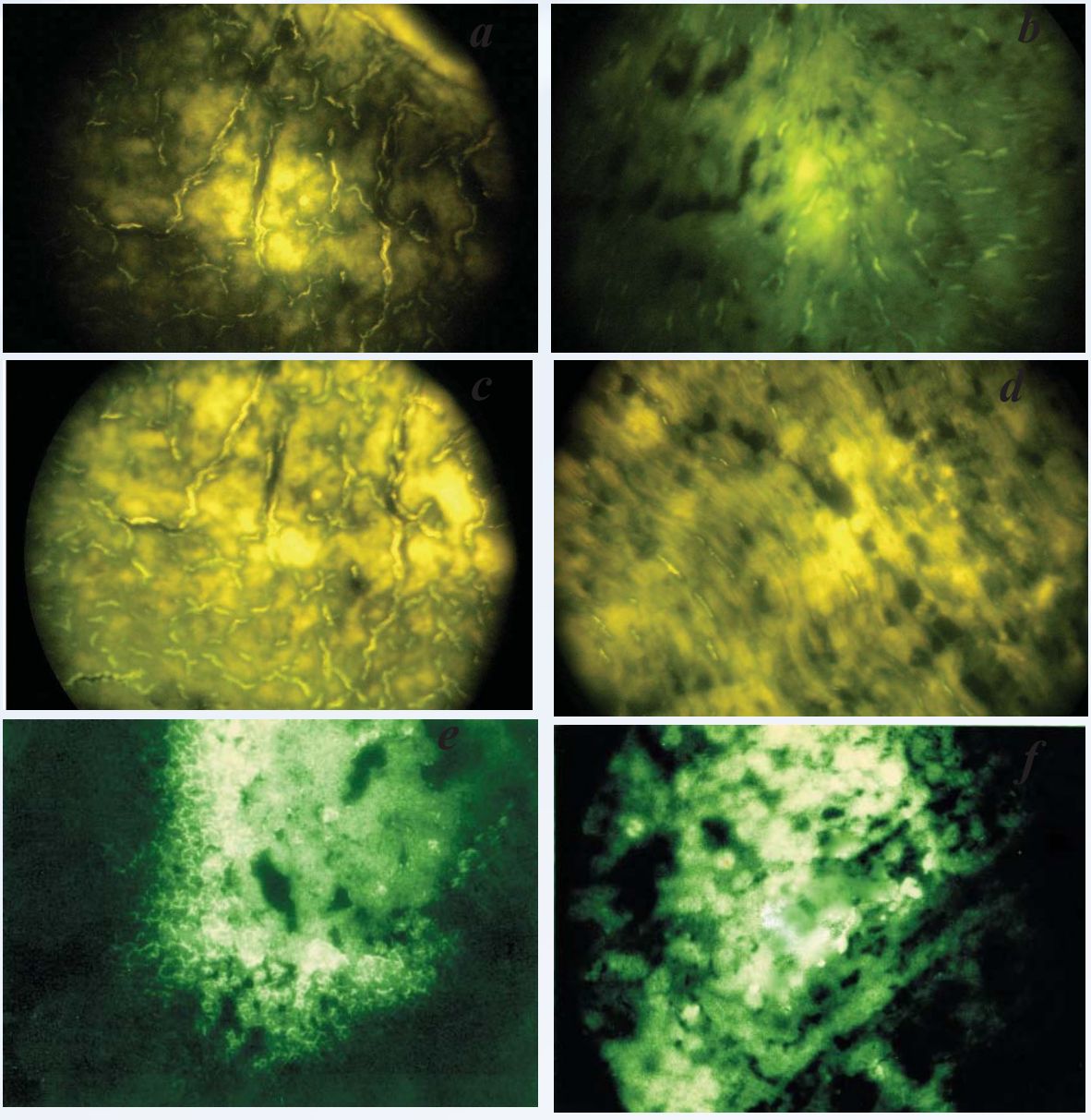


Figure. 6 *Structural alterations and topical features of allocation of catecholamines in cerebral layer of adrenals of experimental animals, in 20 minutes after injection of putrescine.*
a/ *Control group. Cytoangioarchiotectonics of the cerebral layer is preserved. Epineurocytes and adrenoepineurocytes are characterised by uniform orientation in all areas of cerebral matter;* **b/** *Experimental group. Morphological characters of hyperfunction of secretory cells are observed: the number of hypertrophied bright secretory cells is increased considerably, both around sinusoids, and in the remote areas (a and b are hematoxylin eosine stained; 40 x 10);*
c, d / *Control group. Allocation of catecholamines in secretory cells has the focal character. Secretory cells are characterised by moderate and/or weak specific fluorescence;* **e, f /** *- Experimental group. Fields of specific fluorescence of catecholamine dominate in the central and peripheric departments of cerebral matter. Luminescent microscopy (Falck-Hillarp), c, e-about. 10, apprx. 10; d, f - about. 40, apprx. 10.*

tion of concrete structures of effector nerve apparatus in myocardium [*Shvalev V. et al., 1992*]. In myocardium of both ventricles of control group mice the nerve fibrils of different calibers forming glomerular receptors localized close to cytoplasma of cardiomyocytes and walls of blood microvessels, mainly along the intermuscular capillars mostly were more often observed.

The marked decrease in the intensity of luminescence of catecholaminergic neuroplexes and fibers occurred in all sections of heart in the experimental group of mice in 20 and 40 minutes after injection of putrescine. The luminescence had focal minor-clumpy and granular character and was observed in all constituent components of the nervous apparatus of myocardium. As a result, the majority of neuroplexes and adrenergic fibers looked fragmented with the characteristic mosaic distribution of catecholamines. The area with relatively high luminescence of catecholamines alternated with the fields, in which rather low degree of specific luminescence was revealed. In 4 and 8 hours after the injection of putrescine, topical features of distribution of adrenergic structures and the intensity of the specific luminescence along their neuroplexes and fibers practically did not differ from those in myocardium in mice of control group (Figure 4). The interpretation of obtained data will be presented in the "Results and Discussion" section, - the final part of the article.

Processing of fresh-frozen criostate slices with chlortetracycline, already in 20 and 40 minutes after injection of chlortetracycline was accompanied by the marked increase in intensity of specific luminescence, which was of diffuse character and was observed along the sarcolemma and in cytoplasm of the majority of cardiomyocytes of myocardium of the left ventricle (Figure 5 ***c, d***).

Upon processing fresh-frozen criostate-derived slices prepared of adrenals, in 20 and 40 minutes after injection of putrescine, a marked increase in the content of catecholamines in their medulla occurred. The intensive (homogeneous and small-granular) luminescence in the specified period of observation had mostly diffusive character, which was expressed in intensive fluorescence in chromaphinocytes located in both central and peripheral sections of medulla, in immediate proximity of reticular zone of adrenals cortexes (Figure 6 ***e, f***). In 4 and 8 hours after injection of putrescine, the specific luminescence acquired focal character and was traced in cytoplasm of incretory cells focused mainly round the circulatory microvessels of medulla. In all studied time intervals the specific fluorescence was also observed around the sinuoids, in their wall and lumen. Upon morphological treatment (hematoxylin-eozine staining), in 20 and 40 minutes after injection of putrescine, incretory apparatus of medulla of adrenals was basically presented by bright epinephrocytes, thus indirectly testifying to the predominant synthesis of noradrenaline in them (Figure 6 ***a, b***). In 4 and 8 hours after injection of putrescine, the content of the dark, acidophilic cells the cytoplasm of which differed in moderate specific luminescence considerably increased in the medulla of adrenals.

Processing of fresh-frozen criostate-derived sections by chlortetracyclin in order to define the ionized calcium, in 20 and 40 minutes after injection of putrescine, was accompanied by considerable increase of specific fluorescence, from its localization on both surface and in cytoplasm of the majority of intercretor cells, and in walls and lumen of microvessels of medulla of adrenals (Figure 5 ***f***). In 4 and 8 hours there occurred the appreciable decrease in number of secretory and stromal cells (first of all of epinephrocytes), in which the foci of specific fluorescence continued to be revealed.

It is not excluded that biological effects of putrescine on myocardium, in particular intensifying of the contractile ability, are mediated not only by activation of sympathoadrenal system. Endogenously active factors produced in the heart and having modulating influence on contractile ability of myocardium in conditions of normal functioning organism may, in particular, play an important role in this process. In this case IGF-1 and nitrogen oxide (NO) are meant. The expedi-

ency of their determination in heart and blood serum under conditions of putrescine injection has been dictated by following circumstances.

Until now the point of view dominated, according to which growth hormones produced in pituitary gland (prolactin, IGF-1, somatotropin) are the factors responsible for the growth of skeletal mass of muscles and myocardium. Due to clinical and experimental research, a number of authors [*Citadini A. et al., 1999*] express the opinion, according to which the heart is a target organ for the axis of growth hormones - IGF-1, prolactin, somatotropin. According to I. Isgard and co-authors, besides of the adenohypophysis, the heart is also a source of synthesis of polypeptide growth factors [*Isgard I. et al., 1999*]. In this case, the growth hormone is considered a regulator of IGF-1 production in heart. Therefore, it is not excluded that regulation of biological effects of IGF-1 in heart is realized by autocrine-parakrine mechanism. The role of prolactin and IGF-1 in intensification of blood circulation and contractility of myocardium, without the involvement and decrease of its rhythm is also considered to be established already [*Til H. et al., 1997*]. At the same time, the high level of IGF-1 in heart is the reason of vasodilatation and raise of myocardial contractility [*Fratelli et al. 1995; Ross I., 1996*].

It is known that IGF-1, likewise many hormones is exposed to circadian rhythms. Thus, in Z. Ostrowska's research rather informative data was obtained according to which IGF-1 is exposed to circadian rhythms, with the highest level of the hormone in blood serum observed during the period of 11:00 a.m. to 17:00 p.m and the acrophase at 14:00 p.m. [*Ostrowska Z. et al, 2002*]. Therefore, we studied the biological effects of injected putrescine on myocardium of experimental animals, in cases of IGF-1 level determination upon sacrifice of animals at 14:00 p.m., i.e. during the acrophase period. Otherwise, determination of IGF-1 level at night i.e. during the nadir-period, even in cases of hormone content increase in myocardium, these indicators may be within physiological range. Meanwhile, it is considered established that IGF-1 in physiological concentrations does not render the modulating influence on myocardium [*Lembo G. et al., 1996; Reiss K. et al., 1996; Reiss K. et al., 1998*].

At present, it is considered to be established that nitrogen oxide actively participates in realization of many integrative functions of the organism, including those in cardiovascular system.

Thus, it is thought that nitrogen oxide has an important role in the mechanism of the autoregulation of coronary circulation by maintenance of both arterial and arteriolar basal tonus [*Harrison V. et al., 1993; Levin E., 1995; Quyyumi A. et al., 1995; Solodkov A. et al., 2002*]. The reduntant production of NO corresponds to the depression of venous and peripheral resistance, progression of hypotension (the negative inotropic effect connected to local enhanced synthesis of nitrogen oxide in endothelium of coronary vessels and cardiomyocytes is registered) [*Torre-Atoione G. et al., 1996*]. Besides, the high concentrations of NO in many respects activate the development of catabolic processes intensifying "autodestructive" injury of cells and tissues, thus promoting the development of severe systemic reaction of the organism to imparement [*Kuzmin M., 2000*].

The results of the enzyme immunoassay for IGF-1 and NO content in the heart are presented as Table 3.

As it is obvious from the table, IGF-1 level in heart, was within the control values in 20 minutes after injection of putrescine. However, in 4 hours, the tendency to increase of IGF-1 content in heart was definitely traced, while in 8 hours rather high indicaes of the studied hormone were registered in heart of experimental animals; 4-fold exceeding of control level was revealed.

Thus, in relatively later periods of experiment (in 8 hours after injection of putrescine), a marked increase in IGF-1 content took place in heart of experimental animals, which supposes its modulatory influence on processes participating in realization of contractile functions of myocardium. On the 20th minute of the observation, NO level in heart practically did not differ from those in rats of control group (Table 3).

At the same time, single intravascular injection

of putrescine in 4 and 8 hours was accompanied by appreciable decrease in nitrogen oxide level in heart of experimental animals.

Thus, NO content in heart decreased 2.3 times in 2 hours (in comparison with the control group), and was 2.9 times lower in 8 hours.

Rather low indices of nitrogen oxide determined by us in heart of experimental mice testify, to a known extent, that in rather low concentrations putrescine partially inhibits cardiovascular effects *in situ* produced by NO, in particular, its inotropic influence on contractile function of myocardium.

It should not be excluded, that many biological effects of putrescine on heart are also mediated by immune-cytokines, produced in organs of immunity and *in situ* in the myocardium, because cytokines of a proinflammatory spectrum of action play an important role in myocardial remodeling processes; their high concentrations, in a dose-dependent mode, have simultaneously toxic damaging effect on parenchymatous and stromal cells of myocardium [*Til H.P. et al., 1997; Cubria J.C. et al., 1998*].

It is for this reason that we performed an enzyme immunoassay under conditions of injection of putrescine, in order to determine interleikines (IL-1β, IL-2, IL-6) and γ-interferon (IFN-γ) in heart. The selection of concrete terms for determination of cytokines content (in 8 hours and 7 days after injection of putrescine) was made by us, taking into consideration kinetics and increase in their transcripts to mRNA, as well as appearances of cytokines in the culture medium and in organs of immunity under conditions of an antigen stimulation *in vitro* and concrete lymphocytic populations and subpopulations *in vivo* [*Simbirtsev A.S., 1998a; 1998b*].

As demonstrated by the results of the enzyme immunoassay, we failed to register presence of interleikines and IFN-γ in myocardium in animals of both control and experimental groups.

Thus, the research established that under conditions of our experiment, in myocardium of mice there were no structurally functional shifts of alterative order, thus excluding the possibility of direct and/or mediated influence on myocardium by rather low concentrations of putrescine approbated in our study. Otherwise, at preserved cytoangioarchiotectonics of myocardium, in this latter there were no signs of activation of cytokines and NO synthesis, to which an important role belongs in processes of myocardial remodeling under conditions of an acute injury.

Results and Discussion

What possible factors underlie putrescine-dependent cardiomodulatory effect revealed in our study?

It is necessary to specify one aspect of the problem under study. It is known that proinflammatory cytokines immediately participate in myocardial remodeling processes [*Simms M.G., Walley K.R., 1999; Stangl V., 2002; Pasic J. et al., 2003*] and the increase of the nitrogen oxide level

Table 3.

Shifts in IGF-1 and NO content in heart under conditions of putrescine injection to experimental animals

Object of research	Time after the injection of putrescine	Defined indicators	
		IGF-1	NO
Heart	Control group	0.39±0.08	7.2±1.4
	in 20 minutes	0.32±0.07 P>0.5	6.3±1.6 P>0.5
	in 4 hours	0.84±0.1 0.25>P>0.1	3.1±0.9 0.05>P>0.02
	in 8 hours	1.57±0.3 0.002>P>0.001	2.5±0.9 0.02>P>0.01

produces cytotoxic effect on parenchymatous and stromal cells, simultaneously bringing forth a negative inotropic effect [*Kojda G., Kottenberg R, 1999; Iemitsu M. et al., 2000; Wittstein I.S. et al., 2001*]. As we have shown earlier, cytoangioarchitectonics of myocardium in experimental mice was preserved in both studied periods (in 4 and 8 hours after injection of putrescine). The absence of alteration signs in cardiomyocytes and myocardial remodeling (the results of morphological analysis) was confirmed by immune-morphological study, in which it has been established that the processes of cytokines and nitrogen oxide activation were not observed in heart in studied time intervals. The same study testifies that injected concentration of putrescine had no cytotoxic effect on structural components of myocardium. At the same time, it is not excluded that endogenous putrescine directly influenced the processes ensuring raised contractility of myocardium. Scientific literature data also testify in favour of the assumption proposed by us.

Thus, Bordallo C. et al. (2008) studied mechanisms of cardio-stimulating effect of putrescine in model experiments with injection of agonists β-adrenergic receptors to Wistar rats. Due to the research performed, the authors put forward a hypothesis according to which in myocardium putrescine co-operates with β-adrenergic receptors of myocytes, increasing the intracellular activity of cyclic adenylatecyclase, thus conditioning the high contractile activity of myocardium. Other polyamines such as spermine and spermidine failed to possess a similar effect. It is not excluded that in the conditions of our experiment, the similar mechanism of interaction of exogenous putrescine with β-adrenoreceptors of cardiomyocytes is also involved. In our opinion, this is a rather short-term effect, as it should be based on principles of conncurrency with catecholamines, to which, though indirectly, testify our own results of immune-enzyme and fluorescent-microscopy studies for determination of adrenaline levels not only in myocardium, but also in adrenal glands and blood plasma. It is especially worth to mention that, in our opinion, the research of C. Bordallo and co-authors [*Bordallo C. et al., 2008*] has a fundamental character, as they pave the way for establishment of physiological and adaptogene roles of putrescine in the processes ensuring the contractile potencies of myocardium, as well as in cases when expressed disorders in sympathoadrenal system arise in the organism of mammals.

An important role in intensifying the contractile abilities of myocardium, under conditions of our experiment, was devoted to catecholamines; this latter was confirmed by us in immune-enzyme studies, as on the 20th minute after of putrescine injection rather high indices of adrenaline have been registered in myocardium and blood plasma of experimental animals.

In the same period of observation, there was observed the increased content of the ionized calcium in cardiomyocytes; this event was detected by us while processing fresh-frozen (criostate-based) sections of myocardium by means of chlortetracycline probe.

The revealed positive inotropical effect is also mediated by calcium-dependent mechanisms as the direct dependence between contractile potencies of myocardium and levels of extra - and intracellular calcium in cardiomyocytes is considered to be established. It is not excluded that the increase of the ionized calcium in cytoplasm of cardiomyocytes in the conditions of our experiment, to a certain extent, is conditioned by an immediate effect of exogenous putrescine on myocardium. In favour of the proposed assumption, though indirectly, testify also literature data, according to which polyamines (putrescine, spermine and spermidine) act as secondary mediators in the organism of mammals raising levels of ionized calcium alongside with its intracellular mobilisation (in cytoplasm of cardiomyocytes as well); this latter being the final stage of influence exerted to myocardium by many inotropic factors [*Bazzani C. et al., 1988*]. In this concern, possible molecular mechanisms underlying the interaction of putrescine with membranous proteins of cardiomyocytes - in respect of formation of calcium homeostasis in myocar-

dium,- are also established. Thus, Harris S. and co-authors studied the influence of polyamines on the functional state in particular on the contractile ability of protein myofilaments of cardiomyocytes [*Harris S. et al., 2000*]. It has been established that putrescine, spermidine, and spermine raise the concentration of the ionized calcium on membranes of miofilaments stimulating the contractile function of cardiomyocytes. Based on research performed, authors come to a conclusion, according to which polyamines compete with calcium ions while interacting with membranes of myofilaments. At the same time, it should be specially mentioned that calcium-dependent mechanisms underlying the cardiomodulatory action of putrescine are still disputable. Moreover, quite opposite data are also available, according to which biological effects of polyamines on myocardium (their cytotoxic action) are mediated only at optimum concentrations of the ionized calcium. The similar situation is defined as "calcium paradox" [*Busselen P., 1991*]. Thus, using high-performance liquid chromatography, authors have shown that in the conditions of isotonic perfusion of fluid deprived of Ca^{+2}, the levels of putrescine, spermine and spermidine in myocardium remain within the control values. However, these levels decrease, when the perfused fluid contains optimum Ca^{+2} concentration. According to the author [*Busselen P., 1991*], the decrease of polyamines content in myocardium is caused by disturbance of integrity of membranes of cardiomyocytes and "entry" of polyamines to their cytoplasm, which conditions the negative inotropic effect.

Data, according to which polyamines take part in the processes providing the active transport of ions of calcium into cytoplasm of cardiomyocytes also exist.

Thus, in particular, a number of authors [*Lopatin A.N. et al., 2000*] have established that in heart of ODC-transgenic mice rather high level of putrescine participating in processes of modulation of potassium channels in cardiomyocytes is observed.

The analysis of scientific publications including the findings of our research, once again testifies that in this problem there are many debatable moments having intrinsic objective causes (the choice of laboratory animals; various experimental approaches in induction even of the same pathological process in myocardium; various and, as a rule, rather high concentrations of injected polyamines; the modes of their administration to the organism of mammals, etc.), which, in our opinion, are rather negatively reflected in interpretation of the obtained results. Thereupon, for the final interpretation of results of our own research, we are planning to further realize more specified research on indicators of a water-salt metabolism and, first of all, of the ionic homeostasis in myocardium of experimental animals under conditions of rather low concentrations of putrescine.

The results obtained due to the fluorescent-microscopy analysis aimed to define the features of adrenergic innervation of heart, in the conditions of putrescine injection, should be interpreted with a certain degree of caution, first of all, based on availability of concrete literature data reflecting morphofunctional state of the effectory adrenergic system of myocardium in norm and pathology, as well as taking into consideration the development of local involutional processes. Thus, the foci of "desympathisation" of heart with the considerable increase of level of catecholamines in myocardial nerve fibrils arise in a number of experimental states accompanied by expressed changes in the neuromuscular apparatus of an alterative character [*Tsellarius Yu., Semenova L., 1972; Stropus R., 1982; Shvalev V., 1989*].

However, in the conditions of our experiment, upon injection of putrescine to the experimental animals, the cytoangioarchitectonics of myocardium of all sections of heart has been preserved. Therefore, the signs of decrease of catecholamines level found by us in myocardium nerve fibers are neither expression nor a consequence of myocardium injury. On the other hand, the alterations in the maintenance of catecholamines found by us may be considered a consequence of

involutional processes development in myocardium [*Stropus R., 1982*]. However, a relatively young contingent of experimental animals was involved in our experiment in the final period of their puberty. In mice of the control group no changes of involutional character were registered in adrenergic structures of myocardium related nervous apparatus.

In our opinion, the point of view is more acceptable, according to which the marked decrease of catecholamines level in adrenergic nervous structures of myocardium in the conditions of our experiment testifies in favour of activation of its positive inotropic effect.

The assumption put forward by us is also confirmed by experimental research, in which the dose-dependent effect of positive inotropic action of catecholamines on contractile activity of myocardium of the heart ventricles is established, involved in specified conditions of a considerable decrease of catecholamines level in adrenergic nerve structures of myocardium [*Shvalev V. et al,. 1992*].

The authors stipulate that in the given case of induction of a positive inotropic effect, there is a reverse dependence, i.e. the more myocardium is "saturated" by adrenergic plexuses, the less expressed positive inotropic action of catecholamines is observed.

Results of the performed research allow to draw the conclusion, according to which rather low concentrations of putrescine have stimulating effect on myocardium, enhancing its contractile potencies in mechanism of which an important role belongs to catecholamines. As one of possible sources of the enhanced synthesis of catecholamines should be considered secretory cells of medulla of adrenal glands, as in the conditions of our experiment, the hyperfunction of these cells was accompanied by the enhanced entrance of adrenalin into blood and heart.

Our research also established that in 8 hours after putrescine injection to myocardium of experimental animals, on the background of adrenaline levels normalization there was a marked increase in content of the insulin-like growth factor -1. The increase of insulin-like growth factor -1 levels in myocardium of experimental animals has, apparently, an adaptogenic character.

The assumptions are confirmed by data of scientific publications on cardiomodulatory effects of IGF-1. Thus according to G. Lembo and co-authors, IGF-1 leads to the increase of systolic and a diastolic pressure, partial hypertrophy of ventricles [*Lembo G. et al., 1996*]. The research was performed in transgenic mice with IGF-1 deficiency. Based on research findings, the authors come to a conclusion, according to which in regulation of arterial pressure and contractile potencies of myocardium of the left ventricle IGF-dependent mechanisms are also concerned. Moreover, authors express the supposition that there is a functional interrelation between IGF-1 dependent and β-adrenergic receptors in myocardium; however, in normal conditions IGF-1 dependent cardiomodulatory mechanism is apparently not involved. At the same time, we consider it necessary to mention that cardiomodulatory effects of IGF-1 are not ultimately revealed; these aspects are a subject of numerous differently directed discussions [*Stephen B. et al., 2003; Colangelo L., 2004; Sesti G., 2005*].

Considering the circumstance that at present IGF-1 is considered a cardiomodulator, it is not excluded that in the conditions of our experiment the putrescine-dependent mechanism ensuring processes of IGF-1 synthesis activation *in situ*, functioning on autocrine-parakrine principle is involved. Undoubtedly, the proposed assumptions require carrying out special studies aimed, first of all, at alterations in integrated axis of growth hormones - IGF-1, prolactin and somatotropic hormone in various intervals after injection of putrescine, taking into consideration the circadian rhythms of all three growth hormones.

Acknowledgement: The authors express their gratitude to Professor H.S. Sisakian, Professor S.H Sisakyan, Associate Professor N.F. Krasnikov, and laboratory assistant S. Aslanyan for the methodical assistance rendered upon implementation of the current research.

References

1. *Bazzani C., Genedani S., Tagliavini S., Piccinini G., Bertolini A.,* Treatment with polyamine synthesis inhibitors reduces the positive inotropic effect of ouabain, noradrenaline and calcium, Pharmacol. Res. Commun. 1988; 20(1): 23-35.
2. *Bordallo C., Cantabrana B., Velasco L., Secades L., Meana C., Méndez M., Bordallo J., Sánchez M.* Putrescine modulation of acute activation of the beta-adrenergic system in the left atrium of rat. Eur. J. Pharmacol. 2008 Aug 13. [*Epub ahead of print*])
3. *Busselen P.* Polyamines and the calcium paradox in rat hearts. J. Mol. Cell. Cardiol. 1991; 23(3): 237-247.
4. *Cepero M., Cubría J.C., Reguera R., Balaña-Fouce R., Ordóñez C., Ordóñez D.* Plasma and muscle polyamine levels in aerobically exercised rats treated with salbutamol. J. Pharmacol. 1998; 50(9): 1059-1064.
5. *Cittadini A.., Longobardi S., Fazio S.* Growth hormone and heart. Miner. Electrolyte Metab. 1999. 25(1-2): 51-55.
6. *Colangelo L., Liu K., Gapstur S.M.* Insulin-like growth factor, insulin-like growth factor binding protein-3, and cardiovascular disease risk factors in young black men and white men. Am. J. Epidemiol. 2004: 160: 750-757.
7. *Cubria J.C, Reguera R., Balana-Fouce R., Ordonez C., Ordonez D.* Polyamine-mediated heart hypertrophy induced by clenbuterol in the mouse. J. Pharm. Pharmacol. 1998; 50(1): 91-96;
8. *Flamigini F., Rossini C., Stefanelli C., Cardarera C.M.* Poliamine metabolism and function in the heart. J. Mol. Cell Cardiol. 1986; 18(1): 3-11.
9. *Flamigni F., Stanic I., Facchini A., Cetrullo S., Tantini B., Borzì R.M, Guarnieri C., Caldarera C.M.* Polyamine biosynthesis as a target to inhibit apoptosis of non-tumoral cells. Amino Acids 2007; 33(2): 197-202 [Epub 2007 Jun 20].
10. *Fratelli M., Gagliardini V., Galli G.* - Autocrine interleukin-1 beta regulates both proliferation and apoptosis in EL 4-6.1 thimoma cells. Blood, 1995;85(12): 3532-3537.
11. *Harris S.P., Patel J.R., Marton L.J., Moss R.L.,* Polyamines decrease Ca(2+) sensitivity of tension and increase rates of activation in skinned cardiac myocytes, Am. J. Physiol. Heart Circ. Physiol. 2000; 279(3): H1383-391.
12. *Harrison V., Corder R., Anggard E.* Evidence for vesicles that transport endothelin-1 in bovine aortic endothelial cells. J. Cardiovasc. Pharmacol. 1993; 22: 557-560.
13. *Hasegawa S., Nakano M., Hamana K., Taniguchi Y., Iwasaki T., Kanda T., Suzuki T, Nagai R.,* Decrease in myocardial polyamine concentration in rats with myocardial infarction, Life Sci. 1997; 60(19):1643-1650.
14. *Ibrahim J., Hughes AD, Schachter M., Sever P.S.* Depletion of resistance vessel polyamines attenuates angiotensin II induced blood pressure rise in rats. Clin. Exp. Hypertens. 1996; 18(6): 811-830.
15. *Iemitsu M., Miyauchi T., Maeda S., Yuki K., Kobayashi T., Kumagai Y., Shimojo N., Yamaguchi I., Matsuda M.* Intense exercise causes decrease in expression of both endothelial NO synthase and tissue NOx level in hearts. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 2000; 279: R951–R959.

16. *Isgaard I., Tivestor A., Frierg P., Bengtsson B.* The role of GH/IGF-1 axis for cardiac function and structure. Horm. Metab. Res. 1999; 31(2-3): 50-54.

17. *Kojda G., Kottenberg R.* Regulation of basal myocardial function by NO. Cardiovascular Research 1999; 41: 514–523.

18. *Kuzmin M.I.* [A syndrome of system response to inflammation] [published in Russian] Surgery. Moscow (Russia), 2000, №2: 54-59.

19. *Lembo G., Iaccarino G., Vecchione C., Rendina V., Parrella L., Trimarco B.* Insulin Modulation of ß-Adrenergic Vasodilator Pathway in Human Forearm, Circulation. 1996; 93:1403-1410.

20. *Levine E. Endothelins. N. Engl J.* Med. 1995;323: 356-363.

21. *Lopatin A.N., Shantz L.M, Mackintosh C.A, Nichols C.G., Pegg A.E.* Modulation of potassium channels in the hearts of transgenic and mutant mice with altered polyamine biosynthesis. J. Mol.Cell. Cardiol. 2000; 32(11): 2007-2024.

22. *Mackintosh C.A., Feith D.J., Shantz L.M., Pegg A.E.* Overexpression of antizyme in the hearts of transgenic mice prevents the isoprenaline-induced increase in cardiac ornithine decarboxylase activity and polyamines, but does not prevent cardiac hypertrophy. Biochem J. 2000; 350 (Pt 3): 645-653.

23. *Manukhin B.N.*[Physiology of adrenoreceptores] [published in Russian] Moscow (Russia)1968.

24. *Mezl V.A., Villemure C., Garber P.M, Rakusan K.* Aortic stenosis produces hypertrophy of the rat heart without causing an accumulation of N1-acetylspermidine. Biochem. Cell. Biol. 1988.

25. *Morrison R.F., Seidel E.R.* Cell spreading and the regulation of ornithine decarboxylase, J. Cell. Sci. 1995*a*;108(Pt 12): 3787-3794.

26. *Morrison R.F., Seidel E.R.* Vascular endothelial cell proliferation: regulation of cellular polyamines. Cardiovasc. Res. 1995*b*; 29(6): 841-847.

27. *Nakano M., Kanda T., Matsuzaki S., Hasegawa S., Ma H., Imai S., Suzuki T., Kobayashi I.* Effect of losartan, an AT1 selective angiotensin II receptor antagonist, on isoproterenol-induced cardiac ornithine decarboxylase activity. Res. Commun. Mol. Pathol. Pharmacol. 1995; 88(1): 21-30.

28. *Ohta H., Lewis S.J., Talman .T.* Spermidine and cardiovascular control in nucleus tractus solitarii in rat. Brain Res. 1993; 620(1): 72-77.

29. *Ostrowska Z., Kos-Kudla B., Marek B., Kajdaniuk D., Ciesielska-Kopacz N.* The relationship between the daily profile of chosen biochemical markers of bone metabolism and melatonin and other hormone secretion in rats under physiological conditions. Neuroendocrinology Letters 2002; 23 (Nos. 5/6, Oct-Dec).

30. *Panama B.K., Lopatin A.N.* Differential polyamine sensitivity in inwardly rectifying Kir2 potassium channels. J. Physiol. 2006; 571(Pt 2): 287-302.[Epub 2005 Dec 22].

31. *Pasic J., Levy W.C., Sullivan M.D.* Cytokines in Depression and Heart Failure. Psychosomatic Medicine 2003; 65:181–193.

32. *Pshennikova M.G.*[Adrenergic regulation of heart under adaptation to high-altitude hypoxia and continuous high loads] Thesis for the degree of Science Doctor in Medicine, Moscow, (Russia), 1980, 43p.

33. *Quyyumi A., Dakak N., Andews N.* Nitric oxide activity in the human coronary circulation. Impact of risk factors for atherosclerosis. J. Clin. Invest. 1995;95: 1747-1755.

34. *Reiss K., Cheng W., Ferber A., Kajstura J., Li P., Li B., Olivetti, G, Homcy C.J., Baserga R., Anversa P.* Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. PNAS 1996; 93(16): 8630-8635.

35. *Reiss K., Valentinis B., Tu X.., Xu S. Q., Baserga R.* Molecular markers of IGF-I-mediated mitogenesis. Exp. Cell Res. 1998; 242: 361-372.

36. *Ross I., Hongo M.* The role of hypertrophy and GF in heart failure. J. Card. Fail. 1996.: 121-128.

37. *Ruskoaho H., Raunio H.* Altered cardiac polyamine biosynthesis in spontaneously hypertensive rats. Am. J. Physiol. 1987; 253(2; Pt 2): H262-269.

38. *Sesti G., Sciacqua A., Gardellini M., Marini M.A., Maio R.* Plasma concentration of IGF-1 is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. Diabetes Care 2005; 28(1): 120-125.

39. *Shimizu M., Sasaki H., Sanjo J., Ogawa K., Mizokami T., Yagi T., Kato H., Hamaya K., Namiki A., Isogai Y.* Effect of captopril on isoproterenol-induced cardiac hypertrophy and polyamine contents. Jpn. Circ. J. 1992; 56(11):1130-1137.

40. *Shvalev V., Sosunov A., Guski H.* [Morphological bases of cardiac innervation] [published in Russian] Moscow, Russia, 1992. 368p.

41. *Simbirtsev A.S.* [Biology of human interleukine-1 family.Immunology] [published in Russian] Immunology 1998; №3: 9-17.

42. *Simbirtsev A.S.* [Interleukine-2 and receptory complex of interleukine-2 in regulation of immunity] [published in Russian]. Immunology 1998; № 6: 3-11.

43. *Simms M.G., Walley K.R.* Activated macrophages decrease rat cardiac myocyte contractility: importance of ICAM-1-dependent adhesion. Am. J. Physiol. Heart Circ. Physiol. 1999; 277:253-260.

44. *Solani G., Zicchi R., Mariani M., Ronca G.* Poliamines reduce heart injury caused by doxorubicin. Drugs Exp. Clin. Res. 1991:17(10-11): 507-510.

45. *Stangl V., Baumann G., Stangl K., Felix S.B.* Negative inotropic mediators released from the heart after myocardial ischaemia–reperfusion. Cardiovascular Research 2002; 53: 12–30.

46. *Stanic I., Cetrullo S., Facchini A., Stefanelli C., Borzì RM., Tantini B., Guarnieri C., Caldarera C.M., Flamigni F.* Effect of the polyamine analogue N1,N11-diethylnorspermine on cell survival and susceptibility to apoptosis of human chondrocytes. J. Cell. Physiol. 2008; 216(1): 153-161.

47. *Stefanelli C., Stanic I., Zini M., Bonavita F., Flamigni F., Zambonin L., Landi L., Pignatti C., Guarnieri C., Caldarera C.M.,* Polyamines directly induce release of cytochrome c from heart mitochondria. Biochem J. 2000; 347(Pt 3): 875-880.

48. *Stronus R.A.* [Cholinergic and adrenergic innervation of heart and its changes in case of cardiovascular pathology] [published in Russian] Thesis for the degree of Science Doctor in Medicine. Moscow, Russia. 1982. 35p.

49. *Tagliavini S., Genedani S., Bertolini A., Bazzani C.* Ischemia- and reperfusion-induced arrhythmias are prevented by putrescine. Eur. J. Pharmacol. 1991; 194(1): 7-10.

50. *Tantini B., Fiumana E., Cetrullo S., Pignatti C., Bonavita F., Shantz L.M., Giordano E., Muscari C., Flamigni F., Guarnieri C., Stefanelli C., Caldarera C.M.* Involvement of polyamines in apoptosis of cardiac myoblasts in a model of simulated ischemia. J. Mol. Cell. Cardiol. 2006; 40(6): 775-782.[Epub 2006 May 5].

51. *Tantini B., Flamigni F., Pignatti C., Stefanelli C., Fattori M., Facchini A., Giordano E., Clô C., Caldarera C.M.,* Polyamines, NO and cGMP mediate stimulation of DNA synthesis by tumor necrosis factor and lipopolysaccharide in chick embryo cardiomyocytes. Cardiovasc. Res. 2001; 49(2): 408-416.

52. *Til H.P., Falke H.E., Prinsen M.K., Willems M.I.* Acute and subacute toxicity of tyramine, spermidine, spermine, putrescine and cadaverine in rats. Food Chem. Toxicol. 1997; 35(3-4): 337-348.

53. *Tipnis U.R., Haile C., Boor P.J., Gutkowska J.* Polyamine regulation of atrial natriuretic peptide in cultured cardiocytes. Regul. Pept. 1994; 52(2): 75-84.

54. *Tipnis U.R., He G.Y., Li S., Campbell G., Boor P.J.* Attenuation of isoproterenol-mediated myocardial injury in rat by an inhibitor of polyamine synthesis. Cardiovasc. Pathol. 2000; 9(5): 273-280.

55. *Tipnis U.R., Skiera C.* Regional changes in ornithine decarboxylase activity and polyamine levels during thyroxine-induced cardiac hypertrophy. Cytobios 1989; 57(229): 101-108.

56. *Torre-Amione G., Kopadia S., Lee I.* Tumor necrosis factor-2 and tumor factor receptors in the failing human heart. Circulation. 1996; 93(4): 704-711.

57. *Tsellarius Yu., Semenova L.* [Histopathology of focal metabolic lesions of myocardium] [published in Russian]. Novosibirsk, Russia. 1972. 212p.

58. *Vladimirov Yu., Dobretsov G.* In: ["Fluorescent probes in biological research of membrans"] [published in Russian] Moscow. 1980. 320p.

59. *Wittstein I. S., Kass D. A., Pak P. H., Maughan W. L., Fetics B., Hare J. M.,* Cardiac Nitric Oxide Production Due to Angiotensin-Converting Enzyme Inhibition Decreases Beta-Adrenergic Myocardial Contractility in Patients With Dilated Cardiomyopathy Journal of the American College of Cardiology 2001; 38(2): 429-435.

60. *Zhao Y.J., Xu C.Q., Zhang W.H., Zhang L., Bian S.L., Huang Q., Sun H.L., Li Q.F., Zhang Y.Q., Tian Y., Wang R., Yang B.F., Li W.M.* Role of polyamines in myocardial ischemia/reperfusion injury and their interactions with nitric oxide. Eur. J. Pharmacol. 2007; 562(3):236-246. [Epub 2007 Feb 21].