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Chemical Elements and Structural/Molecular Properties of Myocardium in Infants with Transposition of Great Arteries

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1. Introduction

The imbalance of chemical elements (CE) during the prenatal development of a foetus might cause foetal heart abnormalities and even miscarriages (Skalny, 1999; Kudriyn, 2000), while the deficit of many vital CE during the gestation period could lead to congenital heart diseases. The deficiency of Cu in the course of this period might provoke the development of aortic aneurysms and impairment of vessel elasticity (Panchenko, 2004), while the lack of Zn could bring about transposition of the great arteries (TGA) (Shankar & Prasad, 1998; Beerli et al., 2000). The content of Fe, Cu, Zn, Se and Mn in optimal quantities is indispensable for adequate support of the cellular cycle, growth and differentiation of cells, including cardiomyocytes (Ruff, 1999). TGA comprises a special group of congenital heart diseases (CHD) with concordant atrioventricular and discordant ventricular-arterial junctions (Fozzard et al., 1986; Hoffman, 2006). This complicated disease occurs in newborns with CHD in an excess of 10 % of cases, with significant mortality and morbidity (Bokeria & Gorbachevsky, 1996). This is because it is yet unclear why this disease occurs, how this pathology progresses during the growth and development of newborns and, most importantly, which metabolic processes get impaired in cardiomyocytes that lead to the death of myocardium. Nowadays, a high level of immunofluorescent methods allows for identifying the cardiomyocytes that are involved in DNA replication (Re, 1987; Bolli, 2002). The main difficulty encountered in treating this disease is to correctly evaluate the ventricular function providing an adequate cardiac output (Castaneda, 1993, 1998). Age is also an important factor in determining the speed and functional reaction of the myocardium to pressure overload (Isoyama et al., 1987; Re, 1987; Scholzen & Gerders, 2000). Further research is needed to answer the following questions: 1. How is CE distribution disrupted in different parts of the heart and how is this disruption related to pathomorphological abnormalities? 2. How are morphology and the molecular structure of cardiomyocytes changed in the course of growth and development of infants with TGA,

from newborns to 1-year-old babies? 3. What pathomorphological distinctions are typical for 2 anatomical types of TGA: with intact ventricular septum (IVS) and with ventricular septum defect (VSD)?

The purpose of this research is to study the content of chemical elements and the morphological structure of the myocardium in infants with different TGA types. Three tasks were set to achieve this goal: 1. To investigate some features of the content of CE and the structure of cardiomyocytes in 3 age groups: newborns aged 1 to 6 months and babies aged 6 to 12 months. 2. To study the concentration of CE in different parts of the heart in infants with TGA and in patients whose death was not caused by cardiac problems (control group). 3. To compare the features of CE and pathomorphological structure of 2 anatomic types of TGA: with atrial septal defect (ASD) and intact ventricular septum (IVS) and with atrial septal defects (ASD) and (VSD).

2. Methods

A pathomorphological study was carried out using autopsy material of 68 infants aged under 1 who died during the follow-up period, as well as 10 infants of the same age whose death was not caused by cardiac problems. All TGA patients were broken down in 2 groups according to patients' anatomical type: the first group included patients with a simple form of TGA – TGA with atrial septum defect (ASD) and intact ventricular septum (IVS) – 37 patients (19 aged under 1 month and 18 aged 1 to 6 months), while 31 patients having TGA with ASD and VSD (7 aged under 1 month, 13 aged 1 to 6 months and 11 patients aged 6 to12 months) were assigned to the second group.

Biopsy samples were preserved in 4 % phosphate-buffered formalin and then washed off in a distilled water solution followed by processing in cryoprotectans (solutions of saccharose: 5 % for 2 hours, 10 % for 2 hours, 15 % for 12 hours). Fluorometry of histologic specimens was carried out by using an Axioskop 40FL microscope and an AxioCamHRc camera. To get a good computerized image of each histologic specimen, a Zeiss Plan-Neofluar x 40 lens was used for 20 s at a +24 °C room temperature. The images obtained were processed by AxioVision 3.1 software (Carl Zeiss).

Fluorescent probes containing ethidium bromide and chlortetracycline were used to perform fluorometry of the myocardium. Staining myocardium slices with ethidium bromide was done in a phosphate buffer pH=7.4 that contained 5.0 * 10 3 g/l of ethidium bromide for 5 minutes at a temperature of 25 °C, while chlortetracycline, also done in a phosphate buffer pH=7.4 containing 2.6 * 10 2 g/l of chlortetracycline, was applied for 1 minute at a temperature of 25 °C. Fluorometry of histological preparations was then carried out. In the case of ethidium bromide, uptake was equal to 510-523 nm, emission – to 595-605 nm, while in the case of chlortetracycline, those parameters came to 400 nm and 520 nm respectively. In addition, myocardium samples were stained with antibodies for Monoclonal Anti Skeletal Myosin (FAST) Clone MY-32 skeletal myosin. FITS-conjugated secondary antibodies were used as a secondary marker.

The following properties were measured: muscle fibre diameter, relative area of muscle tissue, its apparent density, number of nuclei, mean area of a nucleus, nucleus-cytological relations and number of intramyocardial vessels.

Microsoft Excel 2000 was used to perform statistical processing of the results. T-tests were employed to provide the reliability of differences of mean quantities and correlation relationships. Differences $p<0.05$ were considered as reliable.

The concentrations of CE were determined by X-ray fluorescence analysis with synchronous radiation (SRXRF). All measurements were carried out at the station of X-ray fluorescent elemental analysis in the Siberian Centre of Synchrotron and Terahertz Radiation (Budker Institute of Nuclear Physics SB RAS). The parameters of the storage ring VEPP-3 and experimental station are as follows: E_{ex} = 2 GeV, B = 2 T, I_e = 100 mA; chamber for the analysis is made from elconait; maximum diameter of the sample is 30 mm; the spot size is 1 ÷ 30 mm^2; exposure time is 10 ÷ 1000 c; the excitation energy is from 12 to 45 keV; elements determined: from S to U; X-ray fluorescence from the sample is registered by 10mm^2 Si(Li) detector (OXFORF, Oxford Instruments Inc., USA) with energy resolution 150 eV at 5.9 keV, respectively (Trounova et al., 1998).
The advantages of the application of SR as a primary source of excitation are as follows: the high intensity → the better peak/background ratio → analysis of samples with low masses (down to 0.5 mg, dry weigh); linear polarization → lower background → lower detection limits (down to 0.02 ppm for organic matrices); the wide spectrum of radiation → optimization of excitation energy, the possibility to measure samples, as well as varying the excitation energy.
The concentrations of CE in the samples of heart muscle and vessels were calculated by the external standard method (different certified reference materials [CRM]) were used). The corresponding approaches were elaborated upon, using different certified reference materials with similar matrices: the applicability of different standards and the absorption characteristics of their matrices were investigated (Trunova et al., 2008). All spectra obtained were processed by the AXIL programme (Canberra Packard, Benelux). The samples investigated are the fragments of myocardium tissue with masses from 2 to 10 mg (dry weigh). At one of the steps of the sample procedure they are dried for 48 hours and longer to obtain a dry sample with a flat surface.
The content of CE was measured in 40 samples of myocardium of TGA infants aging from 1 to 4.5 months (mean age 3.0+0.7 months, heart's mass 54.0+5.0 g, body mass 4.2+0.3 kg). The concentrations of the following 14 CE were studied: S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr by SRXRF (Okuneva et al., 2010). By using X-ray fluorescence analysis with synchronous radiation (XFA SR), concentrations of the following 14 CE were studied: S, Cl, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Se, Br, Rb, Sr (Okuneva et al., 2010). Myocardium samples were taken from ventricles and atria not later than 24 hours after death. Overall, more than 270 X-ray fluorescence spectra of CE were obtained. The content of CE was determined on the basis of 1 μg per 1 g of tissue.

3. The clinical examination of infants with TGA

All patients with TGA were broken down in 2 groups: the first group included patients with intact ventricular septum (IVS), while the second one incorporated those with ventricular septum defects (VSD). Two tasks were set; firstly, to study the clinical characteristics of patients depending on their age, for which purpose all of them were classified into 3 age groups: newborns aged 1 to 6 months and babies aged 6 to12 months. The second task was to compare the clinical characteristics of the deceased patients (subgroup I) and patients with favourable outcomes after surgical repair of the disease (subgroup II). Anthropometric measurements of patients with IVS depending on their age in the first and second group are given in Table 1.

Group	Sub group	At birth		By the time of surgery			
		Weight, kg	Height, cm	Weight, kg	Norm	Height, cm	Norm
Newborns	I (n=19)	3.2±0.6	50.4±3.2	3.1±0.1*	4.1±0.2	53.5±0.5	52.8±0.2
	II (n=5)	3.2±0.1	52.0±1.3	3.2±0.1*	4.1±0.2	52.4±1.1	
1-6 months old	I (n=18)	3.2±0.6	51.2±0.2	4.7±0.1	4.9±0.2	57.3±0.4	55.8±0.2
	II (n=5)	3.1±0.2	51.0±1.3	5.3±0.9	4.9±0.2	58.8±3.1	
6 to 12 months old	II (n=5)	3.1±0.1	50.0±0.8	7.4± 0.7	9.5±0.2	70.2±3.0	70.5±0.2

** P < 0.05*

Table. 1. Anthropometric measurements of TGA patients with IVS

As can be seen from Table 1, the weight of patients in all groups was close to the norm. However, by the moment of surgery the delay in body weight gain as compared to the norm in newborns in both subgroups amounted to 1000 g, in infants – 200 g, while in those who survived in the third group it came to 1500 g. Dynamics of height measurements slightly exceeded the benchmark indicators. Similar anthropometric data were also obtained for the VSD group (Table 2).

Group	Sub group	At birth		By the moment of surgery			
		Weight, kg	Height, cm	Weight, kg	Norm	Height, cm	Norm
Newborns	I	3,2±0,2	52,0±1,0	3,3±0,3	3,8±0,2	53,5±1,3	52,8±0,2
	II	3,0±0,1	51,2±0,6	3,5±0,1	3,8±0,2	51,7±1,0	
1 to 6 months old	I	2,8±0,5	51,1±0,8	4,1±0,6*	5,0±0,2	58,1±2,5	55,8±0,2
	II	3,2±0,1	50,8±0,2	4,0±0,1*	5,0±0,2	56,8±0,7	
6 to12 months old	I	3,4±0,4	50,6±1,2	6,0±0,8*	9,8±0,2	63,3±3,0	70,5±0,2
	II	3,0±0,1	50,6±0,3	7,0±0,5*	9,4±0,2	70,5±2,1	

** P < 0.05*

Table 2. Anthropometric measurements of TGA patients with VSD

By the moment of surgery, the delay in body weight gain as compared with the norm in newborns was on average 400 g, in the 1 to 6 months old – 900 g, in 6 to 12 months old infants in the deceased subgroup this difference came to 3800 g, while in those who survived – 2400 g. Dynamics of height measurements slightly exceeded the benchmark indicators in both groups. Based on the data obtained, one might conclude that disruption of metabolic processes in TGA infants evidently manifests itself only by a decrease in body weight, with the height parameters remaining the same. Table 3 looks at echocardiographic (ECHO) measurement data in patients with IVS depending on their age.

Group	Newborns		1 to 6 months old		6 to 12 months old
Subgroup	I	II	I	II	II
End-systolic dimension, cm	1.3±0.7	0.8±0.1	1.1±0.2	0.8±0.1	1.3±0.1
End-systolic volume, ml	1.4±0.7	1.4±0.7	3.8±1.5	1.7±0.5	5.8±1.0
Systolic output, ml	6.0±2.1	4.6±1.0	20.4±13.6	7.3±1.9	16.9±1.7
Shortening fraction, %	44.1±4.9	51.2±2.1	40.7±6.6	47.2±2.2	48.0±5.1
Ejection fraction, %	79.1±4.4	82.4±1.7	77.8±6.8	83.0±1.5	75.6±4.1
LV thickness, cm	0.5±0.1	0.5±0.1	0.6±0.1	0.5±0.1	0.6± 0.1
End-diastolic dimension, cm	1.6±0.2	1.5±0.1	2.0±0.3	1.7±01	2.5± 0.1
End-diastolic volume, ml	8.6±3.0	5.6±1.8	19.1±8.7	6.5± 2.5*	22.7±3.6

Table 3. Echocardiographic measurement data for TGA patients with IVS

As the table shows, the age does not influence the following values of ECHO: RV end-systolic dimension, LV shortening fraction and LV ejection fraction. There was a slight age-related increase in ASD, as well as a decrease in the unclosed ductus arteriosus size. As compared with the newborns, the following ECHO values tended to increase: RV size, end-systolic volume, systolic output, end-diastolic dimension, LV end-diastolic volume and LV thickness, thus indicating a reduction of myocardial contractility. ECHO data on TGA patients with VSD are given in Table 4.

Group	Newborns		1 to 6 months old		6 to 12 months old	
Subgroup	I	II	I	II	I	II
RV size, cm	1.2± 0.2	0.8± 0.1	1.0± 0.2	0.9± 0.2	1.5±0.4	1.4± 0.2
End-systolic dimension, cm	1.1± 0.3	1.2± 0.1	1.5± 0.4	1.3± 0.2	1.4±0.3	1.4± 0.2
End-systolic volume, cm	2.7±0.5	3.5± 0.9	9.4± 5.0	4.8±1.8	6.4± 2.3	6.4± 1.9
Systolic output, ml	10.1±3.5	9.3± 3.1	19.7±9.2	15.7±3.4	18.9±2.0	17.9±1.9
Shortening fraction,%	45.0±4.5	38.5±5.0	35.1±3.0	42.1±15.4	42.3±4.2	48.2±7.2
Ejection fraction, %	69.7±3.4	72.0±5.7	66.5±5.6	75.0±4.6	73.5±4.6	75.6±6.2
LV thickness, Cm	0.51±0.1	0.3± 0.1	0.54±0.1	0.5± 0.1	0.6±0.1	0.7± 0.1
End-diastolic dimension, cm	1.3± 0.3	1.9± 0.2	4.6± 3.3	2.4± 0.2	2.5±0.3	2.5± 0.2
End-diastolic volume, cm	11.6±1.5	13.3±4.6	25.8±11.9	20.6±5.0	24.2±5.9	22.7±4.8

Table 4. Echocardiographic measurement data for TGA patients with VSD

In the 1 to 6 and 6 to 12 months old groups, as compared with the newborn group, the following ECHO values were found to increase considerably: RV size, end-systolic volume, systolic output, end-systolic dimension, end-diastolic volume and end-systolic dimension. In addition, there was a trend toward an increase in the size of ASD, VSD and LV thickness. However, the size of unclosed ductus arteriosus tended to decrease. The shortening fraction (SF) and ejection fraction (EF) values matched the age-related indices. The pressure in the pulmonary artery was elevated in all groups, but it was particularly high in the 6 to 12 months old group.

Clinical/functional examination of TGA patients demonstrated that in terms of basic clinical indicators there were no statistically significant differences between the deceased and surviving infants with TGA. Moreover, average indicators of all 3 age subgroups (newborns, 1 to 6 months old and 6 to 12 months old) in both groups are identical within a time period. From this it follows that negative factors causing the death of infants with TGA are related to molecular disorders of metabolic processes in cardiomyocytes that, in turn, brought us to start studying the content of CE and structural/molecular characteristics of TGA infants' myocardium.

4. Distribution of chemical elements in different parts of the heart and their impact on the development of pathologies in TGA patients

The following mechanisms were identified when analyzing the content of CE in the deceased infants' myocardium (Table 5A,B).

		Intact myocardium		TGA patients' myocardium	
Parts of the heart		Left ventricle (n=5)	Right ventricle (n=5)	Left ventricle (n=15)	Right ventricle (n=20)
Content of CE, μg/g	S	3380±631	3260±335	3268±424	3547±331
	Cl	842±311	624±142	405±45*	435±56
	K	792±257	630±133	508±60	560±55
	Ca	1352±218	1264±94	1256±89	1224±99
	Cr	1.0±0.22	0.9±0.12	0.4±0.15*	0.6±0.23
	Mn	2.4±0.2	2.4±0.2	2.6±0.8	5.1±2.0
	Fe	344±30	422±83	321±42	342±33
	Ni	0.4±0.05	0.6±0.09	0.2±0.03*	0.2±0.05*
	Cu	8.9±0.68	10.1±0.87	14.6±2.99	16.1±2.58
	Zn	360±39	392±43	240±22*	307±42
	Se	0.7±0.1	0.8±0.1	0.2±0.05*	0.1±0.04*
	Br	12±1.6	13±1.6	6±0.7*	8±0.8*
	Rb	1.4±0.23	1.4±0.18	0.8±0.20	0.6±0.08*
	Sr	6.1±0.7	6.3±0.6	3.7±0.7	3.8±0.5*

** $P < 0.05$*

Table 5A. Distribution of CE in infants' ventricle with intact myocardium and TGA infants

It follows from Tables 5A and 5B that in 65 % of TGA patients, as compared to those with intact myocardium, the content of CE was reduced: K was lower, down to 78 %, concentration of Cl, Cr, Sr, Zn decreased to 50 % and the concentration of Br, Ni, Rb was also low. The content of Se equalled to just 25 % of the benchmark value. Three CE: S, Ca and Fe had an appropriate concentration. It was found out that only 2 CE had an increased concentration: Cu – 160 % and Mn – 170 to 200 %. According to the distribution of CE in the heart parts, the lowest concentrations of CE were found in LV and RA myocardium.

		Intact myocardium		TGA patients' myocardium	
Parts of the heart		Left atrium (n=5)	Right atrium (n=5)	Left atrium (n=21)	Right atrium (n=20)
Content of CE, μg/g	S	2560±180	2575±370	2398±300	2505±260
	Cl	504±83	615±158	348±43	290±42*
	K	494±71	580±165	444±58	421±38
	Ca	990±54	1112±96	1148±105	1109±86
	Cr	0.8±0.11	1.1±0.44	0.6±0.25	0.7±0.18
	Mn	2.0±0.2	2.0±0.2	3.1±1.1	4.2±1.4
	Fe	404±101	340±23	338±53	375±38
	Ni	0.3±0.06	0.4±0.09	0.2±0.04	0.2±0.04
	Cu	9.0±0.49	8.9±1.14	13.0±2.18	14.3±2.70
	Zn	344±38	298±57	183±21*	192±27
	Se	0.7±0.1	0.7±0.2	0.2±0.06*	0.2±0.05*
	Br	12±1.2	11±2.2	6±0.7*	6±0.6*
	Rb	1.2±0.17	1.1±0.24	0.5±0.10*	0.5±0.07*
	Sr	5.7±0.6	5.1±0.9	3.2±0.3*	3.5±0.4

** $P < 0.05$*

Table 5B. Distribution of CE in infants' atrium with intact myocardium and TGA infants

Hence, irreversible hemodynamic disorders of the myocardial function and development of cardiac insufficiency might be connected with a low concentration of Cl, Cr, Sr, Zn, Br, Rb, Ni and specifically Se, which in this case drops to 25 % and even beyond the measurement limit. An increased content of Mn and Cu mostly in the right parts of the heart could be explained by an elevated functional load and plays a compensatory role. The content of S, Fe and Ca matches the benchmark values and does not affect the changes in the myocardium. On the basis of the results obtained it may be concluded that in order to maintain normal functional activity of the myocardium in TGA infants, the content of Cl, Zn, Sr, Cr, Ni, Rb, Br and especially Se that protects cardiomyocytes from lipid peroxidation should be optimal. The following relationships were revealed while comparing CE impoverishment in the myocardium of TGA infants in different heart parts (Table 6).

CE	LV		CE	RV		CE	LA		CE	RA	
	Mean	±m		Mean	±m		Mean	±m		M	±m
S	3268	424	S	3547	331	S	2398	330	S	2505	260
Ca	1256	89	Ca	1224	99	Ca	1148	105	Ca	1109	86
K	508	60	K	560	55	K	444	58	K	421	38
Cl	405	45	Cl	435	56	Cl	348	43	Fe	375	38
Fe	321	42	Fe	342	33	Fe	338	53	Cl	290	42
Zn	240	22	Zn	307	42	Zn	183	21	Zn	192	27
Cu	14.6	2.99	Cu	16	2.58	Cu	13	2.18	Cu	14.3	2.7
Br	6	0.7	Br	8	0.8	Br	6	0.7	Br	6	0.6
Sr	3.7	0.7	Mn	5.1	5	Sr	3.2	0.3	Mn	4.2	1.4
Mn	2.6	0.8	Sr	3.8	0.5	Mn	3.1	1.1	Sr	3.5	0.4
Rb	0.8	0.2	Rb	0.6	0.08	Cr	0.6	0.25	Cr	0.7	0.18
Cr	0.4	0.15	Cr	0.6	0.23	Rb	0.5	0.1	Rb	0.5	0.07
Ni	0.2	0.03	Ni	0.2	0.05	Se	0.2	0.06	Se	0.2	0.05
Se	0.2	0.05	Se	0.1	0.04	Ni	0.2	0.04	Ni	0.2	0.04

Table 6. CE impoverishment in the heart parts of TGA infants

It was found that in the hypertrophied myocardium of LV (and RV) there was a decreased content of K, Cl, Zn, while the content of S, Fe and Ca remained at an adequate level (see Fig. 1).

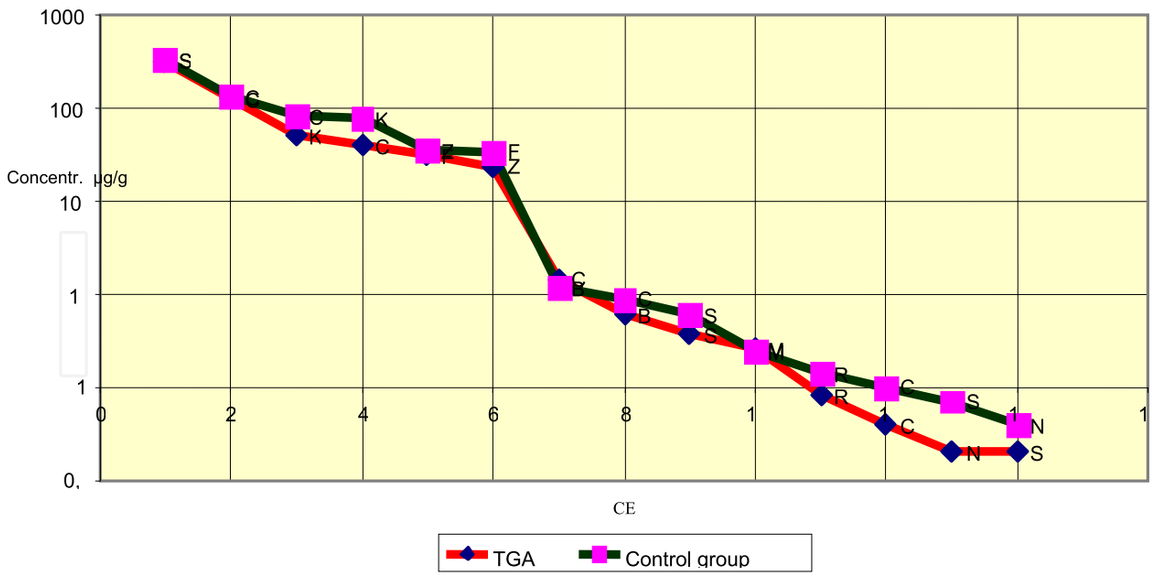


Fig. 1. Distribution of CE in LV of TGA infants, CE are arranged by concentration decrease.

The concentration of microelements Cr, Rb, Ni, Se was notably lowered, while the remaining CE had values that were close to the benchmark ones. The data obtained led us to conclude that the hypertrophied myocardial function in TGA infants was impaired due to a

decreased concentration of microelements. These changes were more pronounced in the left parts of the heart. These results were also confirmed by the morphological examination data. Hypertrophic changes in the heart make rapid strides over age, exceeding the benchmark age values by 2 times at the age of up to 1 month and by 3.5 – 4.5 – at the age of 6 to 12 months. At the same time, the linear dimensions of LV and RV in patients with IVS (first group) were practically identical, while in the VSD group (second group) the thickness of RV exceeded that of LV by 133 % (Table 7).

Group	Heart's mass, g*	Wall thickness, cm		Muscle fibre diameter, μm		Inflow, cm		Outflow, cm	
		RV	LV	RV	LV	RV	LV	RV	LV
1st group	52.6 ±8.31	0.60 ±0.15	0.61 ±0.17	11.65 ±2.12	11.85 ±1.95	2.5 ±0.41	2.6 ±0.24	3.6 ±0.61	3.7 ±0.47
2nd group	48.7 ±9.33	0.60 ±0.12	0.55 ±0.14	11.90 ±3.11	11.60 ±2.55	2.5 ±0.21	2.8 ±0.36	3.6 ±0.52	4.0 ±0.54

* The norm is 24±0.15 g

Table 7. Cardiometric parameters of TGA newborns (first and second groups)

Heart part	Anatomic group	S	K	Fe	Cu	Sr	Zn
RV	1st group	0.15	0.09	0.58*	-0.26	-0.19	0.12
	2nd group	0.31	0.15	-0.29	-0.60*	0.13	0.08
LV	1st group	0.92**	0.75**	0.82**	-0.92**	0.67**	-0.18
	2nd group	0.46	0.47	0.50	-0.33	0.30	-0.25

* - Reliability of correlation relationship ($p<0.05$)
** – Reliability of correlation relationship ($p<0.01$)

Table 8. Correlation relationships (r) between myocardium thickness and content of some chemical elements

A statistically reliable relationship between LV myocardium thickness and the content of S, K, Fe, Sr and a negative correlation relationship with Cu were revealed for the first group with IVS (Table 8).

According to our data, an impaired myocardial function in TGA infants resulting in death might be related to a considerable reduction of metabolism, the markers of which appeared to be a lowered content of Br, Ni, Rb (down to 50 %), Cr, Sr, Zn, Cl (down to 60 %) and particularly Se (down to 25 %). What role do these CE play in myocardium metabolism in TGA infants? Some of these CE are mostly of an endonuclear nature (Cr, Mr, Ni), while others are found outside the nucleus and accumulated in microsomes, mitochondria, lysosomes and Golgi's complex (Cu, Zn, Se, Br, Sr) (Kudrin et al., 2000). Of great importance is Zn, which activates more than 300 enzymes and is part of over 200 metalloproteins (Skalny, 1999; Beerli, 2000). Zinc deficiency results in the development of congenital heart diseases (Panchenko, 2004), Br plays an important role in the development of a foetus and its shortage leads to a greater number of miscarriages. Ni might be a co-factor of many enzymes: urease, hydrogenase, a number of dehydrogenases and methyl-coenzyme M-reductase, while its deficiency affects metabolic processes in the cells. It was found out that

the activity of b-DNA-polymerase directly depends on the content of Cr, a vital chemical element (Panchenko, 2004). Cr deficiency is observed in premature infants, whose mothers do not get enough of it in their diet. Chlorous channels can be found in mitochondrial membranes and muscle tissue. Also, chloride ions regulate the liquid volume and stabilize pH of the cells (Sing & Snow, 1998). Rb is an analogue of K and together with Cl they are very active in redoxreactions. A considerable deficiency of Se, which protects cardiomyocytes from detrimental effects of free radicals, has the greatest impact on cardiomyocyte metabolism. A decrease in muscle mass and a developmental lag were observed in newborns whose mothers were short of Se during pregnancy (Panchenko et al., 2004). In the case of Se deficiency, the cells start dying both in the form of apoptosis and necrosis, which might result in the sudden death of newborns (Azoicai et al., 1997; Bolli, 2002). On the strength of these data, we suggest that a very low content of CE, and Se in particular, in the myocardium could lead to structural disorders in the development of heart parts and, consequently, to deaths among TGA infants.

5. Pathomorphological measurements of myocardium in TGA infants

Data on morphological measurements of myocardium samples of TGA infants and infants of the same age but with intact myocardium are given in Table 9. As is seen from Table 9, the myocardium mass increased by 2.0 – 2.5 times and it tended to increase over age, i.e. in TGA infants the increase in the heart's mass considerably exceeded the normal age-related values for the heart's mass. Morphometric measurement data show that, in comparison with the intact myocardium, the TGA infants' myocardium had a reduced diameter of muscle fibres and a reduced mean area of nucleus and lowered nucleus-cytoplasma ratios in RV. However, the volumetric density and relative area of muscle tissue surface tended to increase.

Morphometric parameters	Heart part	Infants	TGA infants
Muscle tissue diameter, μm	LV	14.6±0.79	12.0±1.47
	RV	13.1±1.13	11.4±1.35
Relative area of muscle tissue surface, μm²	LV	265±22.8	287±27.3
	RV	274±27.3	286±37.6
Volumetric density of muscle tissue	LV	0.78±0.067	0.85±0.08
	RV	0.81±0.081	0.84±0.11
Number of nuclei per field of vision	LV	41±2.5	45±7.1
	RV	53±1.5	34±7.7
Mean area of nucleus, μm²	LV	2358±211.8	2073±107.1
	RV	2534±289.1	2063±355.8
Nucleus-cytoplasmic ratio	LV	0.37	0.33
	RV	0.49	0.26

Table 9. Morphometric parameters of TGA infants' myocardium

Depending on the anatomic type, 2 groups of TGA patients prevail: the first group, the so-called simple TGA form, TGA with atrial septal defect (ASD) and intact ventricular septum (IVS), and the second group, which includes TGA patients with ASD and VSD.

From the point of view of hemodynamics, the first group of TGA patients with IVS features a two-directional shunt, the volume of which, when performing isolated shunting on the level of atria, will depend on compliance of atria, a pressure differential in them during different phases of the cardiac cycle, size of atrial defect and a difference in resistance of the systemic and pulmonary circulation. Since the systemic circulation and pulmonary circulation are separated, the main compensation strategy is to increase the volume of circulating blood, which leads to overflow of the pulmonary circulation system (Adkin et al., 2002). In this anatomic type of TGA, the functional load on the ventricles is practically the same, which is confirmed by the results obtained while staining the myocardium with ethidium bromide. These results indicate that the peak of active synthesis of genetic material uptake in both ventricles in this group, as compared to that in the control group (see Fig. 2A), occurs during the neonatal period and manifests itself as a dramatic drop in colour intensity in fluorescence (see Fig. 2B).

The second group of TGA patients with VSD is hemodynamically characterized by the presence of 2 defects, on the level of atrial and ventricular septa, which improves blood mixing on the ventricular level due to crossed shunting. With VSD size being small, the pressure in pulmonary circulation grows slightly, when the size of VSD is large, the pressure in both circulation systems is levelled out which results in high pulmonary hypertension (HPHT) and augmentation of hypoxemia (Bokeria, 1996; Isoyama et al., 1987). In this anatomic type of TGA, due to an increase in the blood volume, both ventricles are subject to a large functional load as compared with the first group of patients, which makes itself evident in a reduced level of fluorescence in infancy (1 to 6 months old). From our point of view, this phenomenon can be defined as the start of the heart remodelling processes, which at the age of older than 6 months also include hyperplastic processes. These processes are related to polyploidization of nuclear material and subsequent hypertrophic phenomena determined by appropriate hemodynamic conditions developed during the postnatal period.

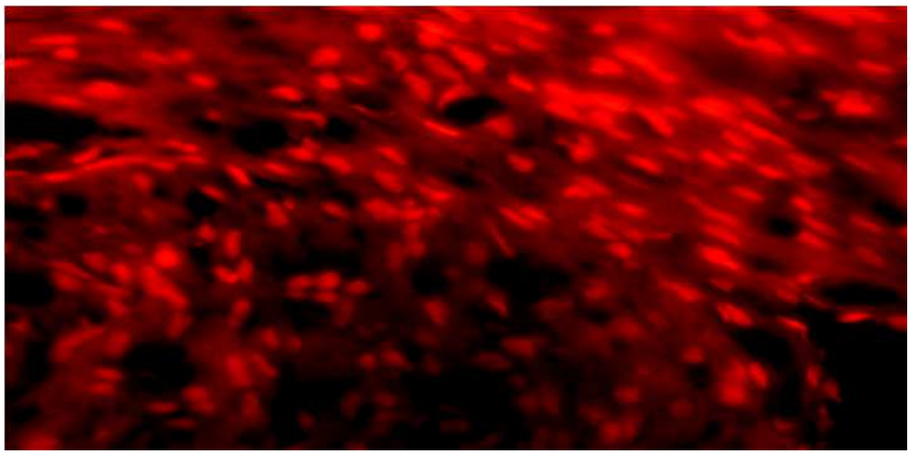


Fig. 2A. Control group (up to 1 month). LV myocardium. Magnification 260. Filter set 14. BP510-560nm. FT580. LP 590nm. Staining with ethidium bromide.

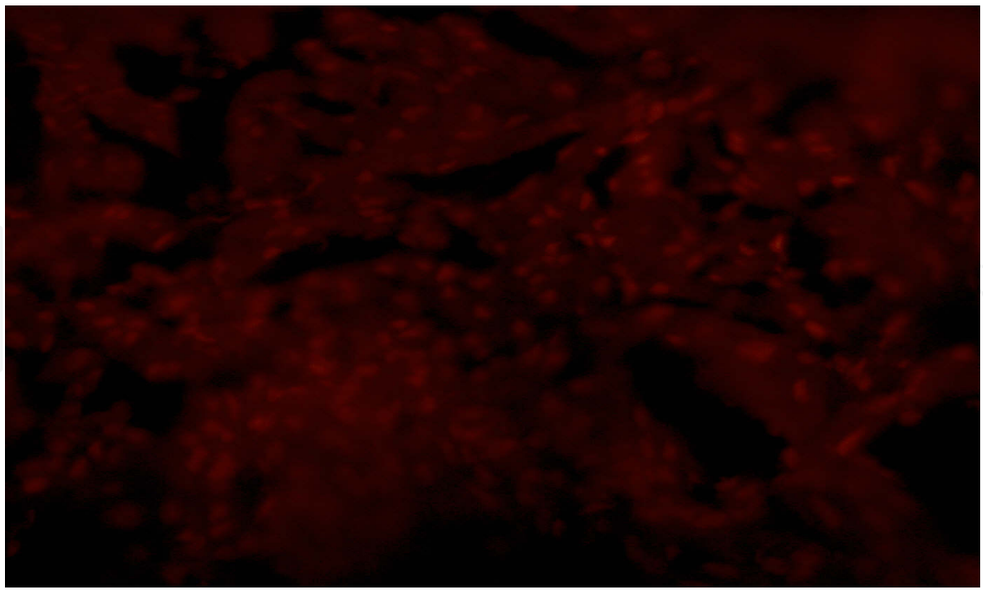


Fig. 2B. TGA with IVS. Lowered fluorescence level. LV myocardium (1 to 6 months). Magnification 260. Filter set 14. BP510-560nm. FT580. LP 590nm. Staining with ethidium bromide.

Clinical examination of TGA patients classified as belonging to the first anatomic type, i.e. with IVS, revealed an increase with age in cardiac insufficiency, respiration rate, liver dimensions, as well as a reduction in blood oxygen saturation on average, down to 60.9+13.5%. Arterial pressure and cardiac rate were within the normal age limits.

All TGA patients from the VSD group had pronounced cardiac insufficiency, increased respiration rate and liver dimensions, and decreased blood oxygen saturation, down to 32. 5+12.5% ($p<0.05$). Arterial pressure and cardiac rate were within the normal age limits. According to echocardiographic data, TGA patients from both groups, as compared with newborns, tended to show with age an increase in the following indicators: RV size (end-systolic dimension, stroke output, end-diastolic volume) and LV thickness. These changes imply a tendency towards a decrease in the contractile potential of the myocardium. The senior group of patients with VSD demonstrated a higher pressure in the pulmonary artery, up to 76.5+2.1 mm Hg.

LV muscle mass was growing faster by 6 to 12 months in the patients with VSD, with this value remaining stable in the IVS group. The mean quantity of nuclei was initially lower than in the control group, while the average area of nuclei was, to the contrary, higher but tended to decrease with age as well. The total area of nuclei in the control group tended to decrease. In the first group with IVS, this process took place faster, while in the second group there was an increase of this value, which is indicative of a compensatory reaction of LV. With age the surface density of cardiomyocytes smoothly grew in all the groups. The nucleus-cytoplasmic ratio in LV gradually decreased with age in infants of all groups. More pronounced was this tendency in RV of TGA patients with IVS. Conversely, a slight increase in this ratio was noted in TGA patients with VSD.

Studying the number density of capillaries revealed their simultaneous changes in both groups, with the highest point occurring at the age of 1 month in LV and RV in patients with

IVS. Measuring the content of CE in groups with IVS and VSD resulted in the following findings. The content of Cu, Zn and Mn in the group with VSD is 1.3 – 1.5 times higher than in the group with IVS. The content of CE in LV and RV of patients with IVS was about the same, except for an increased content of Mn in LV. At the same time, the content of CE in RV of patients with VSD increased, compared with that in LV, notably higher were concentrations of Zn, Mn and, to a lesser extent, Cu, Cr, Br, Rb. These findings are also confirmed by the cardiometric data (see the Table). In the case of the type with IVS, hypertrophic processes in LV and RV develop uniformly, and CE concentrations in LV and RV do not differ essentially. In the second type of TGA with VSD, the right ventricle has to bear a large functional load, therefore, CE concentration in RV is higher than in LV. It agrees with more pronounced structural changes in coronary arteries in the functionally overloaded RV in patients with VSD. However, despite intensive cardiac work in the cases of ASD and VSD, oxygen delivery in this group of patients is worse because of lower blood oxygen saturation down to 32 %.

The reduction of the numeric content of total ions Ca^{2+} is caused by the development of hypertrophic phenomena in the myocardium of patients with congenital heart diseases (see Fig. 3A and 3B). Most probably, these hypertrophic phenomena result from a decrease in the number of myofibrils, which aggravates cardiac insufficiency.

Fig. 3A. Control group. Native sample of LV wall. A high level of fluorescence. Filter set 05. BP395-440nm. FT460. LP 470nm. Magnification 260. Staining with chlortetracycline.

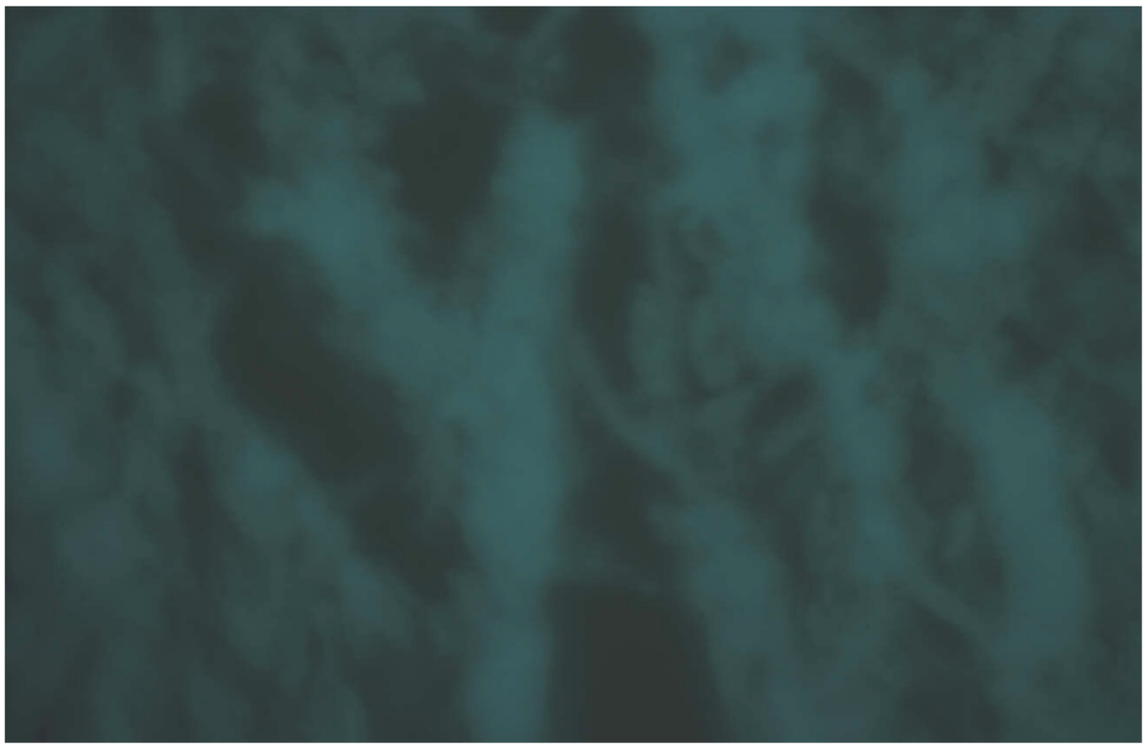


Fig. 3B. TGA with IVS (6 to 12 months). Native sample of LV wall. A lowered level of fluorophore fluorescence. Filter set 05. BP395-440nm. FT460. LP 470nm. Magnification 260. Staining with chlortetracycline.

In the case of hypertrophy not only is the volume (size) of muscle cells changed, but their phenotype as well. In the conditions of overload the contractile proteins in these cells are replaced by protein forms typical for foetuses and newborns. For example, the ß- myosin (ß-MHC) heavy chain is activated and, simultaneously with the suppression of α-MHC gene, the activity is changed over from the genes of the cardial α-actin to the genes of the skeletal one. This results in a reduction of the contractility speed of hypertrophied fibres. As hypertrophy proceeds, a few other genes are activated, including some early growth regulators, genes responding to thermal shock and growth factors, as well as a gene of the atrial natriuretic factor. The latter represents a peptide hormone that facilitates a decrease in hemodynamic overload by regulating blood pressure and salt discharge by the kidneys. Taking into account the preceding, we stained the myocardium samples with antibodies for Monoclonal Anti-Skeletal Myosin (FAST) Clone MY-32 skeletal myosin. As a secondary marker, we made use of FITS-conjugated secondary antibodies. As a result of the technique used, skeletal myosin was found in the myocardium of TGA patients (Fig. 4).

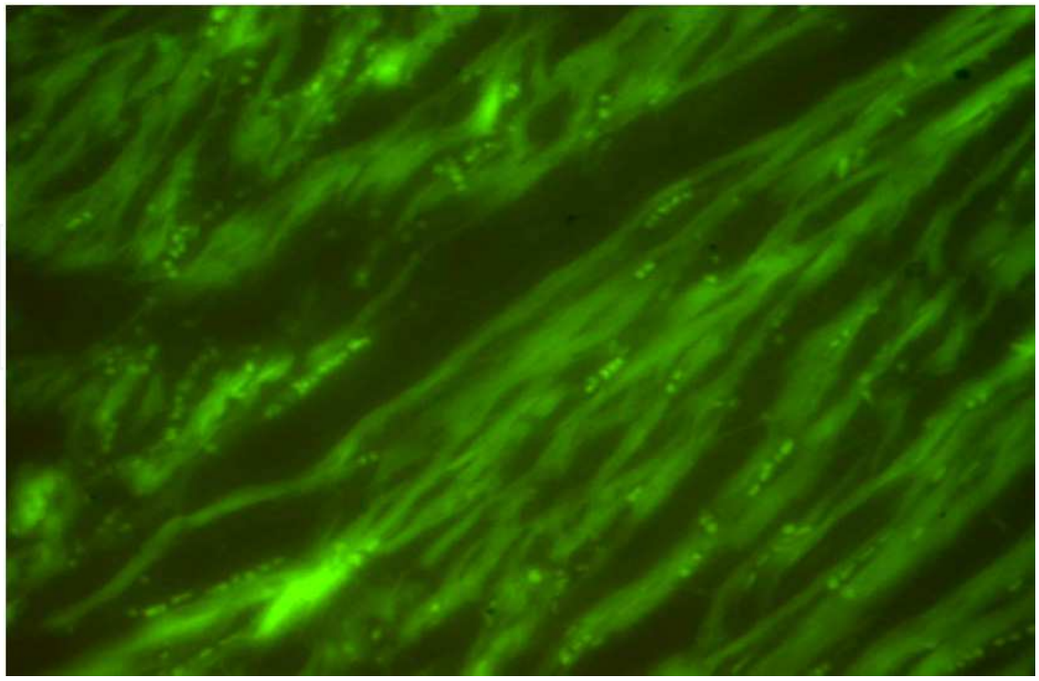


Fig. 4. TGA with IVS (1 to 6 months). Sample of LV wall. Appearance of skeletal myosin granules in the myocardial structure. Filter set 09. BP395-440nm. FT460. LP 470nm. Magnification 260. Monoclonal Anti-Skeletal Myosin (FAST) Clone MY-32, secondary antibody FITS-conjugated.

Thus, considering the dynamics of intensity of the above morphological processes taking place in the myocardium of TGA infants not older than 1 year, it should be noted that hypertrophic changes in TGA patients' myocardium make progress with age. Hyperplastic processes associated with intensive polyploidization of the genetic material and an increase in the quantity of desoxyribonucleic acid play an important role in the remodelling of the heart in patients older than 6 months. On the basis of fluorometric measurement data, the decrease in the level of total calcium ions in cardiomyocytes of TGA patients is dependent on the occurrence of cardiosclerosis zones when hypertrophy of the myocardium is progressing. While adapting to these processes and to chronic hypoxia typical for congenital heart diseases and due to a less energy-consuming mechanism of skeletal muscle contractility, the synthesis changes over from cardiac myosin to a skeletal one, which, in turn, enhances clinical presentations of cardiac insufficiency because of a lowered speed of hypertrophied fibre contractility. In the case of hypertrophy of cardiomyocytes, not only is their volume (size) changed, but their phenotype as well. The synthesis of the ß-myosin (ß-MHC) heavy chain is activated and, simultaneously with suppression of α-MHC, the synthesis of cardio-specific proteins is changed over to proteins specific for skeletal muscles, for example, skeletal a-actin is expressed. This results in a reduction of the contractility speed of hypertrophied fibres. As hypertrophy proceeds, a few other genes are activated including some early growth regulators, genes responding to thermal shock and growth factors, as well as a gene of the atrial natriuretic factor, which facilitates a reduction in hemodynamic overload by regulating blood pressure and discharge of salt by the kidneys. Immunohistochemical examinations of the samples of TGA infants' myocardium made it

possible to observe the appearance of skeletal myosin in the cardiomyocytes. This testifies that, during hypertrophy development, the synthesis changes over from cardiac myosin to a skeletal one. Plain fluorescent microscopy of preparations stained with ethidium bromide revealed a drastic decrease in intensity of the fluorescent marker in infants aged under 6 months, as compared to the control group, and a rapid growth of ethidium bromide incorporation in infants aged 6 months and upward. It indicates a prevalence of the population of cardiomyocytes with diploid nuclei in the hearts of infants aged up to 6 months. In patients aged above 6 months, the heart remodelling process proceeds, with the processes associated with polyploidization of nuclear material and subsequent development of cell hypertrophy dominating. It should be emphasized that the level of polyploidization in LV cardiomyocytes is essentially higher than that in RV. Hence, the hyperplastic processes associated with intensive polyploidization of the genetic material and an increase in the quantity of desoxyribonucleic acid play an important role in the remodelling of the heart in patients aged above 6 months.

6. Conclusion

This study enabled us to come to the following conclusion on the development of a pathological mechanism causing the deaths of TGA patients at an early age. As a result of aorta and pulmonary artery transposition, low-oxygen venous blood flows into the systemic circulation system, limits the growth of newborns. The need for a sufficient volume of oxygen can be met only by increased load on the myocardium, with the development of heart hypertrophy uniform for LV and RV in patients with IVS and more pronounced in RV in patients with VSD. In this case the growth of TGA infants conforms to the age norm and even slightly exceeds it, while the body mass falls far short of the norm by 25-30 %. For hypertrophy and hyperplasia to develop dramatically, there should be an increased supply of nutritional and caloric substances, including CE. As the delivery of CE turns out to be insufficient, or their consumption increases, a 50 % deficiency of such microelements as Cl, Cr, Sr, Zn, Br, Rb and especially Se, which, as an active antioxidant, protects cardiomyocytes from lipid peroxidation, can be seen. As a consequence, structural disorders of the myocardium occur on the morphological/molecular level. Also observed are the following abnormalities: a decrease in the diameter of muscle fibres and the average area of nuclei, a drop in the level of the total calcium ions against the background of intensive polyploidization of genetic material, an increase in the content of the quantity of desoxyribonucleic acid, and change from the cardiac myosin synthesis over to a skeletal one. All these changes lead to alteration of the myocardium, occurrence of cardiosclerosis, development of cardiac insufficiency and reduction of arterial blood saturation down to 32 % and below, which is fatal, and results in death of the organism. This is a picture of TGA development pathogenesis of infants from neonatal age up to 1 year old. In this case, only definitive repair of the disease can break the pathogenetic chain of TGA in an early period, and the best time when effective cardiac surgery could be performed for TGA infants is in the neonatal period.

We suppose, that to prevent the development of congenital heart diseases including TGA, pregnant women and nursing mothers should get the optimum quantity of microelements Cr, Zn, Sr, Ni, Rb, Br and most of all Se, protecting the myocardium from lipid peroxidation.

7. References

Adkin, D.V.; Gnusayev, S.F. & Ilyin, V.N. (2002). Criteria of early detection and tactics of early preoperative treatment of TGA. *Pediatry*, No.3, (2002), pp. 88-91

Azoicai, D.; Ivan, A. & Bredatean, M. et al. (1997). The importance of use of selenium in the role of an antioxidant in preventing cardiovascular disease. *Rev Med Chir Soc Med Nat Lasi*., Vol. 101, No. 2, (1997) , pp. 109-115

Beerli, R.R.; Schopfer, U. & Dreier, B. et al. (2000). Chemically regulated zinc finger transcription factors. *J Biol Chem*., Vol. 275, No. 42, (2000), pp. 2617-2627

Bokeria, L.A. & Gorbachevsky, S.V. (1996). Congenital heart diseases: state-of-the-art in Russia. *Proceedings of Thoracic and cardiovascular surgery*, Moscow, Russia, No. 3, (1996), pp. 31-34

Bolli, R. (2002). Regeneration of the human heart-no chimera? *N. Engl. J. Med,* Vol 346, No. 1, (2002), pp. 55-56

Castaneda, A.R. (1993). Surgical management of transposition of the great arteries. *Acta Chir. Austriaca*, Vol 25, No 2, (1993) , pp. 80-82

Castaneda, A.R.; Trusier, G.A. & Paul, M. H. et al. (1998). The early results of treatment of simple transposition in the current era. *J. Thorac. Cardiovasc. Surg*, Vol. 95, No 1, (1998), pp 14-28

Fozzard, H.A.; Haber, E. & Jennings, R.B. et al. (1986). *The Heart and Cardiovascular System.* New York: Raven Press, ISBN 0881671266, pp. 1491-1495, USA

Hoffman, J. (2006). *Pediatric cardiology*. ISBN 5-89816-071-X, Moscow, Russia

Isoyama, S.; Wei, JY. & lzumo, S. et al. (1987). Effect of age on the development of cardiac hypertrophy produced by aortic constriction in the rat. *J Clin invest*, Vol. 81, No. 6 (1988), pp. 1850-1857

Kudrin, A.V.; Skalny, A.A. & Zhavoronkov, A.A. (2000). *Immunopharmacology of microelements*. ISBN 5-87317-077-0, KMK Publishing House, Moscow, Russia

Okuneva, G.N. et al. (2010). *Chemical elements in the cardiovascular system of cardiac patients.* GEO Publishing House, ISBN 978-5-904682-22-4, Novosibirsk, Russia

Panchenko, L.F.; Mayev, I.V. & Gurevich, K.G. (2004). *Clinical biochemistry of microelements.* GOU VUNMC Publishing House, ISBN 5-89004-077-4, Moscow, Russia

Re, R.M. (1987). Cellular mechanisms of growth in cardiovascular tissue. *Am.J.Cardiol*., No. 60, (1987), pp. 100-104

Reiss, K.; Cheng, W. & Ferber, A. et al. (1996). Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice, *Proc. Natl. Acad. Sci*. USA. Vol. 93, No. 16, (1996), pp. 8630-8635.

Ruff, H.A. (1999). Population-based data and development of individual children: case of low to moderate lead levels and intelligence. *J. Dev. Behev. Pediatr*., Vol. 20, No 1, pp. 42-79

Scholzen, T. & Gerders, J. (2000). The Ki-67 protein: from the known and the unknown. *J. Cell. Physiol*. Vol. 182, (2000), pp. 311-313

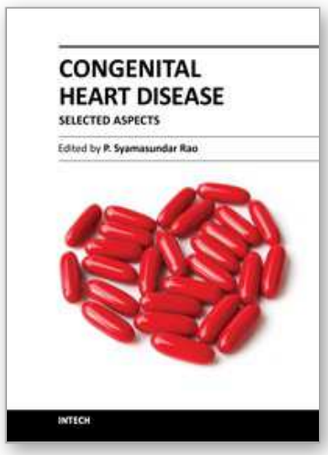
Shankar, A.H. & Prasad, A.S. (1998). Zinc and immune function: the biological basis of altered resistance to infection. *Am. J. Clin. Nutr*. Vol. 68 (Suppl.), (1998), pp. 447-463

Singh, J. & Snow, E.T. (1998). Chromium (III) decreases the fidelity of human DNA polymerase beta. *Biochemistry,* Vol. 37, No. 26, (1998), pp. 9371-9378

Skalny, A.V. & Rudakov I.A. (2004). *Bioelements in medicine.* Moscow, Mir Publishing House, ISBN 5-03-003645-8, Moscow, Russia

Trounova, V.A.; Zolotarev, K.V. & Baryshev, V.B. et al. (1998). Analytical possibilities of SRXRF station at VEPP-3 SR source. *Nuclear Instruments and Methods A*, Vol. 405, (1998), pp. 532-536

Trunova, V. A. & Zvereva, V. V. (2008). Investigation of the distribution macro - and traceelements in human myocardium and vessels by SRXRF. *Journal of Structural Chemistry*, Vol. 49, (2008), pp. 211-216



Congenital Heart Disease - Selected Aspects

Edited by Prof. P. Syamasundar Rao

ISBN 978-953-307-472-6
Hard cover, 348 pages
Publisher InTech
Published online 18, January, 2012
Published in print edition January, 2012

There are significant advances in the understanding of the molecular mechanisms of cardiac development and the etiology of congenital heart disease (CHD). However, these have not yet evolved to such a degree so as to be useful in preventing CHD at this time. Developments such as early detection of the neonates with serious heart disease and their rapid transport to tertiary care centers, availability of highly sensitive noninvasive diagnostic tools, advances in neonatal care and anesthesia, progress in transcatheter interventional procedures and extension of complicated surgical procedures to the neonate and infant have advanced to such a degree that almost all congenital cardiac defects can be diagnosed and "corrected". Treatment of the majority of acyanotic and simpler cyanotic heart defects with currently available transcatheter and surgical techniques is feasible, effective and safe. The application of staged total cavo-pulmonary connection (Fontan) has markedly improved the long-term outlook of children who have one functioning ventricle. This book, I hope, will serve as a rich source of information to the physician caring for infants, children and adults with CHD which may help them provide optimal care for their patients.

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