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PATHOPHYSIOLOGICAL MECHANISMS OF ADAPTATION OF MUSCLE TISSUE OF DESCENDANTS OF IRRADIATED ANIMALS TO ALTERING INFLUENCE OF IONIZING RADIATION

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Abstract

Biochemical processes occurring in a living organism take part in the development of radiation-induced structural disorders, realizing primary damage. As a result, morphological manifestations of radiation damage are preceded by chemical shifts determining them. In the descendants of animals irradiated in different doses, a decrease in physical performance is observed, which is due to a violation of the efficiency of the use of the unique biosubstrate of muscle tissue - creatinephosphate, a change in the ratio of the activity of aerobic and anaerobic metabolism, and processes of trans-deamination of amino acids. The purpose of the work is to investigate the the mechanisms of disruption of metabolic processes in the muscle tissue of the descendants of irradiated animals. It was concluded that, unlike skeletal muscle, the activity of the tricarboxylic acid cycle, in particular the NAD-dependent malate dehydrogenase, in the myocardium is quite significant both in the cytoplasm and in the mitochondria of the tissue, as evidenced by the higher level of tricarboxylic acid cycle metabolites acids - malic and

oxaloacetic, as well as the activity of NADP-dependent malate dehydrogenase, which performs a connecting role between glycolysis and the cycle of tricarboxylic acids in providing them with metabolites and transferring protons from $NADH^{+}H^{+}$ to NADP. The author revealed that myocardium is characterized by a larger pool of adenyl nucleotides due to ATP. Tha data obrtained shiowed hyperglycemia which is observed in the blood of the descendants of irradiated animals. Gluconeogenesis is enhanced in the liver of the descendants of irradiated animals and this explains the hyperglycemia and accumulation of glycogen in the liver. At the same time, the penetration of glucose into muscle cells is weakened, which is associated with a decrease in their glycogen content, and this can be explained by the decrease in adaptation to physical exertion in the descendants of irradiated animals. The author made a cionclusion that the pathophysiological mechanisms of radiation-induced energy supply restructuring are aimed at short-term processes of strengthening the supply of energy to vital organs and systems for destroyed biochemical, physiological, functional and regulatory processes restoration and sanogenetic mechanisms activation.

Key words: ionizing irradiation; irradiated animals; descendents; muscle tissue; adaptation; pathophysiological mechanisms

Biochemical processes occurring in a living organism take part in the development of radiation-induced structural disorders, realizing primary damage. As a result, morphological manifestations of radiation damage are preceded by chemical shifts determining them [1, 2, 14, 17, 19].

It was established that general irradiation of rats in doses of 0.5-4.5 Gy causes irreversible and dose-independent edematous degeneration, intracellular lysis, damage to mitochondria of myocardial endotheliocytes, which may play a role in the development of deterministic, non-stochastic consequences of radiation exposure in small doses. After irradiation, thickened muscle cells with enlarged nuclei appear in the heart muscle, the number of these cells does not decrease for 6 months, which indicates a violation of nuclear-cytoplasmic relations in myocardial cells. The morphometric characteristics of heart mitochondria also change and the contractile capacity of the myocardium is impaired. Blockades of the legs of the bundle of His and violations of the sinus node automatism are noted. In the myocardium of an irradiated organism, the efficiency of oxidative phosphorylation decreases not only in the cytochrome section of the respiratory chain of mitochondria, but also at the points of conjugation to cytochrome enzymes.

It was established that fatigue is accompanied by a change in the activity, affinity with substrate and isozyme spectrum of a number of enzymes (the self-assembly of which is carried out at the epigenetic level) and compartmentalization of enzyme systems. In muscle tissue, gluconeogenesis proceeds along a path different from gluconeogenesis in the liver and kidneys [7, 12, 20, 21].

In the descendants of animals irradiated in different doses, a decrease in physical performance is observed, which is due to a violation of the efficiency of the use of the unique biosubstrate of muscle tissue - creatinephosphate [9], a change in the ratio of the activity of aerobic and anaerobic metabolism [10, 23], and processes of trans-deamination of amino acids [3].

Cardiac muscle differs from skeletal muscle not only in morphological and functional characteristics, but also primarily in the significant content of mitochondria, the speed of protein exchange, the high intensity of aerobic processes, in particular, the reactions of the tricarboxylic acid cycle, creatine phosphokinase. Cardiac muscle, unlike skeletal muscle, uses significant amounts of fatty acids, as well as lactate and ketone bodies, in addition to glucose to obtain energy [6, 17].

The aim of the work is to investigate the mechanisms of disruption of metabolic processes in the muscle tissue of the descendants of irradiated animals.

Material and Methods

The studies were conducted on sexually mature male rats weighing 180-220 g of the Wistar line, which were kept on a standard vivarium diet. Keeping, processing of the animals and manipulations with them were carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the Fifth National Congress on Bioethics (Kyiv, 2013), being guided by the recommendations of the European Convention on the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1985), the methodological recommendations of the State Expert Center of the Ministry of Health Ukraine "Preclinical studies of drugs" (2001) and the rules of humane treatment of experimental animals and conditions approved by the Bioethics Commission of the Odesa National Medical University (protocol No. 32D dated 03/17/2016).

The animals were subjected to total gamma irradiation of Co^{60} on an empty stomach using the “Agat” telegammatherapy unit. The absorbed dose was 6.0 Gy, the dose rate was 0.48 Gy/min, and the distance to the absorption source was 75 cm. The lethality with this method of irradiation was equal to 43.0% of irradiated animals (43 rats) in 1 month.

Studies were conducted on 1-month-old pup rats born to intact animals and 1-month-old pup rats born to animals that were totally irradiated in a dose of 0.5 Gy. To detect the radioresistance of 1-month-old rats, they were totally irradiated once in a dose of 1.0 Gy.

The animals were divided into groups as follows:

1. Intact sexually mature animals.

2. 1-month-old pup rats obtained from intact animals. There were 8-10 animals in each group.

Animals were removed from the experiment by euthanasia under propofol (IV, 60 mg/kg) anesthesia. After the animals were dissected, blood was collected, the heart and the anterior group of thigh muscles were removed. Blood was centrifuged at 3000 g for 10 minutes to obtain serum. The removed cardiac and skeletal muscles were washed with chilled 0.9% physiological NaCl solution, minced and homogenized in a 9-fold volume of 0.32 mol sucrose per 0.05 mol Tris buffer, pH 7.36 in a homogenizer with Teflon surfaces and subjected to differential centrifugation in a refrigerated centrifuge PC-6. Nuclei were precipitated at 1000g for 10 min., then mitochondria at 12000g for 20 min. were resuspended in a homogenizer in isolation medium containing 0.1% triton X-100 solution at the rate of 1 ml of 0.1% triton solution per 500 mg of tissue and left in ice for 30-35 min.

Mitochondria, mitochondrial supernatant of myocardium, anterior group of thigh muscles and blood serum were used for biochemical studies. Our attention was focused on the activity of pyruvate kinase (PK), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), NADP-dependent MDH, phosphoenolpyruvate carboxykinase and the content of lactate, pyruvate, malate and oxaloacetate. To detect the content of biosubstrates in tissues, they were immersed in liquefied nitrogen followed by treatment with perchloric acid [4].

The content of glycogen and glucose was detected in the liver and skeletal muscle of 1-month-old rats, and the content of glucose in the blood. The content of glucose in the blood, glycogen in the liver and muscles was determined by the glucose oxidase method. The total amount of protein in muscles, liver, and blood was determined by the spectrophotometric biuret method [4].

The principle of the method for detecting the activity of pyruvatekinase (PC) is that pyruvate kinase in the presence of ADP converts phosphoenolpyruvate, which in turn, in the presence of reduced NAD and lactatedehydrogenase, turns into lactate, thereby oxidizing NADH [8]. Pyruvate kinase activity was expressed in micromoles of pyruvate per mg of protein in the sample for 1 min of incubation.

MDH activity was determined according to the method [4] and expressed in μmol of formed NADH per mg of protein in the sample in 1 min. incubation The activity of phosphoenolpyruvate carboxykinase was determined according to the method [13] and expressed in nmol of oxidized NADH per mg of protein in the sample for 1 min of incubation.

The principle of the method for determining the activity of lactate dehydrogenase consists of the reduction of pyruvate to lactate in the presence of reduced NAD [4]. LDH activity was expressed in μmoles of used $NADH^+H^+$ per mg of protein in the sample for 1 min of incubation.

LDH isozymes in tissues and blood were detected using polyacrylamide gel electrophoresis at a temperature of +30C (voltage drop of 12 V/cm of block length, current strength of 8 mA/cm^2 of block thickness, time - 2 hrs). Electrophoregrams were painted with a substrate mixture (NAD, tetrazolium nitroblue, sodium lactate, phenazine metasulfate, phosphate buffer). After fixing the electrophoregrams, they were dried in a thermostat for 2 hrs at a temperature of $+50^0$C and densitometered. The content of isoenzymes was determined planimetrically [4].

The principle of the method for detecting the content of lactate and pyruvate consists in an enzymatic reaction catalyzed by LDH in the presence of the oxidized or reduced form of NAD, the accumulation or loss of which is recorded spectrophotometrically at 340 nm against the control, where there is no tissue extract, and expressed in μmol per 1 g of tissue [4]. The protein content in the samples was detected by the biuret method [4].

The content of malate and oxaloacetate was determined according to the method [4] and expressed in μmol per 1 g of tissue (for malate) and in nmol per 1 g of tissue (for oxaloacetate).

The content of adenosinetriphosphate (ATP) was determined according to the method [15]. The content of adenosinediphosphate (ADP) and adenosinemonophosphate (AMP) in tissues was determined in one sample using combined reactions [15]. All indicators of energy metabolism were expressed in μmol per 1 g of the studied tissue.

The obtained data were subjected to statistical processing by the method of estimating the average with the help of "T-tables" using the $\chi 2$ criterion and computer programs. The minimum statistical probability was determined at $p<0.05$.

Results

Irradiation of sexually mature rats at a dose of 0.5 Gy does not significantly affect their fertility. At the same time, the radiosensitivity of the descendants of irradiated animals increases. After irradiation in a dose of 1 Gy, 2 out of 30 intact pup rats died (6.5%) and the

average life expectancy of the dead animals reached 15.5 days, and out of 30 pup rats born from irradiated animals, 5 (16.7%) died and the average the life expectancy of those who died was 12.0 days.

In the descendants of irradiated animals, there are multidirectional changes in the content of glycogen and glucose in comparison with intact animals - a significant increase in the concentration of glycogen in the liver and a decrease in it in skeletal muscle (Table 1).

Table 1

The content of glucose and glycogen in the tissues of 1-month-old pup rats born to intact and irradiated animals

Group of animals	Bloods	Liver		Bone marrow	
	Glucose μmol/l	Glucose μmol/g	Glycogen μmol/g	Glucose μmol/g	Glycogen μmol/g
Pup rats born to intact animals, n=8	4,5±0,3	1,80±0,15	30,0±2,5	0,60±0,08	3,50±0,40
Pup rats born to irradiated animals, n=8	6,80±0,40*	2,70±0,20*	44,0±3,1*	0,35±0,04*	2,10±0.20*

Notes: * - $p<0.05$ - probable differences of the studied indicators compared to the corresponding data in intact animals.

The state of the tricarboxylic acid cycle, assessed by the NAD-dependent malate dehydrogenase reaction and the content of metabolites of this reaction, differs in the myocardium and skeletal muscles (table 2).

First of all, the fact that fact attracts attention is that the activity of the enzyme detected by the formation of oxaloacetate /direct reaction/ is much higher in the myocardium than in the skeletal muscles, and the cell compartments in which the activity is determined are also of great importance. Thus, in the cytoplasm of the heart, the activity of NAD-dependent malate dehydrogenase is 2.4 times higher than in the cytoplasm of muscles, and in the mitochondria of the myocardium it is more than 3.2 times higher than the function of the enzyme in the mitochondria of muscles. It should be noted that the activity of malate dehydrogenase in the heart and muscles is much higher in the cytoplasm than in the mitochondria, and it is more pronounced in skeletal muscles. If the ratio of the activity of the

cytoplasmic form of the enzyme to the mitochondrial form in the myocardium reaches 4.3, then it exceeds 5.6 in the skeletal muscles.

Table 2

Activity of NAD- and NADP-dependent malate dehydrogenases
and the content of metabolites reactions in the tissues of intact animals (n=10)

Enzymes and metabolites	Myocardium		Skeletal muscle		Blood
	Cyto-plasma	Mito-chondria	Cyto-plasms	Mito-chondria	
NAD-MDH (direct reaction)	0,603± 0,014	0,141± 0,009	0,248± 0,008*	43,72± 2,60*	1,874± 0,177
NAD-MDH (reverse reaction)	2,146± 0,125	0,210± 0,013	1,752± 0,095*	65,88± 2,80*	3,777± 0,286
NADP-MDH (direct reaction)	13,43± 0,62		7,299± 0,555*		
NADP-MDH (reverse reaction)	21,41± 1,19		11,94± 0,57*		
Malat	0,405±0,023		0,318±0,028*		0,144± 0,008
Oxaloacet	43,90±1,96		31,94±1,73*		15,54±1,12

Note: * - $p<0.05$ – probable differences of the investigated indicators compared to the corresponding indicator in the myocardium

The study of the NAD-dependent malate dehydrogenase reaction in the direction of oxaloacet - malate /reverse reaction/ showed that the general regularity of the relationship between the activity of the enzyme in the heart and skeletal muscles, as well as between separate compartments of the cell, revealed for the direct malate dehydrogenase reaction, is also preserved for the reverse reaction . This is primarily a higher activity of the enzyme in the myocardium compared to skeletal muscles. If the activity of malate dehydrogenase in the cytoplasm of the heart is 1.2 times higher than in the cytoplasm of skeletal muscles (for a direct reaction, the ratio was 2.4), then in the mitochondria of the myocardium the activity of the enzyme is 3.2 times higher than in the corresponding compartment of the skeletal muscles and does not differ from a similar ratio for the direct reaction, that is, it can be concluded that in the cytoplasm of cardiac muscle, in comparison with skeletal muscle, the conversion of malate into

oxaloacetate prevails over the reverse process. This is also evidenced by the ratio of the direct malate dehydrogenase reaction to the reverse reaction in the tissue cytoplasm. In the myocardium, this ratio is almost 2 times higher than in skeletal muscle. In contrast to the cytoplasm, in the mitochondria of both types of tissues, the ratio of the activity of the direct reaction to the reverse does not differ significantly. The activity of the malate dehydrogenase reaction in the direction of oxaloacet - malate is much higher in the cytoplasm of the heart and skeletal muscles than in the mitochondria of these tissues, and the predominant increase is noted for skeletal muscles.

The concentration of malate and oxaloacetate in tissues is also different. In the myocardium, the content of malate probably exceeds its concentration in skeletal muscles, as well as of oxaloacetate, however, if the heart contains malate approximately 1.3 times more than in muscle, then oxaloacetate is almost 1.4 times, resulting in a malate/oxaloacetate ratio of 9.226 in the myocardium and 9.958 in skeletal muscle.

The ratio of direct to reverse NAD-dependent MDH in blood serum is higher than in the mitochondria of tissues and lower than in the cytoplasm, from which it can be assumed that in the blood of intact animals, enzymatic activity is provided by cytoplasmic forms of the enzyme, while mitochondrial forms are in the matrix of mitochondria and are firmly fixed on them.

The concentration of malate and oxaloacetate in blood is 2-2.8 times lower than in tissues, but the ratio between them does not experience significant changes compared to muscle tissue and makes 9.269.

Skeletal muscle is characterized by high activity of glycolytic processes, and this is reflected in the activity of enzymes that catalyze glycolysis reactions and in the content of metabolites. Thus, determining the activity of pyruvate kinase in the heart and in skeletal muscles, it was established that this enzyme is almost 2.9 times more active in skeletal muscles than in the myocardium (table 3).

All this causes a difference in the activity of lactate dehydrogenase, which catalyzes the terminal stage of glycolysis. In skeletal muscles, its activity is almost 1.3 times higher than in cardiac muscle.

The isoenzyme spectrum of lactate dehydrogenase of the rat myocardium is characterized by a high content of LDH_1 and LDH_2 isoenzymes that migrate rapidly to the anode. They account for 70% of the enzymatic activity of lactate dehydrogenase in this tissue. Much less is contained in the tissue of the third fraction of the enzyme, and the amount of LDH_4 and, especially, LDH_5 is extremely small. If LDH_3 provides almost 25% of the

enzymatic activity in the heart, then LDH_4 is about 5% and LDH_5 up to 1%. The isoenzyme spectrum of skeletal muscle lactate dehydrogenase is mainly represented by the fifth isoenzyme, which reaches almost 75% of the total activity of the enzyme in this tissue. Its activity is more than 5 times higher than LDH_4 and 7 times higher than LDH_3.

Table 3

Activity of glycolysis and gluconeogenesis enzymes and content of metabolites in animal tissues

Enzymes and metabolites	Myocardium	Skeletal muscles	Blood
Pyruvate kinase, n=21	0,097±0,005	0,282±0,015***	10,252±0,899
Lactate dehydrogenase, n=21	1,542±0,076	2,060±0,094***	8,118±0,545
Phosphoenolpyruvate carboxykinase, n=21	17,726±1,151	56,544±1,978***	0,933±0,096
Lactate, n=10	2,768±0,191	3,327±0,165*	1,067±0,072
Pyruvate, n=10	0,310±0,015	0,332±0,018	0,130±0,006

Note: * - $p<0.05$, *** - $p<0.001$ - probable differences of the studied indicators compared to the corresponding indicator in the myocardium

The content of LDH_2 and LDH_1 is approximately 3% and 1%, respectively, of the total activity of the enzyme. If we take into account that fast-migrating LDH isozymes are inhibited by small concentrations of pyruvate and its optimal concentration for LDH_1 is almost 10 times lower than for LDH_5, as well as the fact that the pyruvate kinase reaction, the product of which is pyruvate, is several times higher in skeletal muscles, than in the cardiac ones, the predominant accumulation of lactate in the skeletal muscles becomes clear.

The pyruvate content of muscle of intact animals is only marginally greater than that of rat myocardium, but lactate is likely higher in skeletal muscle than in the heart, resulting in a cardiac lactate/pyruvate ratio of 8.929, whereas in skeletal muscles it reaches 10.021. Therefore, if most of the pyruvate formed in skeletal muscles is used for lactate synthesis, then in the myocardium, pyruvate, undergoing oxidative decarboxylation, enters oxidation reactions in the cycle of tricarboxylic acids.

Phosphoenolpyruvate carboxykinase, which ensures the utilization of cytoplasmic oxaloacetate and its transformation into phosphoenolpyruvate, completes the initial stage of gluconeogenesis and can limit the rate of gluconeogenesis from lactate. A characteristic feature is that the activity of phosphoenolpyruvate carboxykinase in muscles is more than 3

times higher than that in the heart. It should be emphasized that phosphoenolpyruvate carboxykinase is more active in skeletal muscles, where the activity of NAD- and NADP-dependent malate dehydrogenases is lower than in the heart, and the activity of pyruvate kinase and lactate dehydrogenase is increased.

Skeletal muscle contains 1.65 times less ATP than cardiac muscle, but there is1.57 and 1.83 times more ADP and AMP in skeletal muscle than in cardiac muscle. The total pool of adenyl nucleotides in skeletal muscle is 1.46 times less than in cardiac muscle (table 4).

Table 4

The content of ATP, ADP, AMP in the tissues of intact sexually mature animals

Tissue	ATP μmol per gram	ADP μmol per gram	AMP μmol per gram
Skeletal muscle, n=8	3,200±0,260*	0,425±0,050*	0,276±0,030*
Cardiac muscle, n=8	5,290±0,480	0,271±0,030	0,151±0,015

Note: * - $p<0.05$ – probable differences of the investigated indicators compared to the corresponding indicator in the myocardium

Discussion

The effect of ionizing radiation causes instability to harmful environmental factors and increased morbidity in descendants. A decrease in the adaptation of the myocardium to physical exertion was established, as well as a decrease in indicators of general physical capacity and aerobic productivity [14, 18].

Previous studies [5] established that hypercatecholemia in sexually mature animals irradiated in a dose of 0.5 Gy occurs due to an increase in the concentration of adrenaline, and the functioning of the sympatho-adrenal system in rats born to animals irradiated in a dose of 0.5 Gy is characterized by an increase the content of adrenaline in the hypothalamus, norepinephrine in the adrenal glands, which do not dominate in these tissues, hyperadrenalineemia and increased excretion of adrenaline and dopamine.

On the basis of data from the literature, it can be concluded that irradiation causes a number of metabolic disorders related to the function of vitamins [16]. Of particular importance is the disruption of the function of the enzymes of oxidative decarboxylation of ketoacids, NAD-dependent dehydrogenases, which include thiamine pyrophosphate and nicotinamide, and the strengthening of lipid peroxidation processes, which leads to a violation of the structure and function of biomembranes, the antiaggregation activity of vessel walls, and the functional

activity of platelets, which is suppressed by introduction of exogenous tocopherol. In addition, catabolic processes leading to protein destruction and a negative nitrogen balance are enhanced.

Cardiac muscle differs from skeletal muscle not only in morphological and functional characteristics, but also primarily in the significant content of mitochondria, the speed of protein exchange, the high intensity of aerobic processes, in particular, the reactions of the tricarboxylic acid cycle, creatinephosphokinase [12, 17]. Therefore, the anatomical and physiological features of the heart and skeletal muscles and a close connection with the blood supply system ensure a quick reaction of this tissue to the influence of harmful environmental factors [22]. A special place in the bioenergetics of muscles is occupied by the transport of protons from the sarcoplasm, where they accumulate under load conditions, to the cytoplasm, where they are involved in tissue respiration with the release of a significant amount of energy. One of these transport mechanisms is provided by NAD-dependent malate dehydrogenase [6] but the difference between this mechanism in myocardium and skeletal muscle is not known, the relationship between NAD-dependent and NADP-dependent malate dehydrogenase in muscles has not been investigated, which will allow to deepen information about the mechanisms of damaging factors influence on the muscular system.

Thus, it should be noted that the activity of the malate dehydrogenase reaction per mg of tissue protein is much higher in the cytoplasm of the cells of both tissues than in the mitochondria. The ratio between direct and reverse reactions in the mitochondria of the heart and skeletal muscles is approximately equal, but in the cytoplasm of skeletal muscles, the advantage of the reverse reaction over the direct reaction is clearly manifested in comparison with the myocardium.

An important place in the interconversions of malate and pyruvate is occupied by NADP-dependent decarboxylating malate dehydrogenase (NADP-MDH), which plays a connecting role between glycolysis, gluconeogenesis and the cycle of tricarboxylic acids, providing them with metabolites and transferring hydrogen from NADH to NADP. The activity of the enzyme, both for the conversion of malate into pyruvate (direct reaction) and pyruvate into malate (reverse reaction), is more pronounced in the myocardium and is almost 1.8 times higher than the activity in skeletal muscles, which once again indicates a greater intensity of oxidative processes in the heart muscle [11].

It is known that there is a competitive relationship between lactate and malate dehydrogenases for the use of NADH formed in glycolysis, and the ratio of MDH and LDH activity plays an important role in regulating the intensity of aerobic and anaerobic

metabolism. With a high ratio of MDH/LDH in the cytoplasm, the shunting function of MDH is developed, with a low ratio, NADH is mainly used to restore pyruvate [11].

The transfer of reduced equivalents of NADH through mitochondrial membranes depends on the ratio of mitochondrial and cytoplasmic forms of MDH, and the lower the ratio of cytoplasmic MDH to mitochondrial MDH is, the more efficient is the transfer. In addition, the lactate/pyruvate and malate/oxaloacetate ratios characterize the redox potential of the NAD system in the cytoplasm of cells, which can be considered as an indicator of tissue oxygenation.

With insufficient oxygenation, the ratio of lactate/pyruvate and malate/oxaloacetate increases and hydrogen transport from the cytoplasm to the mitochondria is inhibited. At the same time, the malate/pyruvate ratio can characterize the state of the NADP/NADPH redox system. Therefore, the obtained data on the activity of enzymes in tissues and the content of metabolites allow us to characterize the intensity and direction of metabolism in the studied organs [11].

Thus, in the myocardium, LDH is represented by fast-migrating isoenzymes, which, unlike LDH_5, are sensitive to inhibition by pyruvate, lactate, and oxaloacetate, i.e., conditions are created in the myocardium to inhibit the oxidation of NADH by pyruvate in the lactate dehydrogenase reaction [17].

This is facilitated by a higher ratio of MDH/LDH in the cytoplasm of the myocardium compared to skeletal muscles and a lower ratio of cytoplasmic MDH to mitochondrial in the heart muscle. In addition, the ratio of lactate/pyruvate and malate/oxaloacetate is lower in the myocardium than in skeletal muscle and, therefore, the oxidation of the NAD/NADH system is higher in the myocardium.

All this creates conditions for the oxidation of NADH with oxaloacetate with the formation of malate in the heart muscle, and at the same time, the formation of lactate is reduced. In skeletal muscle, in contrast to the myocardium, lactate dehydrogenase is not sensitive to the inhibitory effect of metabolites, the ratio of MDH/LDH in the cytoplasm of cells is lower than in the myocardium, and the ratio of cytoplasmic MDH/mitochondrial MDH is much higher, as well as the ratio of lactate/pyruvate and malate/ocaloacetate. All this leads to the fact that the competition between MDH and LDH for NADH is in favor of LDH and conditions are created for the intensive flow of glycolysis.

Phosphoenolpyruvate carboxykinase, which ensures the utilization of cytoplasmic oxaloacetate and its transformation into phosphoenolpyruvate, completes the initial stage of gluconeogenesis and can limit the rate of gluconeogenesis from lactate. A characteristic feature

is that the activity of phosphoenolpyruvate carboxykinase in muscles is more than 3 times higher than that in the heart. It should be emphasized that phosphoenolpyruvate carboxykinase is more active in skeletal muscles, where the activity of NAD- and NADP-dependent malate dehydrogenases is lower than in the heart, and the activity of pyruvate kinase and lactate dehydrogenase is increased.

It can be concluded that the studied enzyme shows greater activity in tissue characterized by a high intensity of glycolysis and a low capacity for aerobic oxidation. In confirmation of this, it should be mentioned that the liver, which has the highest activity of phosphoenolpyruvate carboxykinase and gluconeogenesis in general, has a great ability to oxidize carbohydrates through the glycolytic pathway [11].

Thus, it can be stated that myocardium and skeletal muscle have a number of distinctive features in carbohydrate metabolism, which consist in a greater intensity of glycolytic substrate phosphorylation and the terminal site of glycolysis in skeletal muscle compared to myocardium. The shunt function of NAD-dependent malate dehydrogenase and the activity of this link of the tricarboxylic acid cycle, as well as NADP-dependent malate dehydrogenase, which performs a connecting role between glycolysis and the tricarboxylic acid cycle, are higher in the myocardium of animals. Compared to the myocardium, skeletal muscles have a greater ability to convert cytoplasmic oxaloacetate into phosphoenolpyruvate, which correlates with the intensity of glycolysis in them and the reversibility of the redox system of nicotinamide coenzymes.

Completing the characterization of metabolic processes in muscle tissue, it is necessary to take into account the relationship of the investigated indicators between tissues and blood. This is important when assessing the depth of the damaging effect, which can have diagnostic and prognostic value. The activity of pyruvate kinase determined in blood serum is almost 9.4 times less than that in the cytoplasm of the heart and 27.5 times less than in skeletal muscle, while the activity of lactate dehydrogenase in blood serum is 190 times less than in heart and 254 times less than in skeletal muscles and is characterized by a significant content of slowly migrating fractions of lactate dehydrogenase, characteristic of skeletal muscles and liver of animals. The content of lactate in blood is 2.6-3.1 times less than in tissues, and pyruvate is 2.4-2.5 times less, as a result of which the lactate/pyruvate ratio in blood is lower than in the heart and skeletal muscles and makes 8.208.

The ratio of the direct to the reverse NAD-dependent malate dehydrogenase reaction in blood serum is higher than in the mitochondria of tissues and lower than in the cytoplasm, from which it can be assumed that in the blood of intact animals, the enzymatic activity is

provided by cytoplasmic forms of the enzyme, and mitochondrial forms located in the matrix of mitochondria, firmly fixed in them. The concentration of malate and oxaloacetate in blood is 2-2.8 times lower than in tissues, but the ratio between them does not undergo significant changes compared to muscle tissue and makes 9.269.

The activity of phosphoenopyruvate carboxykinase in the blood serum of intact animals is very low compared to other enzymes determined in the serum, which is probably related to the low activity of gluconeogenesis enzymes in tissues relative to the activity of enzymes of glycolysis and the tricarboxylic acid cycle.

It is known that there is a competitive relationship between lactate and malate dehydrogenases for the use of NADH produced in glycolysis, and the ratio of activity of malate dehydrogenase and lactate dehydrogenase plays an important role in regulating the intensity of aerobic and anaerobic metabolism. With a high ratio of MDH/LDH in the cytoplasm, the shunt function of MDH is developed, with a low ratio, NADH is mainly used to restore pyruvate. The transfer of reduced equivalents of NADH through mitochondrial membranes depends on the ratio of mitochondrial and cytoplasmic forms of MDH, and the lower the ratio of MDH (cytoplasm) to MDH (mitochondria) is, the more efficient is the transfer. In addition, the lactate/pyruvate and malate/oxaloacetate ratios characterize the redox potential of the NAD system in the cytoplasm of cells, which can be considered as an indicator of tissue oxygenation. With insufficient oxygenation, the ratio of lactate/pyruvate and malate/oxaloacetate increases and the transport of protons from the cytoplasm to the mitochondria is inhibited. At the same time, the malate/pyruvate ratio can characterize the state of the NADP/NADPH redox system. Therefore, the obtained data on the activity of enzymes in tissues and the content of metabolites allow us to characterize the intensity and direction of metabolism in the studied organs.

Conclusions

Summarizing the above, it should be concluded that, unlike skeletal muscle, the activity of the tricarboxylic acid cycle, in particular the NAD-dependent malate dehydrogenase, in the myocardium is quite significant both in the cytoplasm and in the mitochondria of the tissue, as evidenced by the higher level of tricarboxylic acid cycle metabolites acids - malic and oxaloacetic, as well as the activity of NADP-dependent malate dehydrogenase, which performs a connecting role between glycolysis and the cycle of tricarboxylic acids in providing them with metabolites and transferring protons from $NADH+H^+$ to NADP. As a result, the myocardium is characterized by a larger pool of adenyl nucleotides due to ATP.

Hyperglycemia is observed in the blood of the descendants of irradiated animals. Taking into account that irradiation of sexually mature rats in a dose of 0.5 Gy causes hypercatecholemia in them, and in their descendants there is a significant increase in the content of adrenaline in the blood and the phenomenon of hypercorticism, it can be assumed that gluconeogenesis is enhanced in the liver of the descendants of irradiated animals and this explains the hyperglycemia and accumulation of glycogen in the liver. At the same time, the penetration of glucose into muscle cells is weakened, which is associated with a decrease in their glycogen content, and this can be explained by the decrease in adaptation to physical exertion in the descendants of irradiated animals.

In the myocardium and skeletal muscles of rats, the percentage of LDH isoenzymes formed from M-subunits is higher, and with age, as a result of epigenetic changes, the content of H-subunits increases, which affects the direction of carbohydrate metabolism in the tissues of sexually mature animals.

The pathophysiological mechanisms of radiation-induced energy supply restructuring are aimed at short-term processes of strengthening the supply of energy to vital organs and systems for destroyed biochemical, physiological, functional and regulatory processes restoration and sanogenetic mechanisms activation.

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Stepanov G.F. - conceptualization, methodology, formal analysis, data curation, writing—original draft preparation, writing—review and editing, supervision.

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The experimental studies were carried out in the conditions of a chronic experiment in accordance with international standards of humane treatment of vertebrate animals and approved by the Ethics Committee of Odesa National Medical University (N7/21, 11 October 2021)

Informed Consent Statement

The data of experimental studies are given. Written informed consent from the patients was not necessary to publish this paper.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The author declare no conflict of interest.