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The Liver Parameters In The Collagen-Induced Arthritis Rat Model

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Abstract:

The living organisms use defensive mechanisms in their struggle to keep the inner homeostasis and potect themselves from the changes induced by external factors and penetrative agents. The manifestation of these changes depends on the character, the intensity and the duration of the agents' activity, and on the physiological characteristics of the organism (gender, age, health condition etc.).

The aim of our study was to analyze, the effects of collagen-induced arthritis (that is, the autoimmune reaction and the inflammation) on some liver parameters. We determined the content of proteins, DNA and RNA. Animals with collagen-induced arthritis showed decreased relative content of proteins in liver, compared to controls. On the contrary, the relative content of DNA and RNA were increased in animals treated with collagen.

Keywords: rheumatoid arthritis, collagen-induced arthritis, inflamation, Wistar rats, proteins, DNA, RNA, serum.

Introduction:

Joints consist of two or more opposite bone ends, covered with articular cartilage, joined by thick connective tissue and articular capsule. The capsule is covered with intimal layer of different

microscopic structure, called synovium or synovial membrane. Within this closed cavity, there is a small quantity of bright synovial liquid. Around the joints, there are tendons, tendon linings and bursa. These structures are of great importance for the normal mechanical functioning of the joint, which in rheumatoid arthritis, as well as in many other diseases, is primarily or secondarily affected (Moritz, 1942). Joints are anatomically and functionally built in a way to provide quick movement, stability and coordination of the body, but at the same time cartilage surfaces and synovial tissues are exposed to frequent injuries of mechanical, toxic and metabolic character. The articular surface also undergoes important regressive changes during everyday wearing down and natural physiological ageing. These changes may, to a great extent, accelerate the pernicious agents, repeating traumas, impaired mechanics in walking or postural impairment, as well as in inflamatory diseases such as gout or rheumatoid arthritis (Bennett et al., 1942).

Inside the body, tissue damage results in inflamatory response characterized by redness on the affected area, raised temperature, localized edema and pain (Christen, 2005). At the same time, basophiles and mast cells contain and may release substance called histamine (Banham et al., 2006; Ishii et al., 2006). The histamine released in the initial phase of the tissue damage process, is sort of a defense mechanism of the organism which accelerates the healing process. Of special importance to the research on the occurrence of infections and auto-immune reactions is the protective role of T-lymphocytes as well as the function of so called Toll-receptors i.e. transmembrane proteins, that recognize pathogenic molecules when interacting with different ligands acting as their agonists or antagonists (Banham et al., 2006; Ishii et al., 2006).

A common feature of all rheumatic diseases is inflammatory reaction, without pus formation, of the connective tissue, with basic clinical signs, such as inflammation, redness, swelling, local raised temperature, pain and limited motoric movement (Christen, 2005). The activation of immunologic composition in such diseases can be seen through the occurrence of auto-antibodies to one or more autoantigenes, and they occur when the immune system loses control of part of the lymphocytes which are specifically auto-reactive to the cells of their own organism (the functional and tissue antigens) i.e.

the receptors (Banham et al., 2006; Ishii et al., 2006). Inflammation in rheumatoid arthritis begins in the synovium that lines the inner surface of the joint which nourishes the joint cartilage and secretes the synovial fluid. If the disease occurs under the age of 16, it is known as juvenile idiopathic arthritis (Young et al., 2000). The inflammation is caused by the organism's immune system that attacks the tissue wrapped around the joints (Kamradt and Volkmer-Engert, 2004). This results in formation of connective tissue, known as pannus – proliferative tissue from the inflamed synovial membrane that has harmful properties. Namely, according to Kamradt and Volkmer-Engert (2004) pannus is capable of lyzing the cartilage and bone ends within the joint, as well as all the other structures of the joint. All of this causes pain, stiffness, deformation and impairment of the function of the affected joints. Some types of viruses and bacteria may be external factors, after caused infection, and risk-factors for occurrence of the disease, along with other factors such as age, inherited factors (Davidson and Diamond, 2001), then the sex, i.e. the hormonal factors, even smoking, although it hasn't been confirmed with certainty.

There are two hypothetic theories regarding the development of rheumatoid arthritis. According to the first theory, T-lymphocytes, cells that belong to the immune system, come into interaction with yet unknown antigen. T-lymphocytes are responsible for the occurrence of the disease as well as its chronic character. This theory is based on research that has related the rheumatoid arthritis to the composition of HLA-B27 antigen, great number of CD4+ T-lymphocytes and impaired receptors for T-lymphocytes in the joints (Shoenfeld, 1989; Cohen, 1993). According to the second theory, T-lymphocytes are responsible only for triggering the onset of the illness, whereas the chronic inflammation is a result of the activity of the macrophages and the fibroblasts. The most frequently applied therapeutic measures are non-steroid antirheumatic or anti-inflammatory (NSAIL)-antirheumatics. Glucocorticoids are the most powerful drugs in preventing the inflammatory processes of the organism, but, their usage should be time-limited, due to the fact that during longer application it may lead to undesired effects, such as osteoporosis, and during a treatment of juvenile idiopathic arthritis it may lead to growth impairment.

Materials and Methods:

During the experimental work, albino lab rats of the Wistar strain were used. For the experiment, we chose healthy male animals at the age of around seven weeks. They were placed in cages (Hulskamp Alkamar Holand) under constant control, at the lab animal farm of the Department of Physiology and Bio-chemistry at the Faculty of Natural Sciences and Mathematics in Skopje. They were fed with food produced by Animal Food Factory - Radobor from Bitola (standard food for lab animals). Food and water were given ad libitum. Around sixty animals were used. All the rats were sacrificed on the 21-st day of the post-immunisation with collagen type-II.

Collagen preparation and process of its application

For the purposes of this experiment, highly refined collagen type-II was used, prepared by a defined protocol, since the collagen quality has impact on arthro-genicity (Michaelsson, 1994). The preparation was carried out by taking 2-4 mg/ml and then putting it into 0.05M of Hydrochloric acid, mildly stirring it during the night, at + 4°C temperature. The collagen was used the following day after the preparation of the solution for application. The preparation of the collagen emulsion was carried out according to a defined protocol by using electric homogenizer.

- A homogenizer with small blade (with a diameter of 5mm or less) was used for mixing IFA and the collagen solution. It was suitable to use 5ml or 10 ml plastic syringe that can be cut into two equal parts. The syringe was placed into the holder and was put into cold water in order to prevent a denaturalization of the collagen, since collagen’s temperature increases while it is being stirred, and denaturalized collagen will not cause arthritis.

- One IFA dosage (2.5ml maximum) was added into the syringe by valve into 3 directions. Then, equal quantity of collagen solution (2mg/ml in 0.05M hydrochloric acid) was added by dropping while being mixed at small speed.

- The mixing continued until there was a thick emulsion at maximum speed (30000 rounds in 2-3 minutes average). Then, we waited for it to get cool in cold water, before starting to mix it again.

- The emulsion stability was tested by adding one drop into test tube with water. If the emulsion was stable, the drop would remain hard and it would not be lost, i.e. it would not dissolve.

- The emulsion was transferred into a syringe.

The prepared collagen was applied by small, thin insulin needles and syringes to the knee joint of the back right leg. We would place the needle a little bit subcutaneously and then carefully, pushing the needle forward, we would enter the joint and apply the collagen. The injected quantity (0.1ml) was from the collagen emulsion with small quantity of physiological solution, and we paid special attention not to lose the liquid during the insertion of the needle. After the injection, the rats were placed back into the cages and, naturally, were given food and water ad libitum.

Blood-taking from experimental animals for analysis purposes and production of serum

Blood was taken from all animals from the tail in the ammount of about 1ml in special-ependorf test-tubes. After the retraction of coagulum and separation of serum, the marked test tubes were centrifuged, in order to produce serum, and then the serum was separated into special test-tubes without blood coagulum. Appropriately marked test-tubes with the separated serum were centrifuged once again, and serum analyses were performed on computerized, automated COBAS Integra bio-chemical analyzer.

Determined parameters

Determination of absolute and relative liver content, absolute and relative liver protein content, DNA and RNA absolute and relative content in liver tissue, enzyme activity as well as the hystological processing of analyzed samples, were performed according to appropriate methods in laboratories at the Faculty of Natural Sciences and Mathematics in Skopje.

Determination of total proteins in tissue

The concentration of total proteins in tissue was determined by Goa method (1953). The principle of this method is based on the ability of copper salts in an alkaline environment to react with compounds that have at least two peptide bonds and form violet-coloured complex. Intensity of staining, given by this complex, is directly proportional to protein concentration and is measured photometrically at 546 nm. 4 ml of biurethic reagent were added to 1 ml of examined solution. The mixture was stirred and left for 30 minutes at room temperature. Then, measurement of light absorption at wavelength of 546 nm (green filter) was carried out. Parallel to the test, calibration curve of standard solution with starting concentration of 60 g/l was prepared. Operating biurethic reagent was prepared by dissolving basic biurethic reagent (60 mmol/l CuSO x $5H_2O$; 160 mmol/l KNaCHO x $4H_2O$; up to 27 mmol/l KJ and up to 0.25 mol/l NaOH) with dissolving reagent (30 mmol/l KJ; 0.25 mol/l NaOH) with 1:5 ratio. Concentration of proteins was directly read from the standard calibration curve, and values were expressed in mg proteins in 100 mg of tissue (mg %), as well as total proteins in the complete organ.

Determination of total DNA content in tissue

The principle is according to the method of Cerioty (1952), with strictly specific binding of indole with the deoxyribose, which needs a strong acidic environment of solution and high temperature, at which the intensity of solution's staining, which comes from the formed yellow-brownish complex, is directly proportional to concentration of the deoxyribose i.e. to the DNA content. After sacrificing of the animal, a piece of tissue is taken and is put into a test-tube with 4 ml of hot 0.1 mol/l NaOH solution. With the purpose of complete homogenizing of the tissue, it is left to stay during the night at room temperature or at 37°C, and then it is boiled in water bath for 20-30 minutes. After the tissue is homogenized and cooled, we add 2 ml 0.04 % indole solution and 2 ml concentrated HCl. The content is well stirred and closed test-tubes with rubber lids with opening for a glass tube are taken to water bath with boiling water, where they are left exactly 10 minutes. The reaction is ceased by transferring of test-tubes into a cold water tub. After complete cooling off, 4 ml of chloroform is

added. The content is mixed in an automatic mixer until it forms milky white colour. The mixture is left for 10 minutes in cold, then the test-tubes are centrifuged for 10 minutes at 2500 rounds per minute. Afterwards, by an aspirator or vacuum-pump, the lower layer (chloroform) of the test–tube is removed. Solution's staining intensity is measured in cuvette on a colorimeter with wave length of 490 nm. Parallel to the analysis, a standard DNA solution is prepared.

The values obtained for DNA tissue concentration were expressed as mg DNA/100 mg tissue (mg%), or as a total DNA content in a certain organ.

Determination of tissue total RNA content

Determination of RNA tissue content was carried out according to the method of Munro (1963). Right after the sacrifition of the animal, a piece of tissue is taken and put into cold distilled water of 1:20 ratio. The piece is macerated until complete tissue homogenization. From the homogenate, 5 ml are taken (it should be equal to around 250 fresh tissue mass) into a 15 ml centrifugal test-tube and 2.5 ml ice-cold 0.6 N $HClO_4$ solution is added; then it is carefully stirred and left for 10 minutes at 0°C temperature; it is centrifuged; the supernatant fraction (into acid soluble fraction) is gathered; and the precipitate is washed two more times with ice-cold 0,2 N $HClO_4$. After centrifugation, the excess, through filter-paper, is transferred into volumetric flask, into acid soluble fraction. To the precipitate are added 4 ml 0,3 N KOH, and the content is stirred and left to incubate for 60 minutes at 37°C (in water- or air-bath). After the incubation, 2.5 ml 1,2 N $HClO_4$ is added to the complete content and is left to stay for 10 minutes in cold. The content is centrifuged, after which DNA and proteins precipitate, whereas RNA stays in supernatant. The supernatant with RNA is collected, and the precipitate is washed two more times by using 5 ml 0.2 N of $HClO_4$ solution. Washed supernatant is collected together with previously separated RNA faction, to which another 10 ml of 0.6 N of $HClO_4$ solution are added and up to 100ml distilled water is added into the volumetric flask. In this way, a solution of ribonucleotides in 0.1 N of $HClO_4$ solution is produced. By measuring the absorption at 260nm of ribonucleotide solution, the RNA content may be measured, provided that

extinction of 1.000 at 260 nm corresponds to 32 μg RNA in ml. Values obtained on RNA tissue concentration were expressed as mg RNA/100 mg tissue (mg%), or as total RNA content in the organ.

Hystological processing of material

For the purposes of histological analysis, immediately after the sacrifition of the animals, a piece of tissue was taken from the liver. After cleaning the material from the surrounding tissue, it was fixated into buffered neutral formaline (formaline-100 ml, Na_2HPO_4-6,5 g, NaH_2PO_4 x H_2O-4,0 g and 900 ml distilled water). In order to perform tissue dehydration, standard procedure and rinsing by alcohol and xylol were carried out. The moulding was performed in paraffin, while paraffin slices with thickness of 4 to 7 μm were made on microtom in citohystologic laboratory. For staining of histologic slices, hematoxylin-eosin and azure II-eosin methods were used. Hystologic processing of analysed samples was performed in accordance with appropriate cyto-hystological methods in appropriate laboratories at the Faculty of Natural Sciences and Mathematics in Skopje. Cytomorphologic and hystologic analyses of all examined groups were performed by light microscopy. On the histologic slices of the liver, the number of nuclei was determined (nuclear index -Ni) on 50 visual fields with a surface of 1249.5 mm^2 as well as the nuclear volume-Vn of the aforementioned on 50 nuclei of each individual in mm.

Statistical analysis

Using an appropriate formula, the obtained individual values of the results were reduced to average values, while the standard error was calculated according to another appropriate formula. The significance was determined by Student’s t-test, during which, one formula was used for comparison of groups with same number of animals, while another formula was used for comparison of groups with different number of animals. The level of significance was defined at $\alpha = 0,05$.

Results:

Protein content in liver

The results of our examinations on absolute and relative protein content in liver in the control group of animals and the group of animals with induced rheumatoid arthritis are given in Figures 1 and 2. Average value of absolute protein content in liver in control group of animals was 1663.98 mg, whereas in arthrosed group, it was 422.01mg. The results show that induced rheumatoid arthritis causes decrease of absolute protein content in arthrosed group of animals in relation to absolute protein content in the control group of animals. This can also be seen from the chart in Figure 1, where the effect of liver protein content in arthrosed group of rats is visible. The differences observed, in relation to the control group of animals, are highly significant (A:B, $p< .001$). Hence, induced rheumatoid arthritis also conditions the maintanence of the relative protein mass in liver at extremely lower level compared to the level of the control group (Fig.2, A:B, $p< .001$). The value of relative protein content in liver in control group was 27.62 mg %, whereas in the treated group was 18.27 mg%, i.e. the concentration of proteins in animals with induced arthritis is significantly lower compared to the concentration in the control group.

The changes manifested can be seen best by the relative protein content, which means that the induction of rheumatoid arthritis in rats exerts suppressing effect on protein content

in arthrosed group in relation to the control group (Fig. 1 and 2, A: B, $p < .001$).

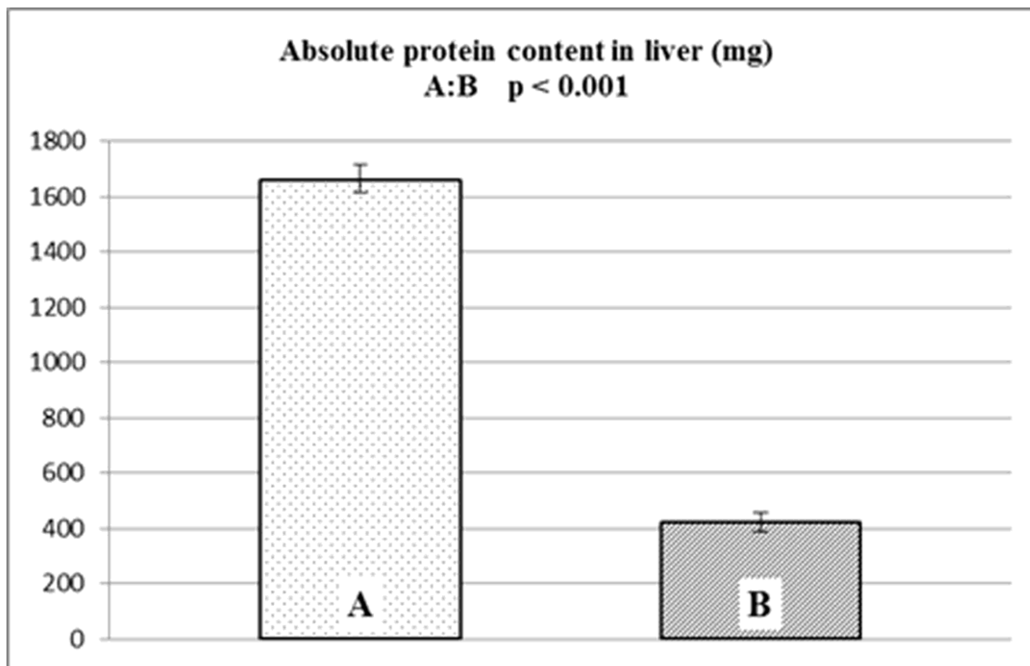

Figure 1. Absolute protein content in liver (mg) in control and arthrosed groups of animals (means±SE).

- control, group A, (animals from this group during the whole experiment period were on ad libitum food and water diet,with no treatment and in standard breeding conditions; (n=31)- at around fifty days of age at the beginning of the experiment).

- animals with collagen type-II induced arthritis, group B, (the animals from this group were also on ad libitum food and water diet, but at the beginning of the experiment we induced arthritis in them; (n=30) at around fifty days of age at the beginning of the experiment).

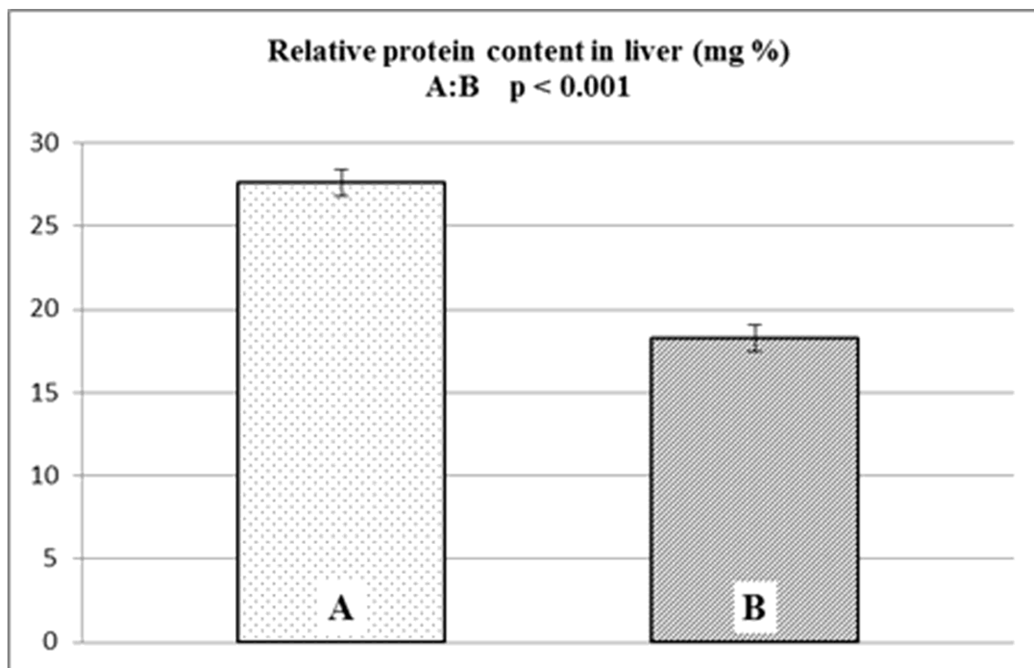

Figure 2. Relative protein content in liver (mg %) in control and arthrosed groups of animals (means±SE).
(The legend is the same as in Figure 1).

DNA content in liver

During our examination of the impact of induced rheumatoid arthritis upon some liver parameters, an important factor was also the DNA content. Results are shown by absolute and relative liver DNA content in control group (n=31) and in arthrosed group of rats (n=30), and obtained values are graphically shown in Figures 3 and 4.

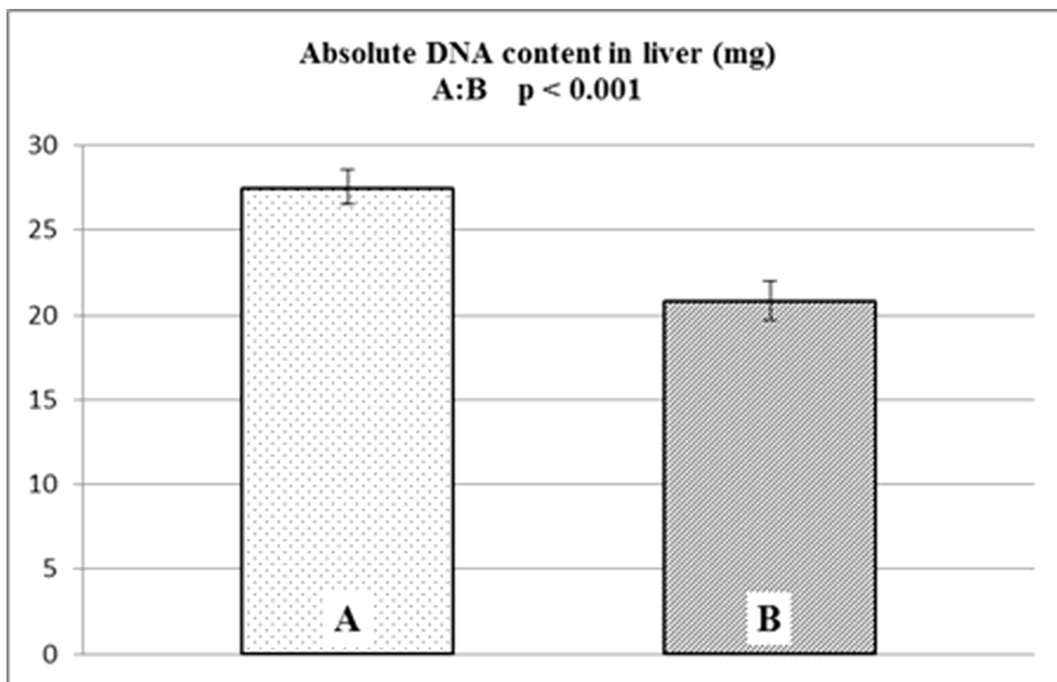

Figure 3. Absolute DNA content in liver (mg) in control and arthrosed groups of animals (means±SE). (The legend is the same as in Figure 1).

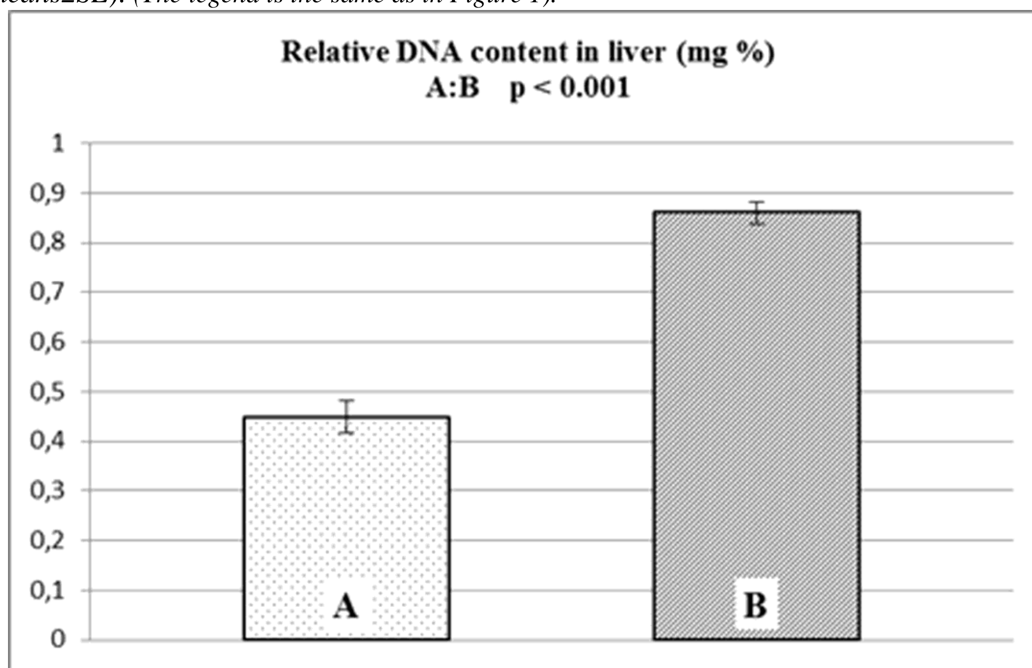

Figure 4. Relative DNA content in liver (mg %) in control and arthrosed groups of animals (means±SE).

(The legend is the same as in Figure 1).

Average absolute liver DNA content in control group was 27.54 mg, whereas the value of the same parameter in arthrosed group was 20.85 mg. From the diagram in Figure 3, where the absolute liver DNA concentration is shown, the decreased absolute DNA content in treated animals in relation to the absolute DNA content in control group (A:B, $p< .001$) can be clearly seen. This is, most probably, due to the suppressing effect of the arthritis upon the metabolism in the liver, or more specifically, at the hepatocyte level, as well as upon the normal function and development of the organ. The results on the relative DNA liver content are graphically shown in Figure 4. Relative liver DNA content in the control group is 0.45 mg %, and in the treated group 0.86 mg %. It can be seen from Figure 4 that in the arthrosed group of animals, there is highly significant increase of the relative DNA content, compared to the control group (Fig. 4, A:B, $p< .001$). It can be seen from this that the expression in percentage of the DNA on 100 mg on hepatic tissue in the arthrosed group has an increase of the DNA concentration by almost 100% in relation to the control group. It is probably a compensating mechanism due to the suppressing effect of the arthritis upon the metabolism in the liver.

RNA content in liver

The impact of induced rheumatoid arthritis was also tested on the total and relative RNA content in liver in the experimental group in relation to the control group, and the results are graphically shown in Figures 5 and 6.

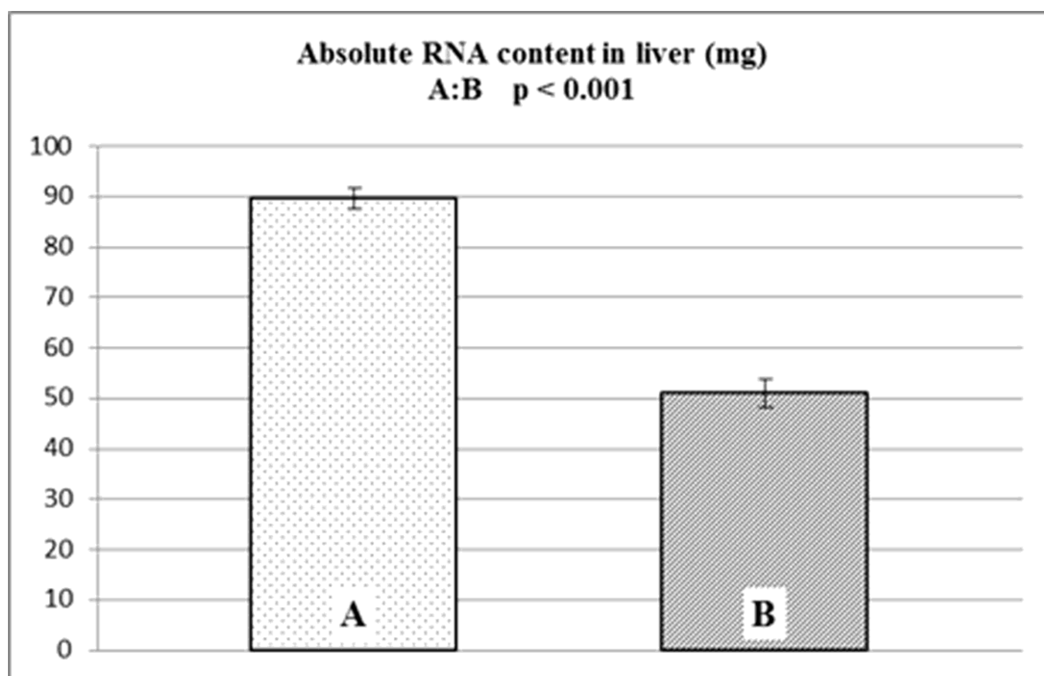

Figure 5. Absolute RNA content in liver (mg) in control and arthrosed groups of animals (means±SE). (The legend is the same as in Figure 1).

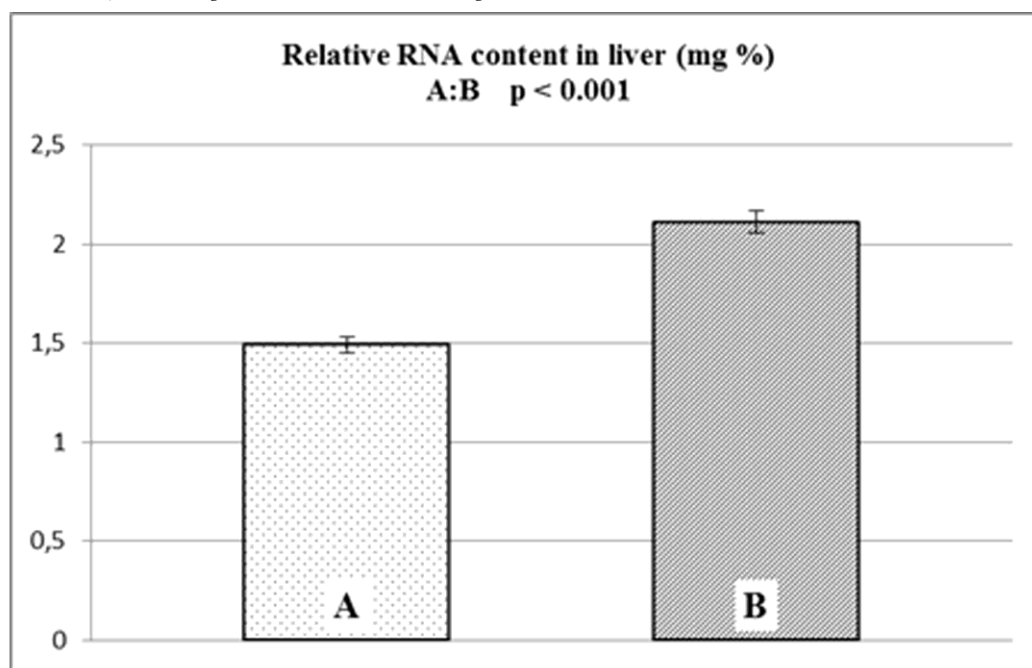

Figure 6. Relative RNA content in liver (mg %) in control and arthrosed groups of animals (means±SE).

(The legend is the same as in Figure 1).

From the results on RNA concentration in liver, it may be observed that the changes are very adequate in relation to the results on DNA. Our results have shown that inducement of rheumatoid arthritis in albino lab rat causes significant decrease of absolute RNA content in liver in the arthrosed compared to the control group, which can be clearly seen in Fig.5, (A:B, $p< .001$). Average value of absolute RNA content in liver in the control group of animals was 89.75 mg, whereas the value of the same content in the treated group was 51.00 mg. Regarding the treated group of animals, an increase of relative RNA content can be clearly seen, in relation to the control group (Fig.6, A:B, $p< .001$). Relative RNA content in liver in control animals was 1.49 mg %, in comparison to arthrosed animals where the content was 2.11 mg %. It can be clearly seen from the chart that the relative RNA concentration in liver is at significantly higher (41 % higher) level in the arthrosed compared to the control group of rats, which means that the relative RNA concentration expressed in mg % in treated animals increases after the advancement of arthritis, and the value is significantly higher, in relation to the one in the control group (Fig.6, A:B, $p< .001$). Qualitative differences in the charts on the absolute and relative RNA concentration in liver in the control group in relation to the arthrosed group of experimental animals (shown in Fig. 5 and 6), are manifested as a result of the calculation of both concentrations, upon the mass of the entire organ and upon tissue unit.

Discussion:

There were no changes in the diet of the experimental animals, in terms of increasing or decreasing the food quantity after the treatment. Collagen-induced arthritis caused reaction in almost 100% of the immunized rats. The results from our examinations on average absolute values, and especially on average relative values, suggest that the effect of induced rheumatoid arthritis is manifested through significant decrease of absolute and relative liver mass in arthrosed animals in relation to control animals.

Induced rheumatoid arthritis shows efficient impact upon the decrease of liver protein content. Namely, in arthrosed animals it is manifested in significantly lower values compared to control group of rats, clearly represented also by the absolute and relative liver protein content (A:B, $p < .001$). It may be observed that the induced rheumatoid arthritis is stressor for the albino rat, which leads to certain physiological and biochemical disorders in the liver. Our results on the effect of induced rheumatoid arthritis on the absolute liver protein content in albino lab rat unambiguously confirm the same through their significant decrease (A:B, $p < .001$). Such changes may be explained, on one hand, by the usage of proteins for defensive purposes against imported external agent and their spending in terms of emergency situation in the organism for defense against infiltration or import of foreign agent into organism, and on the other hand, by their decreased synthesis, as a result of lesion and disintegration of hepatocytes (Bethesda, 1999). Namely, in the acute phase of the inflammatory process, the first responce is the incease in the proteins from the acute phase, which stimulate cascade process for synthesis of immune-mediators, cytokine- and interleukine-type molecules. During our examinations, we also observed that induced arthritis has suppressing effect on synthesis and protein content in the liver, shown by the percentage of proteins in this organ. Thus, the tested parameter in arthrosed compared to control animals, shows significantly lower values (A: B, $p < .001$). All of this led us to conclude that induced rheumatoid arthritis has significant suppressing impact on liver protein content. Decreased liver protein content may also be a result of the impact of rheumatoid arthritis on other organs and organic systems. It is assumed that cell immune response to different antigens may lead to tissue damage at the spot where secondary reaction occurred, followed by inflammation and fibrosis, which leads to tissue disintegration, decrease of their function and protein synthesis (Markelevic et al., 2006). This may be consequence of temporarily impaired regulation of feedback controlling functions of the neuro-endocrine and microvascular system, regarding the coordinated activity of different genes that mutually control expression of certain genes in charge of activation of humoral and cellular immune system components and leads to inflammatory processes (Davidson and Diamond, 2001). Also, cellular immune response to different microorganisms and other antigens,

infiltrated or imported into the organism, may lead to tissue damage on the spot where an infection occurred, or it was induced and spread in the body, during which inflammation and fibrosis may lead to greater tissue disintegration and decrease of their function and protein synthesis (Markelevic, 2006). Such teratogen effect of the arthritis on liver has been confirmed by scientific data which has shown that during arthritis followed by hepatocyte disintegration there is a release of many enzymes from the lysosomes of lost cells, such as hydrolases and proteases (Banham et al., 2006; Ishii et al., 2006).

In rheumatoid arthritis condition, hepatocytes' activity increases in the battle for maintaining normal biochemical processes in the liver, such as nucleic acid synthesis, protein synthesis, as well as other products required for normal function of the organism as a whole (Griffiths, 1988). According to the research by Christen (2005), there is an increased blood flow in the area where the injured tissue recovers as a result of histamine release. For example, IgE anti-bodies are related to hypersensitive reactions and they cause degranulation of mast cells, releasing histamine and increasing the nucleic acids synthesis (Christen, 2005). This can be seen also from our examinations and from the results on relative DNA and RNA content, which provide the best representation of the real condition of increased nucleic acids synthesis in hepatocytes. According to Christen (2005), in autoimmune reaction and loss of one part of the cells of the affected organs, compensatory with increased activity of functionally healthy cells of the same organ, the ribosomes in those cellsincrease their activity, and there is also increased synthesis of ribosomal subunits in the nucleus and their faster transport to the cytoplasm and increased RNA synthesis.

The impact of induced rheumatoid arthritis can be most clearly seen from the relative DNA content in liver of the treated group compared to the control group of animals (A:B, $p< .001$).. Similar, if not the same, are the effects on absolute and relative RNA mass in hepatic tissue. Especially important is the relative RNA content in the liver tissue, because it shows the real change in RNA concentration on 100 mg hepatic tissue. Our results, for the state of collagen-induced arthritis, show significantly higher values of relative RNA content in arthrosed rats compared to control rats (A:B, p<

.001). The stimulating effect of induced rheumatoid arthritis on relative RNA content in liver is expressed by significant increase in the treated in relation to the control group (A:B, p< .001). It has been proved that induced arthritis may cause autoimmune reaction and increased nucleic acids synthesis, but it is not clear how the autoimmune reaction is induced or inhibited and how the process is propagated (Markelevic, 2006; Davidson and Diamond, 2001).

The main cells, which comprise 72-78% liver constitution, are of parenchymal origin, and they are so called hepatocytes. Beside these cells, there are also non-parenchymal cells (endothelial cells, Cupffer-cells and Pit-cells) which account for about 5 %. There are disproportionally distributed on the cell membrane of the hepatocyte, and almost always non-fixed, hormonal receptors, among which most significant are the ones for insulin, glucagon, plasma proteins and glycoproteins (De Meyts and Hanoune, 1982). At the end of the experiment we took a piece of liver from the experiment and arthrosed groups and processed it histologically, and after appropriate preparation procedure, we measured the nuclear index (Ni) and nuclear volume (Vn). From the findings it can be seen that hepatocytes in the liver in control group show normal histological pattern, i.e. liver cells are distributed in groups and possess one or two euchromatic nuclei with clearly visible nucleoli. Nuclear index and nuclear volume values of the results from the examinations of the liver in arthrosed animals show significant changes in relation to the control group. It can be seen that there are significant changes ($P < 0.005$) at the hepatocyte level. The number of nuclei has reduced by 17 %, and their volume has increased by nearly 13 %.

The effect of induced rheumatoid arthritis, according to the results we obtained, upon AST activity in control and arthrosed groups of animals show that the level of enzyme activity in arthrosed group of rats, has been increased significantly (A:B, p< .001). The enzyme activity in the treated group of animals is 135.6 U/l, which compared to the 96.8 U/l, measured in control group, is by 40 % higher value of enzyme activity. It may be noticed from the results on the ALT that the level of “normal” ALT and the level of “arthrosed” ALT differ, and in the most part there is significant deviation. This may be asserted from obtained values from our examinations in which average ALT value in control

group of animals ammounts 69.15 U/l, whereas average ALT value in arthrosed group of animal amounts 78.11 U/l, which is by 13 % higher value. It may be concluded that there has also been an impact of induced rheumatoid arthritis on the activity of ALT (A:B, $p< .005$). In processes in which there is lesion of cells and tissues rich with AST, the enzyme transfers into the circulation and this results in his increased activity in blood serum (Wroblewski, 1958). Since ALT is typical cytoplasmic enzyme, during small tissue damage or change in cell membrane's permeability, it exits into the intercellular space, i.e. into the circulation, which increases its serum activity.

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