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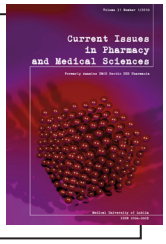

Assessment of structural and functional condition of rats bone tissue under the influence of various parameters of vibration

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ABSTRACT

Whole body vibration involves the exposure of the entire human body to direct contact with environmental vibration. Chronic mechanical vibrations, combined with the physical attributes of the human body, can amplify the incoming energy and present the potential for negative health effects. Vibration exposure can, thus, result in adverse health effects such as spinal injuries, abdominal neurological and cardiovascular disorders. These can manifest indirectly as an accident causal factor. The aim of our research is to study the impact of vibration fluctuations of different frequencies on the structural and functional condition and mechanisms of bone remodelling. An experimental study was, therefore, conducted on mature male rats. For assessment of bone metabolism in the venous blood of rats, osteocalcin level was determined, while fragments of rats' lumbar vertebrae were subsequently taken for histologic examination. Our work revealed that with the increase of vibration frequency, an increase of osteocalcin level in the blood of experimental animals comes about. Moreover, we noted after terminating vibration fluctuations on the 56th day of the experiment, osteocalcin levels are gradually reduced. In addition, in the course of histological study of specimens of lumbar vertebrae bone tissue, even as early as of the 28th day of the experiment, evidences of acute impairment of the bone tissue and initial signs of its remodelling are clearly traced. Indeed, on the 56th day, the remodelling processes represented by enhanced regeneration in the zone of the cartilage plate, increased in proliferation activity. We also saw hyperplasia of chondrocytes, hypertrophy of the respective zones of cartilage tissue, zones of forming immature bone tissue on the areas of previous damage, focal replacement fibrosis and angiomatosis. Hence, with increasing vibratory acceleration of 0,5 g, the rate of bone metabolism grows, osteoblast activation processes are accelerated and the impairment of collagen and calcium loss is increased. All this leads subsequently to the occurrence of osteoporosis.

INTRODUCTION

Bone tissue is a metabolically active system, its regular functioning is ensured by a remodelling process which involves continuous replacement of bone plates, as well as formation of new osteons and trabecules at the place of the resorbed ones. Modelling, reconstruction and reparation of bone tissue are possible due to maintaining balance between the processes of resorption and bone formation [1-5]. Since bone tissue is mechanically sensitive, its state depends on many factors such as genetics and immunity status, dietary habits and lifestyle, as well as exogenous influences. The last include vibration fluctuations [6-9].

Literature presents data which reveals the continuous influence of mechanical fluctuations on bone metabolism. This has a catabolic effect and induces loss of calcium by the bones, notwithstanding the age. Recent immunological studies confirm that the chronic influence of mechanical vibrations on the body leads to activation of CD4-lymphocytes, increased synthesis of pro-inflammatory (TNF, IL-1, IL-6, IL-8) and decreased synthesis of anti-inflammatory cytokines (IL-4, IL-10, IL-13). This leads to loss of balance in the RANKL/RANK/OPG system, as well as

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differentiation and activation of osteoclasts with subsequent incensement of bone resorption [10-15].

In modern literature, much research is devoted to studying the influence of the medium and high frequency vibration on the body, with the speed of vibratory acceleration ≤ 0.5 g. In particular, Rubin and McLeod have demonstrated high bone sensitivity to mechanical stimuli. By modelling general vibration fluctuations with the frequency of 30 Hz and vibratory acceleration of 0.3 g for 5 m daily within 30 days, the researchers determined bone mass acquisition in the trabecular layer of turkey shin bone [13]. Much later, Rubin *et al*. revealed the anabolic effect of the aforementioned fluctuations, including the deceleration of bone tissue remodelling by means of retardation of osteoclastogenesis processes (downregulation of RANKL and cytokines related to osteoclastogenesis) [16]. Radicular syndrome, which is a main reason for chronic back pain, accompanies bone mass loss in the trabecular layer of lumbar vertebrae in drivers. This is caused by the general vibration fluctuation transmitted through the support points of the body, and, in most cases, leads to disability [17].

It is also now realized that a significant number of people working in the industry is exposed to a general vibration that has resulted in an increase in the number of patients with disorders of the musculoskeletal system [18,19]. This engenders muscle hypertrophy, osteoarthrosis, osteoporosis and formation of hypercalcinosis in the vertebrae. This may subsequently lead to the development of secondary radicular syndrome with trophic disorders of the limbs. Such changes may generally occur even within two to five years of enduring conditions of continuous vibration exceeding maximum permissible levels [20-23].

Considering the data of professional literary sources related to the research of the vibration effect on bone metabolism, the need arises to study the structural and functional changes that may occur in the bone tissue of the body's musculoskeletal system under experimental conditions.

MATERIALS AND METHODS

An experimental study was conducted on 60 mature male rats weighing 180-220 g. The animals were placed into 5 groups, each consisting of 12 individuals. Animals of the control group and all experimental groups were kept under the same conditions of the vivarium. All experiments on animals were conducted in compliance with bioethical principles according to the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986), Council Directive 86/609/EEC (1986), the Law of Ukraine № 3447-IV On the Protection of Animals from Cruelty, General Ethical Principles of Animal Experiments, approved by the First National Congress of Ukraine on Bioethics (2001).

The rats of the four study groups were exposed to heave vibration oscillations of the frequencies of 15, 25, 50 and 75 Hz, correspondingly, with an amplitude of 2 mm, twice a day for 20 minutes, for 28 days. Vertical vibrational oscillations were modelled using a vibrating pump with a power of 250 W and a maximum pressure of 7 bar. A vibrating platform with a container wherein a test group of rats was placed, was attached to the vibration pump stem. Vibration acceleration of the device was controlled using the remote control AFC-120 [24].

Six animals from each group were removed from the experiment on the 28th day of investigation by rapid decapitation with blood collection and subsequent preparation of histological specimen of lumbar spine (L_1-L_6). The remaining animals continued to be maintained under standard vivarium conditions over the next 28 days, without exposing them to vibration.

For assessment of bone metabolism, in the venous blood of the experimental rats, osteocalcin level was determined. Herein, 3 ml of rat's blood were placed in each test tube with gel phase, and the obtained plasma was analyzed by immunochemical methods with chemiluminescence detection. The research was conducted with the use of a Cobas 6000 analyzer, a RocheDiagnostics machine (Switzerland) and an Immulite test systems (Siemens AG, Germany). For generating the chemiluminescence reaction, derivatives of luminol with peroxydase and hydrogen peroxide were applied, and as a potentiator, n-iodophenol was used [25-27].

Fragments of experimental rats' lumbar vertebrae (L_1-L_6) were also taken for histologic examination. For this purpose, they were purified from muscle tissue and fixated with 10% formalin solution during a 24 hour period in an airtight container for storage of biological material. Decalcification of vertebrae was then performed utilizing the solution RAPID DECALCIFIER KALTEK s.r.l. (Italy) and they were covered with a paraffin-wax mixture. Longitudinal and cross cut-offs of tissue with the thickness of 7 μm were made with MS-2 sledge microtomes (Ukraine). For staining the obtained cut-offs, standard hematoxylin and eosin solutions were used. Description of the histological specimens as fixed on a glass slide were conducted by use of a Nikon Eclipse E200 microscope, photos were taken with Nikon D5000 camera.

RESULTS AND DISCUSSION

The experimental study was performed on 60 mature male rats initially weighing 180-200 g. At the end of the experiment, the weight of laboratory rats of the first and second groups remained virtually unchanged, while in the third and fourth experimental groups, insignificant weight loss came about ($p > 0.05$).

The conducted blood test revealed significant differences in the osteocalcin levels of the experimental groups of rats compared to the control. On the 28th day of the experiment, the level of free osteocalcin in the control group was 39.52 ± 0.78 ng/ml. In the first experimental group, the rate amounted to 48.55 ± 1.31 ng/ml, while in the second and third groups, the figures were 59.60 ± 1.21 ng/ml and 70.80 ± 1.79 ng/ml, correspondingly. In the fourth group, the rate increased twice, being 85.75 ± 1.92 ng/ml ($p < 0.05$).

With regard to the 56th day of the experiment, osteocalcin levels in the control group remained virtually unchanged at 41.07 ± 0.62 ng/ml. In the first experimental group, the average was 42.82 ± 0.71 ng/ml ($p > 0.05$), in the second, third and fourth groups, the corresponding figures were

46.18 ± 0.70 ng/ml, 50.78 ± 1.19 ng/ml and 63.75 ± 0.95 ng/ml ($p < 0.05$), respectively (Tab. 1, Fig. 1.). Bone tissue consists of an organic matrix and a mineral phase, the structural units of which are hydroxyapatite crystals. Osteocalcin is mainly located in mineralized tissue, serving as an intermediary in matrix mineralization, and has a high affinity for calcium. After segregation from the osteoblasts, osteocalcin is deposited in the matrix of bone tissue and released into the bloodstream, thus, this marker may indicate the speed of bone tissue remodelling [25,26]. Nearly 70-90% of all osteocalcin synthesized by osteoblasts is included into the bone matrix, while the rest is in the blood stream. Therefore, osteocalcin can be considered to be the most specific protein of bone tissue.

The rapid increase of osteocalcin level in venous blood of rats in the second, third, and fourth experimental groups on the 28th day of the experiment testifies to the influence of the medium and high frequency vibration on bone metabolism. Herein, the increased activity of osteoblasts is a response to the acceleration of collagen catabolism which leads to a loss of calcium and reduced bone tissue mineral mass. On the 56th day, the bone metabolism gradually reduces to the original values, but in the third and fourth groups, the rates remain high, which testifies to the continuation of loss of the bone mineral component after the termination of the vibration fluctuations.

Table 1. Osteocalcin level (ng/ml) in the blood of experimental rats on the 28th and 56th days of the experiment

Experimental group	Osteocalcin level (ng/ml)				
	28th day of experiment			56th day of experiment	
	M	± m	σ	M	± m
Control	39.52	0.78	1.40	41.07	0.62
I	48.55	1.31	1.58	42.82	0.71
II	59.60	1.21	1.56	46.18	0.70
III	70.80	1.79	2.66	50.78	1.19
IV	85.75	1.92	2.13	63.75	0.95

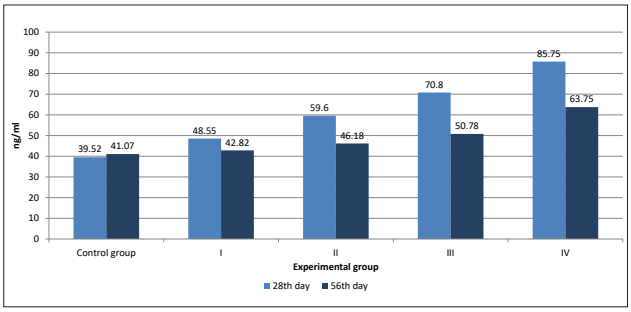

Figure 1. Osteocalcin level (ng/ml) in the blood of experimental rats on the 28th and 56th days of the experiment

As a result of the conducted histological examination of the rat bone tissue, a dependence of the progressive loss of mineral density on the frequency of vibration has been determined. In particular, on the 28th day of study, in the lumbar vertebrae of the rats of the first group, moderate alternative and adaptive changes in bone tissue have been observed. This was indicated by the focal atrophy of the periosteum along with the formation of a thin linear osteogenic layer in which single groups of osteoblasts have been visualized. The layer of bone tissue is also sophisticated, with massive and widespread petrificates and a limited number of osteocytes in the lacunae within bone matrix. What is more, the total area of bone matrix is slightly reduced in comparison with the control group. In addition, the cartilage plate displays evidence of atrophy, and shows a reduction of chondrocytes number, as well as violation of zoning (Fig. 2).

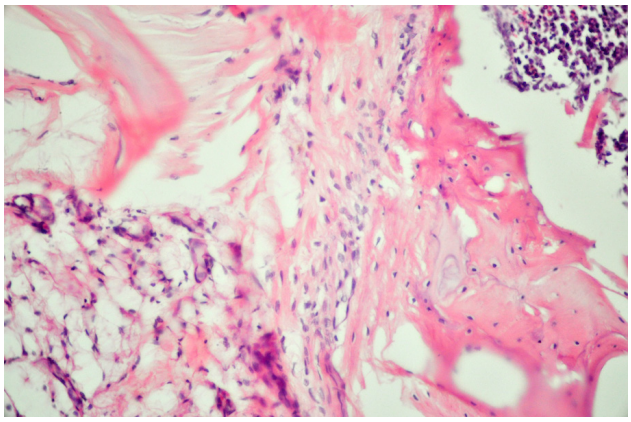

Figure 2. Hematoxylin and eosin staining. Increase ×400. Vertebra, cartilage area. Strongly pronounced atrophy of the cartilage plate, with a total violation of zoning, with small isolated chondrocytes surrounded by eosinophilic extracellular matrix. At the boundary with the soft tissue, there is a thin layer, represented by osteoblasts

The rat vertebrae bone tissue in the second experimental group is characterized by the growing evidence and dominance of morphological changes peculiar to impairment. These changes are represented by the formation of isolated zones of osteolysis deep in the bone tissue of the vertebral body, the emergence of foci of myxomatosis, as well as alternations of very thin, atrophied bone areas with others which are irregularly thickened. Furthermore, the cartilage plate has the signs of violation of zone stratification and contains disseminated small foci of alteration in the form of discomplexation of columns, as well as random location of polymorphic and slightly enlarged chondrocytes. Moreover, it is the foci of chondroid matrix accumulation (Fig. 3).

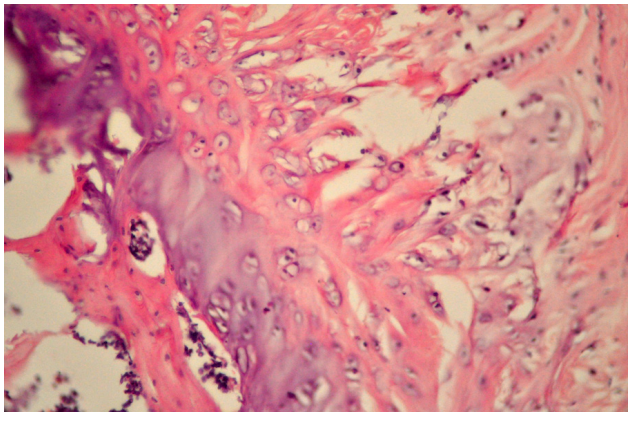

Figure 3. Hematoxylin and eosin staining. Increase ×400. Vertebra, cartilage area. Numerous foci of cartilage tissue impairment. Randomly located small groups of polymorphic chondrocytes among the chondroid matrix

In the vertebrae bone tissue of the third group of animals, exposed to vibrations with the frequency of 50 Hz in the course of histological study, morphological evidences of strongly marked impairment are also evident. The compact vertebral bone tissue is very thin, is sometimes missing as a result of total osteolysis and is replaced with cartilage tissue. What is more, the bone trabecules are small, sharply refined, atrophied, and display significant deposits of calcium salts. In relatively preserved small areas of bone tissue, insignificant focal intraosseous foci of vascularisation are also seen. In addition, chondrocytes in the areas of vicarious proliferation are located extremely compact, with signs of increased proliferative activity, and are multiple, as well as variably polymorphic and multilocular. Moreover, only a few places in the exterior layer show small isolated differentiated chondrocytes. In these samples, the cartilage plate has the most severe evidences of the impairment, with a total violation of zoning and full disorientation of chondrocytes (Fig. 4).

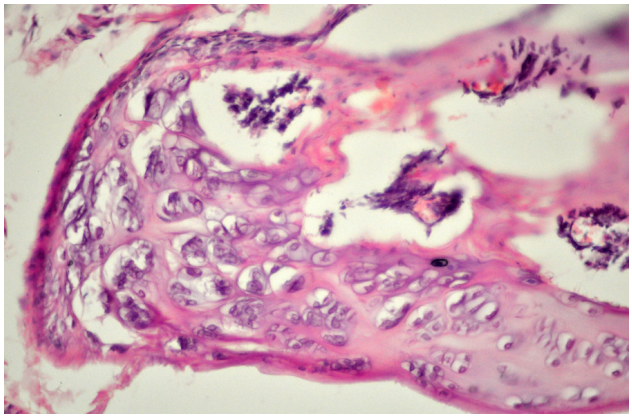

Figure 4. Hematoxylin and eosin staining. Increase ×400. Vertebra, cartilage area. Cartilage plate, with a total violation of zoning and disoriented location of polymorphic chondrocytes

The vertebral bone tissue of the fourth group, that which received the maximum vibration influence, has strongly pronounced, disseminated and diverse evidences of impairment. In addition, various areas of the vertebral bone tissue display vicarious foci of proliferating cartilage tissue represented by densely arranged groups of chondrocytes of different shapes and sizes, a focal proliferation of fibrous connective tissue with pronounced fibrinoid changes (fibrinoid swelling and fibrinoid necrosis), focal angiomatosis, small focal macrophage infiltrates (appearance of local inflammatory reaction in response to the maximally pronounced tissue damage) and numerous zones of accumulation of calcified focus. In the area of the cartilage plate of the femoral bone, there is also total impairment and absence of mature bone tissue, atrophic changes within the cartilage, total violation of zoning and disordered arrangement of large, polymorphic chondrocytes. Finally, the bone trabecules are sharply thinned, focally fragmented, with few osteoids (Fig. 5).

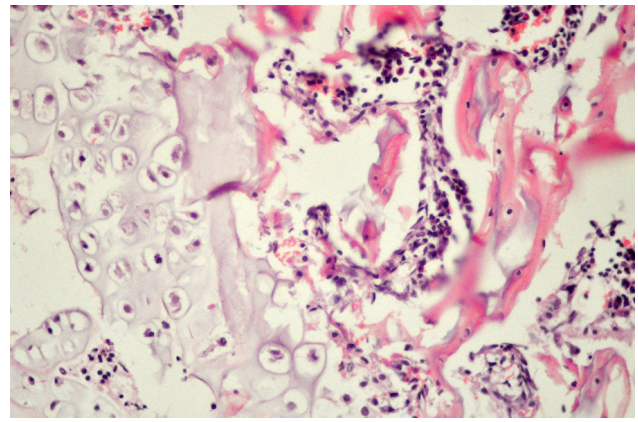

Figure 5. Hematoxylin and eosin staining. Increase ×400. No mature bone tissue. Atrophic changes of cartilage plate with total violation of zoning

By the 56th day of the experiment, the first experimental group of animals showed a predominance of bone tissue reparation processes and remodelling. This was especially pronounced in the area of the cartilage plate. The cartilage tissue still evidenced the signs of plate zoning violation, however. Moreover, thinning could be seen, as well as the sometimes almost full absence of zone of reserve cartilage. In contrast, expansion of the proliferation zone, represented by the large number of small sized proliferating chondrocytes with hyperchromic nuclei and column arrangement, was observed. There is, furthermore, local thickening of the zone of hypertrophy due to the hyperplasia of large cells. These have rounded hyperchromic nuclei and enlightened cytoplasm. The cells are also arranged randomly, compactly, in small groups of 2-3. Finally the zone of hypertrophy borders directly with the zone of resorption, which is thickened and hypercellular (Fig. 6).

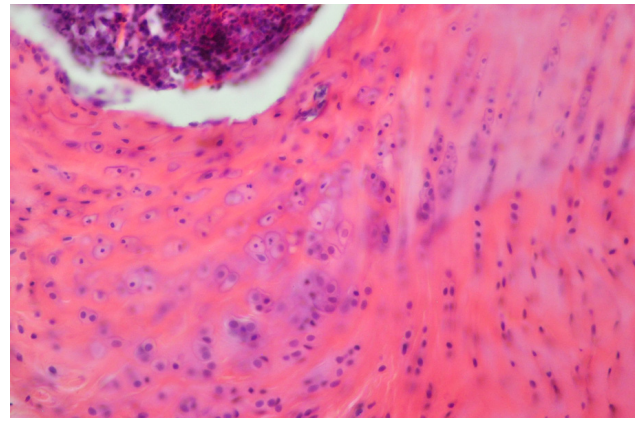

Figure 6. Hematoxylin and eosin staining. Increase ×400. Zone of cartilage plate. Expansion of the zone of proliferating cartilage, increased number of small polymorphic chondrocytes with enlarged, hyperchromic nuclei and pronounced arrangement of cells in columns

In the second group of experimental animals, an expanding area of cartilage plate with violation of its zoning due to the expansion of the chondrocyte proliferation zone, can be seen. The indicated layer is hypercellular, and is represented

by multiple, small chondrocytes forming elongated and, in some places, vortex column structures. What is more, the quantity of matrix between them is rather significant. In addition, the zone of hypertrophy is absent, and, in some places, there is a thin layer of calcified cartilage that is presented by separate small groups of polymorphic chondrocytes surrounded by a basophilic chondroid matrix. Nearby, foci of immature bone tissue with chaotic arrangement of osteocytes are noticeable (Fig. 7).

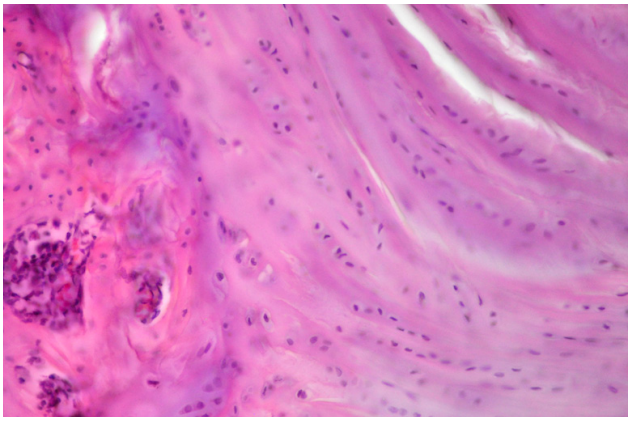

Figure 7. Hematoxylin and eosin staining. Increase ×400. Vertebra, cartilage plate area with insignificant violation of zoning: expansion of proliferation zone with the preserved arrangement of cells in columns, compact column location (on the right side in the specimen); in the centre of the specimen – zone of calcified cartilage can be seen, with small groups of larger, slightly polymorphic chondrocytes

The bone tissue of the third and fourth groups of animals, on the 56th day of the experiment, is also characterized by morphologic evidences of remodelling and reparation processes. In contrast to the first two experimental groups, in the area of the cartilage plate of the vertebrae, the extended zone of hypertrophy, is evidenced by large groups of vacuolated chondrocytes with enlightened, almost optically transparent cytoplasm. There are also initial indications of osteogenesis in the form of small areas of immature bone tissue located directly next to the zone of calcified cartilage. Finally, bone trabecules are unevenly thickened and built mostly of immature bone tissue (Fig. 8, Fig. 9).

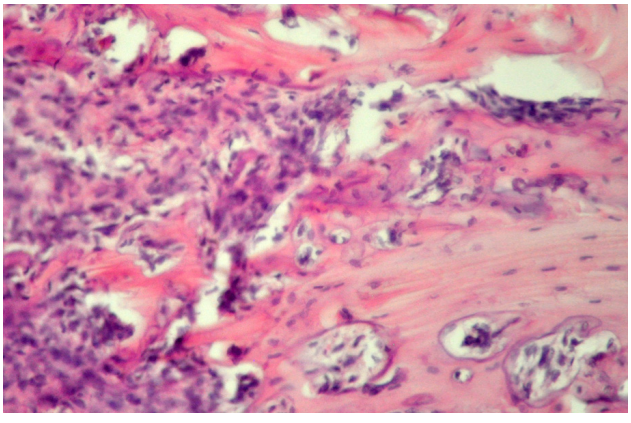

Figure 8. Hematoxylin and eosin staining. Increase ×400. Cartilage plate area with expansion of proliferation zone and atrophic changes of cartilage plate

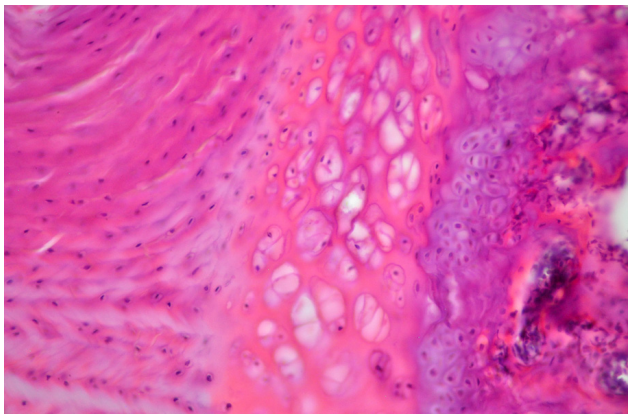

Figure 9. Hematoxylin and eosin staining. Increase ×400. Cartilage plate area with insignificant violation of cartilage zoning: expansion of proliferation zone (on the left side in the specimen); a wide zone of hypertrophy (in the centre of pthe specimen) and a pronounced zone of calcified cartilage (on the right side of the specimen)

As a result of the conducted research, the conclusion may be drawn that the chronic influence of whole body vibration can affect bone tissue and the organism in general, by intensifying the development of pathological processes induced by the negative effects of the environment [10,24]. Because of our work, the dependence of the osteocalcin concentration in the blood of experimental animals on bone tissue metabolism levels, as well as the degree of manifestation of alternative changes in its structure, has been defined. In all experimental groups, the relationship between power of vibration stimulus and the level of bone tissue metabolism can be traced. Herein, pathological changes in the bone tissue in the form of various types of impairment show a tendency to increase in a correlation with indicators of osteocalcin level in the blood. Finally, remodelling processes of bone and initial evidences of osteogenesis reach maximum manifestation in animals after vibration effects cease.

CONCLUSION

We determined that a positive correlation exists with the increase of vibration frequency (from 15 Hz to 75 Hz), and the increase of osteocalcin level in the blood of experimental animals.

In our work, after terminating vibration fluctuations on the 56th day of the experiment, a gradual reduction of osteocalcin level can be observed, especially in the second, third and fourth experimental groups, while in the first experimental group, the results were insignificant in terms of statistics in comparison with the control group.

In the course of our histological study of specimens of lumbar vertebrae bone tissue, on the 28th day of the experiment, the evidences of acute impairment of the bone tissue and the initial signs of its remodelling are already clearly traceable. On the 56th day, moreover, the remodelling processes reach a maximum degree of manifestation. Herein, they are represented by enhanced regeneration in the zone of cartilage plate, increased proliferation activity and hyperplasia of chondrocytes, hypertrophy of the respective

zones of cartilage tissue, as well as zones of immature bone tissue formation accompanied by the creation of isolated Haversian canal areas within the areas of previous damage, and, finally, focal replacement fibrosis and angiomatosis. Thus, we have established that with increasing vibratory acceleration (> 0,5 g), the rate of bone metabolism grows, osteoblast activation processes are accelerated, and the impairment of collagen and calcium loss is increased, leading, subsequently, to the occurrence of osteoporosis.

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