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EFFECT OF SIX-MONTH HYPOKINESIA IN DOGS ON MINERAL COMPONENT, RECONSTRUCTION AND MECHANICAL PROPERTIES OF BONE TISSUE

A. I. Volozhin, M. P. Pavlova, I. Sh. Muradov,
G. P. Stupakov, V. A. Korzhen'yants

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A. I. Volozhin, M. P. Pavlova, I. Sh. Muradov, G. P. Stupakov, V. A. Korzhen'yants

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16. Abstract

Ca^{45} incorporation into the bones of the limbs, particularly in the area of the muscle attachment increased in dogs as a result of 6-month hypokinesia. There were no phenomena of osteoporosis in the cortical layer of the diaphyses; however, changes in the form of osteons, an increase in the number of anastomoses between the channels and the thinning of the subperiosteal layer pointed to disturbances of the bone tissue reconstruction. Mineral saturation of the bone microstructures of the experimental dogs had a tendency to rise.

No changes in the mechanical properties of the long bones occurred as a result of hypokinesia in dogs.

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EFFECT OF SIX-MONTH HYPOKINESIA IN DOGS ON MINERAL COMPONENT, RECONSTRUCTION AND MECHANICAL PROPERTIES OF BONE TISSUE

By

A. I. Volozhin, M. P. Pavlova, I. Sh. Muradov, G. P. Stupakov, V. A. Korzhen'yants*

As a consequence of the prolonged reduction in the functional load on the support-motor apparatus the bone tissue is exposed to atrophic changes. Demineralization of the bones and a negative calcium balance were found in paralyzed patients [11, 14], as well as in practically healthy human volunteers under conditions of hypokinesia for several weeks and even months [4,10, 15]. In experiments on rats and rabbits restricted movement induced changes in the calcium, phosphorous and protein metabolism [1, 5,7], as well as a slowing down of the renewal of calcium salts [2,3]. There are no published data on the state of mineral saturation of the bone tissue microstructures and the mechanical properties of the bone in relation to the peculiarities of its reconstruction with restricted motor activity. /34**

Technique

Experiments were set up on 16 dogs of both sexes in age 1½-2 years weighing 8-12 kg. Hypokinesia lasting 6 months was created in 8 animals by placing them in specially designed metal cages whose volume roughly corresponded to the size of the dog. Eight dogs were the controls. During the entire period of hypokinesia the dogs were in the lying position, and thorough sanitary and hygienic care was

*Department of Pathological Physiology of the N. A. Semashko Moscow Medical Stomatological Institute and the Laboratory of Biophysics of the N. N. Priorov Central Institute of Traumatology and Orthopedics.

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taken of them. The calcium metabolism in the mineralized tissues was studied in 6 experimental and 6 control dogs with the help of Ca^{45} that in the form of a chloride salt diluted in an isotonic solution of NaCl was administered intravenously in a dose of 100 mCi per 10 kg of animal weight on the 180th day of the experiment. Within 24 h after administration of Ca^{45} the dogs were killed by ether vapors and chloroform vapors, the maxillary, humeral, femoral and tibial bones were removed from the right side, they were cleaned of the soft tissues and fixed in 10% neutral formalin. The incisors, premolars and molars were extracted from the jaw. With the help of a fine metal cutter fragments were sawed off the middle of the diaphyses /35
of the long bones, heads of the humerus and femur, trochanter major of the femur and tubercle major of the humerus. The bone fragments were dried to a constant weight, weighed, incinerated in a muffle furnace, the ash was again weighed and the content of ash residue in percentages of dry weight of the tissue was calculated. After pulverization in mortar standard suspensions were made of the ash of 20 mg each that were placed in a target; radiometry was conducted on the unit "Tesla" with the help of a window counter T-25-BFL. The radioactivity was computed in impulses per 1 mg of ash.

In the cortical bone measurements were made of the average mineral saturation by a direct method--by determination of the volume of diaphysis fragment (by weighing on torsion scales in air and in water) with its subsequent incineration. From the microsections the mineral saturation was determined in the microstructures of the subperiosteal, intermedial and subendosteal zones of the cortical layer in the bone diaphyses with the help of the method of quantitative microroentgenography [6,12]. The results of the analysis of average mineral saturation and in the bone microstructures were expressed in grams of mineral salts per 1 cm^3 of tissue.

For histological study the fragments of the bone diaphyses were decalcinated in 10% nitric acid, and poured into celloidin. Sections 10 μm thick were stained with hematoxylin-eosin. From the histological preparations on the cross sections of the diaphyses the width of the osteons and the haversian canals was determined. The average dimensions and frequency of distribution of these structures with respect to width were determined.

The left femoral and humeral bones of the dogs were fixed in a 1% solution of neutral formalin and x-rayed in the sagittal and frontal projections without a magnification screen. From the x-ray photographs with the help of a MBS-1 microscope in the middle third of the bone diaphysis the external diameter, width of the medullar canal and the thickness of the cortical layer were determined. No later than in 48 h after the extraction the mechanical properties of the whole femur and humerus were studied in 4 dogs by testing them for static bending on a standard machine with maximum force 500 kg-f. The error in computing the indices of the strength-measuring device is about 1% of the measured load. The load conditions: distance between the supports 65 mm, axis of the bending knife coincided with the middle of this distance and with the middle of the length of the bone; the rate of loading was 10 mm/min. The tensile strength was computed according to the formula: $\frac{M_x}{W_x}$, where M_x--bending moment, W_x--moment of resistance. The magnitude of the moment of inertia necessary to calculate the moment of resistance was determined with the assumption that in the cross section the external shape of the bone and the shape of the medullar canal represent a regular ellipse.

The numerical data were statistically processed according to Fisher-Student.

Results and Discussion

After hypokinesia the inclusion of Ca^{45} (see table) was increased in the diaphysis of the femur and trochanter major, while it was reduced in the diaphysis of the humerus. The trend towards the increase in inclusion of Ca^{45} was revealed in the head of the humerus and femur, and a considerable increase--in the tubercle major of the humerus. Ca^{45} assimilation in the lower jaw of the experimental animals was reduced, and in the upper jaw was not altered. Inclusion of the calcium isotope was considerably reduced in the lower premolars and molars, and in the other teeth corresponded to the control. No significant changes were revealed in the content of the ash residue in all the mineralized tissues of the experimental animals as compared to the control.

Hypokinesia in the dogs did not affect the ratio of the thickness of the cortical layer, as well as the medullar canal to the external width of the bone diaphyses.

As a result of the morphometric study it was shown that the average width of the osteons in the diaphyses of different long bones in the control dogs was 191-264μm, and of the haversian canals--38.9-55.0 μm. In dogs after hypokinesia the average width of the osteons corresponded to the control, while the average diameter of the haversian canals rose insignificantly.

About 50% of the haversian canals in the diaphyses of the long bones in the control dogs have a diameter up to 40 μm, 50-55%--up to 80 μm (fig. 1). The number of wider canals is 5-8%, which apparently reflects the process of reconstruction of the osteons. Under the influence of hypokinesia in dogs the number of vascular canals over 80 μm wide is increased and with width 30-40 μm--decreased. The distribution of the diaphyses in the bones of the control and experimental dogs according to width of the osteons was the same.

The average mineral saturation of the fragment of the humeral diaphysis in the control dogs was 1.113 ± 0.021 g/cm^3, and the femoral--1.160 ± 0.017 g/cm^3. After hypokinesia practically the same amounts were obtained, respectively 1.118 ± 0.014 /36
and 1.159 ± 0.019 g/cm^3.

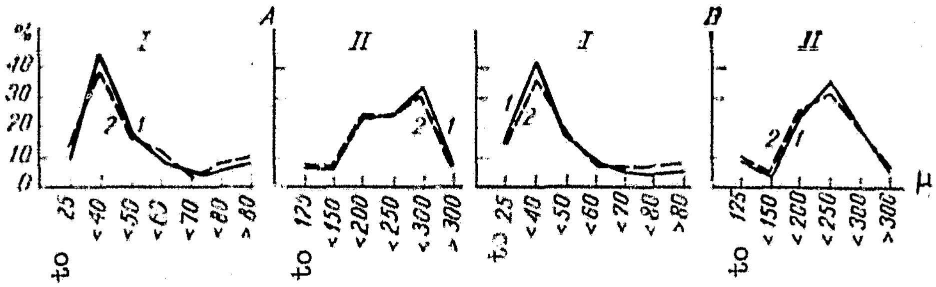

Figure 1. Frequency of Distribution of Haversian Canals (I) and Osteons (II) according to Width in Diaphyses of Bones of Dogs
A--humerus; B--femur; 1--control; 2--hypokinesia

The microroentgenographic study of the long bones in the control dogs revealed the nonuniformity in the mineral saturation of different zones of the diaphysis: the intermedial zone was the most mineralized (1.39±1.50 g/cm^3) as compared to the subperiosteal and subendosteal (1.22-1.39 g/cm^3, $P < 0.05$).

During hypokinesia an increase was found in the indices of mineral saturation of the microstructures of all the zones of the diaphysis of the humerus to 1.36, 1.52 and 1.43 g/cm^3 (in the control 1.28, 1.50 and 1.24 g/cm^3 respectively for the subperiosteal, intermedial and subendosteal zones). In the femur of the experimental animals the mineral saturation was increased in all the zones, except the

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Group	Tissue	Radioactivity of impulses per 1 mg of ash	% of control	Tissue	Radioactivity of impulses per 1 mg of ash	% of control
	Teeth of upper jaw:			Bone diaphyses:		
Control	incisors	43.8±2.6		femur	63.0±4.7	
Experiment		44.5±4.9	101.6		72.7±4.1	115.4
Control	premolars	39.3±2.3		tubercle major	113.2±5.4	
Experiment		49.2±6.4	125.2		145.5±7.2	128.5*
Control	molars	41.7±2.5		humerus	93.5±9.2	
Experiment		44.4±5.6	106.5		74.7±3.7	79.9
	Teeth of lower jaw:			Heads of bones:		
Control	incisors	26.7±2.1		femur	405±26.0	
Experiment		27.7±1.1	103.7		451±23.0	110.4
Control	premolars	36.0±2.7		humerus	519±37.6	
Experiment		27.7±1.7	76.9*		577±29.2	111.2
Control	molars	30.3±2.2		trochanter major of femur	298±21.9	
Experiment		25.0±1.1	82.5*		353±27.2	118.5
Control	lower jaw	174±23.7		tubercle major of humerus	270±22.9	
Experiment		130±13.5	74.9*		385±39.8	142.8*
Control	upper jaw	186±16.2				
Experiment		182±28.9	94.0			

*$P < 0.05$

subperiosteal. In contrast to the humerus and femur in the tubercle major the corresponding indices in all zones were somewhat reduced.

On the histological preparations the thickness of the layer of the general plates of long bones in the experimental animals was smaller than in the control, especially in the subperiosteal zone of the femur. The osteons were characterized by a less regular form; among them anastomoses were observed more often than in the control, and the area occupied by the intercalative plates was increased (fig. 2).

In the control dogs the magnitude of tensile strength of the femur fluctuated from 15.03 to 18.45 kg-f/mm^2, and the humerus--from 16.34 to 22.65 kg-f/mm^2. The fluctuations in the tensile strength of the bones of the dogs in the experimental group were roughly the same.

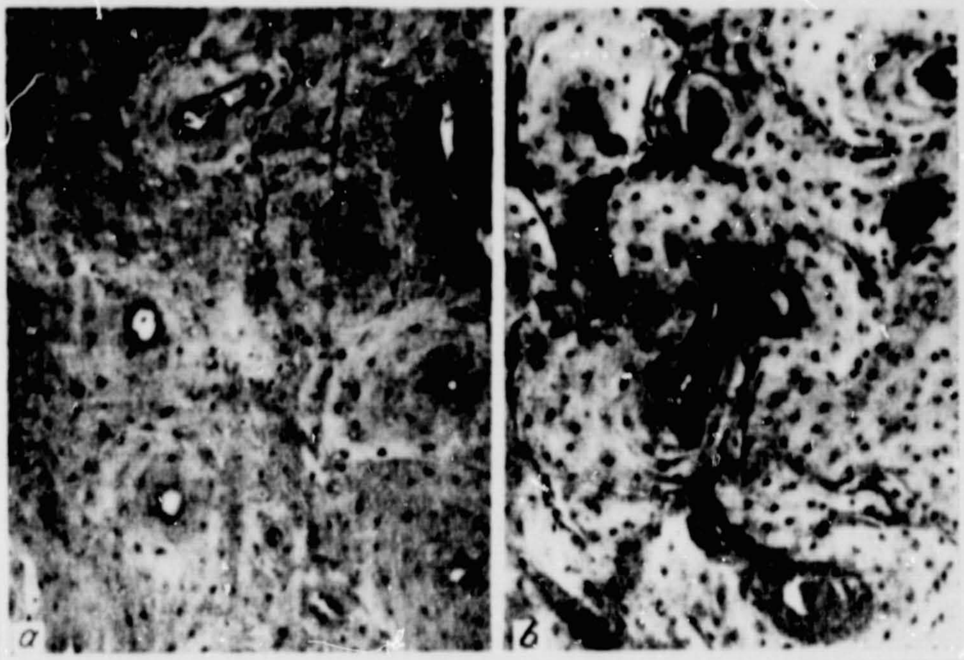

Figure 2. Diaphysis of Femur
a--control; b--after 6-month hypokinesia: anastomoses are visible between the haversian canals in the cortical layer, irregular shape of the osteons. Staining with hematoxylin-eosin. Mag. x 120.

Totally the tensile strength of the femur and humerus in dogs of the control /37 group was 17.56-0.76 kg-f/mm^2, and after 6-month hypokinesia--17.37-0.55 kg-f/mm^2, i.e., practically was not altered.

Thus, as a result of 6-month hypokinesia in dogs a change occurs in the calcium metabolism in the bones of the extremities consisting of an intensification of Ca^{45} assimilation in the zones of muscle attachment. It is possible that this fact indicates the development in the indicated regions of osteoporosis. In the mineralized tissues of the tooth-jaw system incorporation of Ca^{45} primarily is reduced, which can be explained by its redistribution between different sections of the skeleton under conditions of hypokinesia.

In dogs as a result of the restricted movement no intensified resorption of bone tissue occurs and the average dimensions of the osteons and haversian canals are not significantly altered. However the data of the histological study make it possible to consider that during hypokinesia certain disorders develop in the process of bone tissue reconstruction. Here the mineral saturation of the microstructures has a tendency to increase almost in all zones. Based on our studies one can draw the conclusion that under conditions of hypokinesia demineralization is intensified in the compact substance of the dog bones. /38

As a result of hypokinesia no decrease occurs in the strength of the long bones of adult dogs, which agrees with the published data that the mechanical properties of the bone depend not on the dimensions of the haversian canals but are linked to the width of the osteons [9, 13, 17] and mainly with the mineral saturation of the bone tissue [9, 16]. The main factors on which the strength of the bones depends are not significantly changed as a result of hypokinesia.

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