EFFECT OF HYPOKINESIA ON STRUCTURE AND MINERAL CONTENT OF THE LONG BONES IN PUPPIES

M. N. Pavlova, A. I. Volozhin, and I. Sh. Muradov

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The effect of hypokinesia for 2 months on the structure and mineral content of the cortical layer of the humerus, femur, and tibia was investigated in experiments on 6 puppies aged 2 months. The mineral content of the microstructures of the subperiosteal, subendosteal, and intermediate zones of the cortical layer of the long bones of the puppies, investigated by contact microroentgenography, increased under the influence of hypokinesia as a result of predominance of more mature structures resulting from delayed osteogenesis. An increase in the mean diameter of the Haversian canals was found as a result of increased osteoclastic absorption of bone tissue. Resorption also was intensified in the subperiosteal zone, as the result of which this zone and the whole cortical layer became thinner.

KEY WORDS: *hypokinesia; long bones; mineral content.*

Prolonged hypokinesia in rats and rabbits delays growth of the skeletal bones and modifies calcium and protein metabolism in bone tissue [1-3, 5]. No data are available on the effect of hypokinesia on the mineral content of the microstructures and reorganization of the bone tissue of the limbs in the period of their growth and development.

EXPERIMENTAL METHOD

Experiments were carried out on six noninbred puppies aged 2 months. Hypokinesia was produced in 3 puppies (experiment) for 2 months by keeping them in a cage measuring 45 X 45 X 45 cm; 3 puppies were used as controls. The puppies were then killed, their humori, femora, and tibiae removed, and x-rayed in the lateral view without an intensifying screen. The length of the bones (l) was determined from the roentgenograms, after which the external diameter (d), the thickness of the cortical layer on the anterior and posterior surfaces of the bone (d_{ca} and d_{cp}), and the width of the medullary canal (d_{mc}) were measured at the mid-diaphysis of the bones on the MBS-2 microscope; the ratios d/l, d_{ca}/d and d_{cp}/d, and d_{mc}/d were calculated. The area of cross section of the whole bone (s_b) and of the cortical layer of the diaphysis (s_c) also were determined from the roentgenograms and the ratio s_c/s_b was found.

The degree of mineral saturation was determined by Engström's method [6] in Polyakov's modification [4] in the microstructures of the subperiosteal, intermediate, and subendosteal zones in transverse thin sections taken from the middle part of the diaphyses of the bones. The results were expressed in g/cm^2 relative to the density

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TABLE 1. Effect of Hypokinesia in Puppies on Osteometric Indices (in mm) (M ± m)

Group of animals	Indices		
	l/d	s_c/s_b	$d_{c(a+p)}/d$
Humerus			
Control	11,8±0,92	0,131±0,0029	0,397±0,014
Experiments	11,6±0,53	0,106±0,0017	0,285±0,0092
Percent of control	98,2	80,9†	71,8†
Femur			
Control	14,2±0,96	0,170±0,0042	0,388±0,018
Experiments	13,3±0,15	0,150±0,0038	0,305±0,024
Percent of control	93,7*	88,3*	78,5*
Tibia			
Control	16,4±0,23	0,225±0,0054	0,546±0,016
Experiments	15,8±0,12	0,140±0,0041	0,499±0,041
Percent of control	96,2*	62,3†	91,3

Group of animals	Indices		
	d_{mc}/d	d_{cp}/d	d_{ca}/g
Humerus			
Control	0 639±0,033	0,183±0,029	0,213±0,051
Experiments	0,729±0,048	0,130±0,0060	0,154±0,0096
Percent of control	111,4	71,0	72,4
Femur			
Control	0,648±0,0024	0,205±0,0040	0,183±0,0088
Experiments	0,699±0,041	0,149±0,011	0,157±0,0071
Percent of control	109,3	72,7†	85,8*
Tibia			
Control	0,471±0,028	0,294±0,011	0,251±0,015
Experiments	0,509±0,029	0,237±0,013	0,229±0,022
Percent of control	108,0	80,5*	91,4

Values of P shown for significant difference between data for experimental and control series

*$P < 0.05$.
†$P < 0.01$.

of hydroxyapatite. Fragments from the middle third of the diaphyses for histological investigation were decalcified in 10% HNO_3; transverse sections 7-10 μ in thickness were stained with hematoxylin—eosin. The mean diameter of the osteons and the width of the Haversian canals were determined in the preparations. The numerical data were subjected to statistical analysis by the Fisher—Student method.

EXPERIMENTAL RESULTS

In puppies as a result of hypokinesia the ratio l/d was reduced in all bones compared with the control (Table 1). The value of s_c/s_b fell sharply, indicating a relative decrease in the volume of the cortical layer of the bone. The relative thickness of the cortical layer of the bones (d_{cp}/d and d_{ca}/d) also decreased, and the decrease in thickness of the posterior surface of the femur was much greater than its decrease in the anterior surface.

Morphological investigation of the bone tissue in the experimental puppies revealed active resorption of the Haversian canals, the mean width of which was 2-3 times greater ($P < 0.01$) than that in the control puppies. For example, the width of the canals in the femur was from 36.3 ± 2.5 to 41.1 ± 4.1 μ in the control and from 75.6 ± 4.7 to 135 ± 1.1 μ in the experimental series. The width of the Haversian canals in the humori of the puppies of the experimental group was increased by the same degree as in the femora, whereas in the tibiae the increase was less marked.

Resorption of bone tissue was characterized by lacunar and sinusoid absorption

Fig. 1. Photomicrograph of humoral diaphysis of puppy after hypokinesia. Dilated vascular canals and foci of resorption in layer of outer Haversian lamellae. Hematoxylin—eosin, 120X.

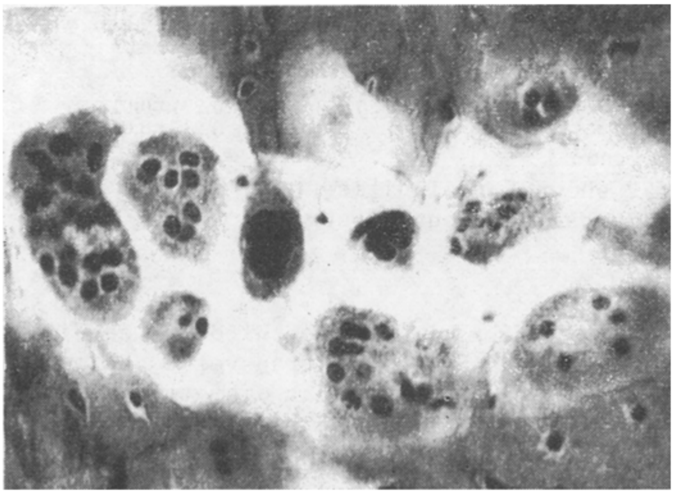

Fig. 2. Photomicrograph of femoral diaphysis of puppy after hypokinesia. Multinuclear osteoclasts in focus of resorption of bone tissue. Hematoxylin—eosin, 500X.

(Fig. 1a, b); some sinuses filled with "liquid bone" were seen. Absorption was accompanied by the formation of many multinuclear giant osteoclasts (Fig. 2). The osteons were mainly irregular in shape, whereas in the control they were cylindrical. The thickness of the subperiosteal layer was slightly reduced, but in some places on the side of the subendosteal layer it was reduced to zero.

In the cortical layer of all long bones of the control puppies the distribution of mineral saturation was irregular. In the femur, for instance, this index reached its minimum in the subendosteal and subperiosteal zones (1.05 ± 0.023 and 1.06 ± 0.027 g/cm^3, respectively) and its maximum in the intermediate zone (1.20 ± 0.017 g/cm^3). Differences in the values of the mineral saturation of these zones are significant.

In the puppies after hypokinesia the mineral saturation of the microstructures of the femur showed a tendency to decrease in the intermediate zone to 1.25 ± 0.020 g/cm^3 and in the subperiosteal zone to 1.15 ± 0.024 g/cm^3, in the tibia the tendency was observed in all zones, but in the humerus the values corresponded to the control.

These results showed that as a result of hypokinesia in puppies the cortical layer becomes thinner but the medullary canal of the long bones becomes longer relative to their external diameter. These changes arise through inhibition of oppositional growth on the side of the subperiosteal zone of the bone and resorption of the bone tissue on the side of the medullary canal. The small increase in mineral saturation of the bone tissue is evidently the result of relative predominance of the older, completely mineralized microstructures over newly created structures on account of the reduced osteogenesis.

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