EFFECT OF VARIOUS TYPES OF REHABILITATION ON SKELETAL MORPHOLOGY IN INBRED RATS AFTER HYPOKINESIA

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Statistically significant correlation was demonstrated between physical exertion and drug therapy in hypokinesia and adaptation of the bones. The various types of correction studied differed in their manifestations in rats of different inbred lines, evidence that growth and morphogenesis of the limb bones are genetically determined.

KEY WORDS: inbred animals; hypokinesia; rehabilitation; genetic determination.

Recent investigations [1-3] have shown that hypokinesia causes a combination of polymorphic disturbances in man and animals. The organization of concrete measures aimed at preventing the adverse effects of prolonged restriction of movement is an essential task. At the same time, it can now be accepted [4] that the degree of these changes depends not only on exposure to hypokinesia itself, but also on individual differences between animals. It has not yet been settled whether this rule applies also when measures of rehabilitation and prophylaxis are applied in conjunction with prolonged restriction of movement. The object of the present investigation was to study the morphological aspects of rehabilitation in hypokinesia in inbred animals of different strains.

EXPERIMENTAL METHOD

Experiments were carried out on 160 inbred August and Wistar rats, all sexually immature males aged 2 months. The animals of each strain were divided into five equal groups. The animals of group 1 served as the control. The rats of group 2 were exposed to hypokinesia by keeping them in small closely fitting cages. The animals of groups 3-5 were kept under identical conditions of hypokinesia but, in addition, received daily rehabilitation measures. Training of the animals of group 3 started with running on a treadmill moving at a constant speed of 30 m/min for 3 min from the 1st day of hypokinesia. On alternate days the period of training was increased by 1 min, so that at the end of the experiment it was 30 min. Animals of group 4 were trained in the same way, but the duration of training was increased daily by 1 min, so that at the end of the experiment it was 60 min. The rats of group 5 were kept under the same conditions as those of group 4. For stimulation they were given a daily intraperitoneal injection of amphetamine (2 mg/kg body weight). The experiment lasted 2 months and covered the period of most intensive growth of the animals until sexual maturity. The animals were chosen to be similar as regards sex, age, and experimental conditions within the individual experimental groups and they differed only genetically. The rats were decapitated. The bones of the left hind limbs were macerated chemically in a 1.5% solution of caustic potash at 45°C. Osteometry was carried out by Durst's method. The bones were weighed on torsion scales. Their linear dimensions were determined with calipers with an accuracy of 0.1 mm. The bones of the right hind limbs were cleaned manually for histological investigations, then fixed in 10% neutral formalin solution, decalcified with nitric acid, and embedded in celloidin. Sections 5-10 μ thick were stained with hematoxylin-eosin and with picrofuchsin by Van Gieson's method. In preparations of the bones of the hind limbs the knee joint and the bones forming it were studied. By means of an ocular micrometer the mean thickness of the distal epiphyseal plate of the femur, the proximal epiphyseal plate of the tibia, and the thickness of their articular cartilages was determined from three measurements in the center and two at the periphery. The mean number of cells in the cartilaginous columns in the zone of young growing cartilage was determined by counting the cells in 10 columns from each section studied. The results were subjected to statistical analysis and also to mathematical analysis on the Mir computer.

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TABLE 1. Thickness of Proximal Epiphyseal Plate of Tibia Depending on Experimental Procedure and Strain of Animals (M ± m)

Strain of rat	Groups of experimental animals						
	group 1 (M)	P_2	group 2 (M)	group 3 (M)	group 4 (M)	group 5 (M)	P_3
August	190,2±1,4						
		<0,001	219,0±3,0				<0,01
		<0,001		207,5±1,3			<0,01
		<0,05			197,1±2,1		<0,01
		<0,002				175,0±3,2	<0,01
P_1	<0,001		<0,001	<0,001	<0,01	<0,02	
Wistar	176,4±2,3						
		<0,001	203,8±1,5				<0,001
		<0,01		188,3±2,1			<0,001
		<0,05			169,8±1,5		<0,001
		<0,01				185,6±1,4	<0,001

Legend. P1) Differences between strains, P2) differences within same strain relative to control; P3) differences within same strain relative to hypokinesia.

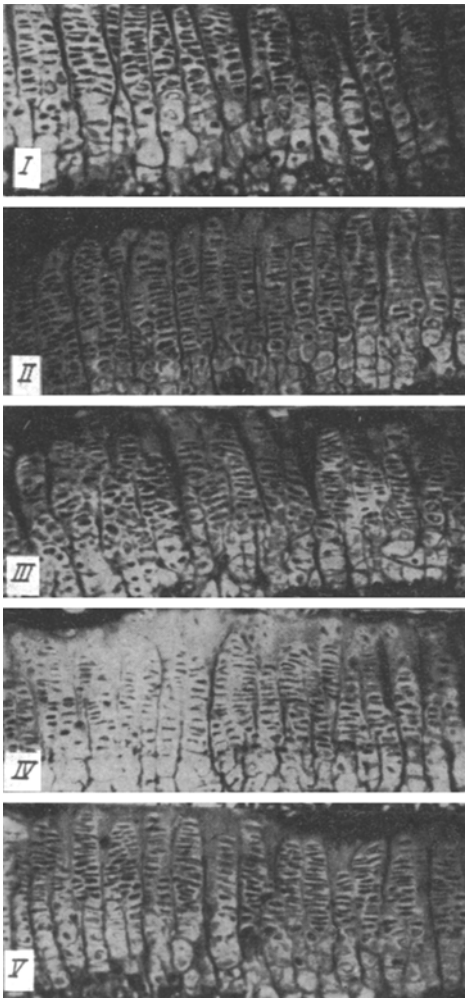

Fig. 1. Thickness of epiphyseal plates of proximal tibial epiphysis of August rats. Here and in Figs. 2 and 3: I) control, II) hypokinesia, III) hypokinesia plus running on treadmill for 30 min by end of experiment, IV) hypokinesia plus running on treadmill for 60 min by end of experiment, V) hypokinesia plus running on treadmill for 60 min by end of experiment plus amphetamine. Stained by Van Gieson's method, 70×.

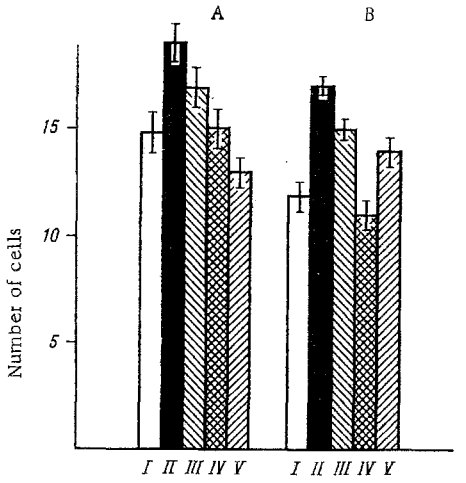

Fig. 2. Number of cells in columns of zone of young growing cartilage of proximal tibial epiphyseal plate in August (A) and Wistar (B) rats.

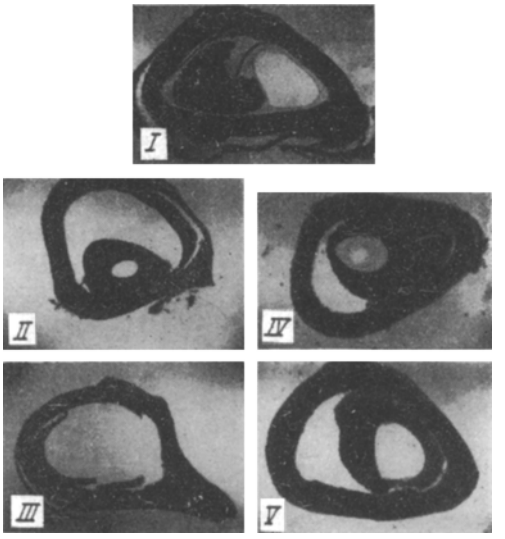

Fig. 3. Thickness of compact layer of femur of August rats.

EXPERIMENTAL RESULTS

Analysis of the experimental results showed a significant decrease in body weight after hypokinesia for 60 days. The body weight of the August rats fell from 145.5 ± 1.0 g in the control to 114.6 ± 0.8 g after hypokinesia. In the Wistar rats this index fell from 217.6 ± 0.9 to 162.1 ± 0.9 g respectively. When rehabilitation measures were used the body weight was close to that in the control, namely: in August rats in group 3 134.7 ± 0.4 g, in group 4 125.2 ± 0.9 g, in group 5 135.2 ± 0.9 g; in Wistar rats in group 3 186.0 ± 0.5 g, in group 4 190.3 ± 0.9 g, and in group 5 169.1 ± 0.1 g. The differences are significant at $P < 0.001$. A similar tendency was observed when the length of the femur and tibia was studied. For instance, the length of the tibia in August rats was: 30.4 ± 0.1 mm in group 1, 28.8 ± 0.07 mm in group 2, 29.1 ± 0.08 mm in group 3, 29.7 ± 0.09 mm in group 4, and 30.1 ± 0.07 mm in group 5; in Wistar rats the corresponding values were 35.0 ± 0.09, 31.4 ± 0.08, 32.5 ± 0.05, 32.9 ± 0.05 and 32.3 ± 0.04 mm.

The differences are significant at $P < 0.001$ (for group 5 of August rats $P < 0.05$). By contrast with the control rats, in animals exposed to hypokinesia ossification was severely disturbed. The cancellous substance of the epiphyses of the femur and tibia had a wide-looped structure, the bony trabeculae were very thin, and they consisted of only a few ill-defined layers. The bone contained very few osteocytes which were reduced

in size. Examination of microscopic specimens of the femur and tibia of inbred animals after rehabilitation measures revealed adequate development of both the cancellous and the compact bone. By contrast with hypokinesia alone, rehabilitation measures led to the almost complete ossification of the proximal epiphyses of the tibia and distal epiphyses of the femur. The degree of these changes was a linear function of time whether after hypokinesia alone or after hypokinesia combined with rehabilitation measures, and this could be attributed to differences in the genotype of inbred rats of different lines.

The results of osteometry were particularly interesting. After exposure to hypokinesia the thickness of the proximal epiphyseal plate of the tibia (Table 1) and the distal epiphyseal plate of the femur was significantly increased in the rats of both strains exposed to hypokinesia. The changes discovered also were connected by a linear relationship and were combined with an increase in the number of cells in the columns of the zone of young growing cartilage. The thickness of the articular cartilages also was significantly increased. By contrast to this, in animals exposed to hypokinesia the compact layer of the femur was significantly reduced in size, to a different degree in the August and Wistar rats. In August rats receiving rehabilitation measures, unlike those exposed to hypokinesia alone, the thickness of the epiphyseal plates (Fig. 1) and the number of cells in the columns of the zone of their young growing cartilage (Fig. 2) decreased gradually but significantly from group 3 to group 5, whereas in the Wistar rats the maximal effect of rehabilitation was manifested in the animals of group 4. The pattern thus revealed also was characteristic of the articular cartilages. As regards the thickness of the compact layer of the femora of the animals receiving rehabilitation measures, the changes observed were in the opposite direction (Fig. 3). In the August rats the increase in thickness took place from group 3 to group 5, whereas in Wistar rats the maximal rehabilitation effect was observed in group 4. Consequently, after hypokinesia for 60 days development of the skeleton was delayed. The rehabilitation measures diminished and, in some cases, completely abolished the changes constituting the "hypokinesic syndrome," and the degree of these changes, both during hypokinesia and after the various forms of rehabilitation, was determined by genetic differences between the experimental animals.

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