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EFFECTIVENESS OF USING THYROCALCITONIN FOR THE PREVENTION OF A CALCIUM METABOLIC DISORDER IN THE MINERALIZED TISSUES OF RABBITS WITH 30 DAYS OF HYPOKINESIA

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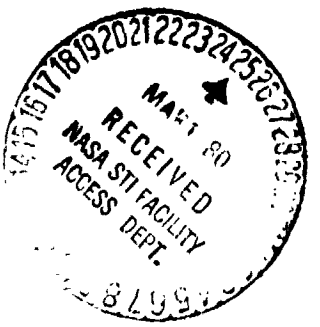

EFFECTIVENESS OF USING THYROCALCITONIN FOR THE PREVENTION OF A CALCIUM METABOLIC DISORDER IN THE MINERALIZED TISSUES OF RABBITS WITH 30 DAYS OF HYPOKINESIA

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During a 30-day hypokinesia in rabbits, a sharp lag occurs in the /42* growth of skeletal bone weight, Ca^{45} uptake is reduced, and the isotope resorption rate in the "rapidly metabolized fraction" of the bones of the extremities increases. Ca^{45} content in the teeth and maxillary bones, on the other hand, increases, which may be explained by the redistribution of isotope among assorted mineralized tissues. Injection of thyrocalcitonin (50 IU/day) has a distinct normalizing effect on the uptake of Ca^{45} in the mineralized tissues of rabbits and its resorption during restriction of motor activity.

Calcium excretion increases and osseous tissue resorption intensifies during weightlessness on space flights, as well as during protracted limitation of motor activity (hypokinesia) in humans and animals. Regular alterations of calcium and protein metabolism in osseous and dental tissues resulting from protracted hypokinesia have been demonstrated in experiments on laboratory animals [2, 5, 6].

Among the various measures used to correct calcium metabolism during movement restriction, use of the thyroid hormone thyrocalcitonin (TCT), injection of which stimulates the formation of osseous tissue and prevents osteoporosis caused by various factors, is worthy of note [10, 11]. It has been shown that TCT normalizes calcium metabolism in bones during hypokinesia in rabbits [3]. In this work we studied some aspects of calcium metabolism in the bones and teeth of rabbits following 30 days of hypokinesia without prophylaxis, and also following use of TCT.

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*Numbers in the margin indicate pagination in the foreign text.

Materials and Methods

Four series of experiments were performed on 98 chinchilla rabbits having initial weights of 2500 ± 220 g. Calcium metabolism in the bones and teeth was studied using Ca^{45}, in the form of a chloride salt dissolved in an isotonic NaCl solution, which was injected in 50 μcurie doses into the marginal vein of the ear, regardless of the animal's weight. In series I, Ca^{45} was injected into the rabbits on the 29th day of hypokinesia and the animals were sacrificed after 24 hours. In series II, the isotope was injected 24 hours before the beginning of the experiment, in III -- 30 days before, and in IV -- 60 days before, i.e., prior to placing the rabbits in cages which restricted their motor activity. Conditions were identical in all series of experiments.

Using differentiated labeling, the arrangement of experiments permitted study of calcium metabolism in the mineralized tissues according to Ca^{45} uptake indices (series I), its removal primarily from the rapidly metabolized (series II) and slowly metabolized (series III) "fractions", and following the establishment of an equilibrium between these "fractions" (series IV).

The rabbits of each series were divided into 4 groups of 5-7 animals: group 1 -- control, group 2 -- control + TCT, group 3 -- hypokinesia, group 4 -- hypokinesia + TCT. Hypokinesia was brought about by placing the rabbits in special cages with movable walls permitting their internal volume to be adjusted. Diet consisted of pelletized feed with a supplement of hay, cabbage, and carrots. Water intake was not limited.

The TCT preparation, dissolved in distilled water, was injected subcutaneously twice a day (morning and evening) at a rate of 30 MRC (IU) of hormone per day for each rabbit. The preparation used was obtained from bovine thyroids at the Department of Endocrine Preparation Technology, All-Union Antibiotic Research Institute [8].

The rabbits were sacrificed by air embolism and the calcanei, /43 tibiae, femora, humeri, scapulae, parietals, 2nd cervical vertebrae, and upper and lower maxillae were removed. Molars and incisors were extracted from the upper maxillae. Removed bones were thoroughly washed in running water for a day, carefully divested of soft tissue, desiccated to a constant weight, and incinerated in a retort furnace at 700° for 20 hours. The ashes were weighed, ground in porcelain mortars to a fine powder, from which 20 mg. weighed specimens were prepared, and transferred to metal plates having 3.95 cm^2 areas. After equal distribution of powder on the plates, radiometry was performed using an end-type counter on a "Tesla" installation. By registering the half-life of Ca^{45}, the radioactivity of all the ash from the tissue being studied was calculated and Ca^{45} content in the bones and teeth was determined in percent of injected isotope dose, which was taken as 100%, according to the formula:

$$\%Ca^{45} = \frac{A_s \cdot P_{at} \cdot 100 \cdot K}{20 \cdot A_i}$$

where A_s = activity of specimen by weight (in imp./min.); P_{at} = weight of ash (in mg.) for the entire tissue being studied; A_i = activity of isotope injected into one animal (in imp./min.; calculated according to a standard prepared from the operant solution of calcium isotope); K = a correction factor, considering the value for the half-life of Ca^{45}; 20 = weight of sample (in mg.).

Ash content in the teeth and bones (in % dry weight) and absolute and relative tissue weight (desiccated) were calculated (*in toto* for all series).

Numerical material was worked out according to the Fischer-Student method. Discrepancies were considered reliable at $P \leq 0.05$.

Results

In 30 days of observation, body weights for the control rabbits (group 1) and those which were given TCT (group 2) were the same and increased up to 130% of initial ($P < 0.001$).

In the same period, the weights of rabbits kept in hypokinesia (groups 3 and 4) did not essentially vary from initial data. TCT injection had no effect on the weights of the free-moving rabbits or those kept hypokinetic.

Basic data on the effects of TCT on calcium metabolism in mineralized tissue during hypokinesia are presented in the table.

As a result of TCT injection into group 2 animals, the weight of molars, incisors, and all skeletal bones investigated increased (an average of 6-15%; $P < 0.05$), with the exception of the parietals and maxillae, which had a tendency to decrease in weight (by 4-6%; $P < 0.1$). With hypokinesia (group 3) bone weight was considerably lower than in the controls: the weight of calcanei amounted to 81.1%, and that of other skeletal bones 83-86% of control values ($P < 0.05$). Molar weights increased 17%, while incisors remained unchanged. TCT injection into animals kept hypokinetic (group 4) led to an increase in weight for the lower extremity, vertebral, and parietal bones, relative to the same bones in "pure" hypokinetics. A particularly noticeable effect on the animals in this group was obtained in the tibiae and vertebrae, the weights of which were the same as in the controls.

The relative weight of mineralized tissues in free-moving rabbits increased relative to control values, depending upon the increase in absolute weight of bones and teeth without noticeable change in carcass weight. Relative bone weight in group 3 animals sharply increased: calcanei, femora, humeri, and maxillae -- 13 to 16%, tibiae, scapulae, parietals, and vertebrae -- 21 to 28%, molars -- 77%, and incisors -- 43% ($P < 0.01$). Calcaneal, femoral, tibial, and humeral weights in rabbits of group 4 differed only slightly from controls.

Ash content (content of ash in percents of tissue dry weight) for the free-moving rabbits did not change under the influence of TCT in all mineralized tissues under study, except for the parietal bone, where ash residue was 86.6% of control ($P < 0.05$). Interestingly, content of ash increased in the tubular bones of the extremities during hypokinesia (104.0-105.8% of control; $P < 0.05$). A tendency for ash /44 content to increase was noted in the calcaneus and vertebra (102% of control; $P < 0.01$), while in the facial and skull bones -- parietal and maxillary -- there were different alterations -- residual ash content decreased 5-10%. No changes were noted in the ash content of molars and incisors from these animals.

EFFECTS OF HYPOKINESIA AND TCT INJECTION

[Results are expressed in percents of injected isotope dose, taken as 100%, on Ca^{45} metabolism in the teeth and bones of rabbits ($M \pm m$).]

Mineralized Tissue	Group	Series of experiments I	II	III	IV
Molars	1	0,112±0,011	0,115±0,012	0,158±0,007	0,081±0,005
	2	0,129±0,009	0,138±0,006	0,140±0,008	0,074±0,00[illegible]
	3	0,095±0,010	0,186±0,021*	0,161±0,008*	0,084±0,00[illegible]
	4	0,122±0,017	0,136±0,009	0,161±0,011	0,078±0,00[illegible]
Incisors	1	0,065±0,004	0,061±0,006	0,080±0,005	0,020±0,001
	2	0,060±0,007	0,068±0,002	0,070±0,008	0,020±0,001
	3	0,049±0,009	0,038±0,016	0,080±0,011	0,017±0,002
	4	0,047±0,009	0,0790±0,007*	0,085±0,008	0,020±0,001
Scapula	1	0,317±0,011	0,291±0,024	0,433±0,040	0,118±0,006
	2	0,342±0,017	0,321±0,024	0,338±0,029	0,124±0,008
	3	0,238±0,013**	0,393±0,023*	0,625±0,043*	0,121±0,007
	4	0,270±0,007*	0,270±0,016	0,593±0,031*	0,105±0,003
Tibia	1	0,589±0,026	0,360±0,037	0,541±0,047	0,503±0,036
	2	0,915±0,049**	0,293±0,029	0,421±0,025	0,465±0,023
	3	0,441±0,023**	0,446±0,062	0,559±0,037	0,392±0,019*
	4	0,979±0,130*	0,426±0,106	0,557±0,036	0,446±0,024
Calcaneus	1	0,137±0,008	0,158±0,015	0,516±0,059	0,180±0,013
	2	0,177±0,066	0,161±0,021	0,419±0,046	0,132±0,010*
	3	0,049±0,020*	0,118±0,016	0,402±0,075	0,145±0,014
	4	0,092±0,030	0,160±0,007	0,493±0,021	0,187±0,018

N.B.: Values of P are cited at reliable divergences of data in experimental and control:
*$P < 0.05$
**$P < 0.01$

In all tissues under study (teeth and bones) of rabbits kept hypokinetic and given TCT, ash residue content increased relative to that observed with "pure" hypokinesia. The ash content of weight-bearing skeletal bones correspondingly increased to 106-109% of controls ($P < 0.05$), while the maxillary and parietal bones reached control values.

The radiometric studies indicated that TCT injection leads to an increase in Ca^{45} uptake (series I) in the molars, vertebrae, scapulae, calcanei (108-129% of control values) and particularly in the tibiae (155% relative to controls; $P < 0.01$). Contrary changes occurred in the femora, parietals, maxillae, and incisors, in which isotope uptake weakened. In the rabbits of group 3, the capability of mineralized tissues to assimilate Ca^{45} was sharply reduced. The extent of these changes depends upon the typological features of the tissues under study and their functional condition; in the bones of the lower extremities (calcanei, femora, and tibiae) which were kept practically motionless, depression of isotope uptake was more pronounced (36, 75, and 61%/45 of controls; $P < 0.01$) than in the maxillary bones and molars (92-85%; $P < 0.05$), where functional load corresponded to the norm. Nonetheless, the reduction in Ca^{45} uptake in molars, maxillae, and especially in the incisors and parietal bones (75 and 65% of control values) is quite significant, in that it is evidence of generalized alteration in calcium metabolism for the entire skeleton. The mineralized tissues of the TCT rabbits in group 4 incorporated more Ca^{45} than those in the "pure" hypokinetics. This mainly refers to the lower extremity bones, which were most strongly affected by hypokinesia, and the parietals. Uptake of Ca^{45} in these bones for group 4 rabbits exceeded that of the respective ones in group 3 by 88, 39, 22, and 78% ($P < 0.01$). There was a similar trend in the molars and maxillae, but Ca^{45} uptake in the humeri of group 4 rabbits was considerably lower than in group 3.

In series II it was shown that injection of TCT into rabbits of group 2 led to accelerated resorption of the Ca^{45} given 24 hours before the beginning of the experiment, in the maxilla, humerus (isotope content amounted to 88 and 79% relative to controls) and particularly in the

femur (71% of control; $P < 0.05$). Isotope content in the teeth and scapulae, on the other hand, increased (by 10-20%; $P < 0.05$), and did not change in the remaining tissues. In group 3 animals, isotope content in the bones of the extremities decreased considerably, amounting to 59, 63, and 75%, respectively, of control values ($P < 0.05$) in the femora, calcanei, and humeri. Ca^{45} resorption also increased in the incisors and vertebrae. Contrary changes were noted in the molars, scapulae, and maxillae: isotope content increased to 162, 132, and 135%, respectively, relative to controls ($P < 0.05$). The resorption rate in rabbits of group 4 decreased (in comparison with group 3) in those tissues in which isotope resorption was elevated in response to "pure" hypokinesia (in the calcanei, humeri, vertebrae, and incisors). On the other hand, where isotope resorption decreased during hypokinesia (in the tibiae, scapulae, maxillae, and incisors), TCT injection accelerated resorption. The exceptions were the femora, in which isotope content did not alter under the influence of TCT.

In series III, resorption of Ca^{45}, given 30 days before the beginning of TCT injection, from all tissues under study intensified, particularly in the bones of the lower extremities (by 20-30%; $P < 0.05$), but less significantly so from the other osseous tissue and teeth (by 7-12%). As a result of hypokinesia (group 3) in the animals of this series of experiments, the Ca^{45} resorption rate decreased in all tissues under study except the calcanei and femora, in which isotope content was reduced somewhat ($P > 0.1$). With TCT injection into rabbits of group 4, calcium metabolism indices had a tendency to normalize in those tissues in which decelerated Ca^{45} resorption was noted during hypokinesia (except in the incisors and parietals).

In series IV, TCT injection into rabbits of group 2 increased resorption of Ca^{45}, given 60 days before the beginning of the experiment, in the femora, parietals, and especially in the calcanei (73% relative to controls; $P < 0.02$). The increased isotope resorption in the vertebrae, molars, and tibiae (7-8%) was unreliable. There were no changes in other tissues. Hypokinesia resulted in increased Ca^{45} resorption in the incisors, maxillae, vertebrae, and lower extremity bones (74-90% relative to controls; $P < 0.05$). There were no changes in the humeri,

parietals, and scapulae. TCT injection into rabbits of group 4 elevated Ca^{45} content in those tissues in which isotope resorption increased under the influence of hypokinesia.

Hence, it has been established that hypokinesia in rabbits leads /45 to cessation of animal growth and a sharp lag in weight increase in skeletal bone tissues, including the maxillae. Assimilation of Ca^{45} from the blood by all mineralized tissues abruptly decreases here. This process occurs simultaneously with a considerable increase in extremity bone resorption rate of Ca^{45} injected 24 hours before hypokinesia, i.e., in the rapidly metabolized fraction of the bones, and accumulation of isotope in the molars, maxillae, and parietal bones also increases. This may be explained by its redistribution among assorted osseous tissue during hypokinesia, which corresponds to our previous data [2]. The resorption rate of Ca^{45} from the slowly metabolized fraction of mineralized tissues is lowered during hypokinesia, except in the bones of the lower extremities, which may be explained by more rapid renewal of their mineral components. These data confirm our previously expressed opinion [3] that accelerated resorption of calcium from the labile fraction and decelerated resorption from the stable fraction of osseous tissue occurs during hypokinesia.

When movement is unrestricted, TCT accelerates calcium resorption from osseous tissue in rabbits, with the exception, however, that during hypokinesia the preparation causes a considerable increase in calcium assimilation by osseous tissues, as a result of which, apparently, a negative calcium balance does not develop in the skeletal bones.

Injection of TCT into rabbits kept hypokinetic has a sharply normalizing effect on all indices characterizing calcium metabolism in osseous tissues.

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