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Studies of the Calcium Accretion Rate of Bone during Immobilization in Intact and Thyroparathyroidectomized Adult Rats

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In order to study the role played by the thyroid and the parathyroid glands in the development of osteoporosis induced by immobilization, 153 male adult Sprague-Dawley rats allocated in two groups were used. The animals of one group were thyroparathyroidectomized; those of the other group were left intact. Each group was then divided into two subgroups; in one the right hind leg of the animals were immobilized by an elastic adhesive bandage. The animals were observed for varying periods of time up to 16 weeks. Studies of bone mass and ^{45}Ca accretion rate of the right and left femur and tibia were performed in all animals. The results indicate that disuse osteoporosis occurs in rats in the virtual absence of the thyroid and parathyroid glands. The bone loss of the immobilized femur and tibia was less pronounced in thyroparathyroidectomized rats compared to intact rats. The uptake of ^{45}Ca in bone treated by immobilization as compared to the non-immobilized bone was found to be increased to the same proportion in thyroparathyroidectomized and in intact rats. The calcium-accretion rate of non-immobilized bone was lower in thyroparathyroidectomized than in intact rats. The observations indicate that in the rat the parathyroid and thyroid hormones influence the rate of bone turnover and thereby the rate of development of disuse osteoporosis.

Key words; ^{45}Calcium — Immobilization — Osteoporosis — Parathyroid glands — Thyroparathyroidectomy.

Introduction

The role of the parathyroid glands in osteoporosis has been dealt with in several earlier investigations. At least in disuse osteoporosis opinions about their effect are somewhat controversial. In 1967, Burkhart and Jowsey reported that no signs of disuse osteoporosis developed in dogs after prior resection of their parathyroid or/and thyroid. Later, however, it was stated that no difference occurs in the development of disuse osteoporosis between intact and parathyroidectomized rats (Ziegler et al., 1973). Partial protection against disuse osteoporosis by parathyroidectomy in rats was reported by Conaway et al. (1973).

Earlier studies provided evidence of increased calcium accretion rate in bone developing disuse osteoporosis (Heaney, 1962; Karmosh and Saville, 1963). This pattern might be altered, reflecting disturbed bone metabolism, after thyroparathyroidectomy.

The present investigation was undertaken in order to study further the influence of thyroparathyroidectomy on the reactions of bone to immobilization.

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Method

A total of 153 male adult Sprague-Dawley rats divided in two groups were used in this investigation. At the beginning of the experiment, the animals were between 8 and 10 months old. Their mean body weights were 524.1 g in one group and 454.5 g in the other. The former group was subjected to thyroparathyroidectomy. Each group was then divided into two subgroups: (a) animals treated by immobilization of their right hind leg and (b) non-immobilized rats to serve as controls.

Eighty-one rats were thyroparathyroidectomized under ether anaesthesia. Ten rats died shortly after the operation, most of them because of respiratory failure. The criterion of successful parathyroidectomy was a total serum calcium level of 4.1 Eq/l or lower at sacrifice (Johansson and Segerström, 1972).

The right hind leg of 48 intact and 48 thyroparathyroidectomized rats was immobilized as described below. Immobilization of the rats of the latter started 3 days after thyroparathyroidectomy. Groups of 8 rats each were immobilized for 1, 2, 4, 8, 12 and 16 weeks, respectively. Groups of control non-immobilized animals, 23 thyroparathyroidectomized and 24 intact rats, were observed in groups of 3 or 4 also for 1 to 16 weeks, respectively.

With the rat fixed in a simple device, a strip of polyvinyl wool was applied around the pelvis and the right hind leg, which was then fixed between two layers of elastic adhesive bandage (Lindgren, 1974). Anaesthesia was not necessary for the procedure.

Isotope (calcium=45) was injected i.p. 72 h before killing the rats, the dose being 20 μCi ^{45}Ca in 1.0 ml physiological saline solution.

When the animals had been killed, the femora and the tibiae of both hind legs were thoroughly dissected free. The wet weight of all dissected bones was recorded using a Mettler balance with an accuracy of 0.05 mg. To determine the dry defatted bone weight, the bones were defatted and dehydrated in six changes of acetone for 3 days. Thereafter they were allowed to dry at 50° C for 48 h, and their weights were then recorded (Robinson, 1960). Bone powder was prepared from the dissected and dehydrated bone fragments and about 200 mg of the powder was subsequently ashed in a muffle furnace at 650–700° C for 16 h and then weighed.

A known quantity of whole bone ash, approximately 130 mg from each bone, was dissolved in 1.2 N HCl and the activity of each sample was determined in a Packard Tri-Carb liquid scintillation spectrometer. The activity was expressed as counts per min per mg ash.

Blood was collected at 6, 24 and 48 h after isotope injection and when killing the rats 72 h after administration of the isotope. Determination of serum Ca was made using a Unicam SP 90 atomic absorption spectrometer (Willis, 1961). The ^{45}Ca activity of the blood samples was determined as for the bone ash and expressed as counts per min per mg Ca in serum. The total amount of faeces produced after the injection of the isotope was also collected and the ^{45}Ca content was determined as for bone.

The calcium content of bone ash was taken to be 38.09%, according to the determinations made by Larsson (1969). The accretion rates, calculated by the method of Bauer et al. (1955), were expressed as mg Ca per h per mg Ca of bone.

Student's t-test was applied to the results.

Results

Ten rats died during the experimental period and 21 thyroparathyroidectomized rats were excluded because their serum calcium levels tended to be restored. Studies of body weight showed that intact non-immobilized rats did not change their body weights significantly during the experimental period. In the intact immobilized animals on the other hand, the weight decreased and reached an average loss of 10–15%. Thyroparathyroidectomized non-immobilized rats showed body weight reductions which averaged a maximum of 15% in the fourth postoperative week. Thyroparathyroidectomized immobilized animals reduced their body weights by about 25% in the 4–16 week groups.

The results from the investigation of bone mass and composition have been described in detail elsewhere (Lindgren, 1974). There was no difference in ash,

Table 1. Bone loss of the immobilized extremity as percentage of the values from the non-immobilized extremity

	n	Femur (mean SE)	Tibia (mean SE)
Intact immobilized rats	43	6.5±1.0 ($p<0.01$)	5.9±0.8 ($p<0.01$)
			$p<0.01$
Thyroparathyroidectomized immobilized rats	32	2.5±0.6 ($p<0.01$)	3.8±0.9 ($p<0.01$)

Table 2 Accretion rate of nonimmobilized bone (10^{-5} mg Ca/h × mg Ca)

	n	Left femur (mean ± SE)	Left tibia (mean ± SE)
Intact control rats	21	3.78±0.50	3.62±0.49
Intact immobilized rats	40	3.56±0.26	3.14±0.27
		$p<0.01$	
Thyroparathyroidectomized control rats	15	2.29±0.29	2.08±0.24
Thyroparathyroidectomized immobilized rats	25	2.38±0.30	2.07±0.25

i.e., inorganic content of the bone substance, between the bones from immobilized extremities and the corresponding bones from the non-immobilized extremity either in intact or in thyroparathyroidectomized rats. The same would then hold true for the contents of organic material, and in this respect the bone composition appeared not to be changed as a result of immobilization. However, the bone mass measured as the weight of dry defatted whole bone was significantly ($p<0.01$) lower in the bones of the immobilized than in the corresponding bones of the non-immobilized extremity, both in intact and thyroparathyroidectomized rats (Table 1). This difference increased with the time of immobilization. When the bone loss of the right tibia and femur together was compared between intact and thyroparathyroidectomized rats, the loss was significantly ($p<0.01$) less in the latter group. The wet weight of the bones was only little affected by immobilization. This might indicate that bone tissue resorbed because of immobilization had to some extent been replaced by fat and water, a condition also reported by Klein et al. (1968).

The 2-week group of thyroparathyroidectomized immobilized animals was excluded from the accretion rate analysis because of a technical error in the preparation of the samples. Another five rats were excluded because ^{45}Ca was suspected to have been injected in the gastrointestinal tract. A high ^{45}Ca activity in faeces and a very low serum level were observed in these rats.

The basic accretion rate as found in the bones from the non-immobilized leg in each group of animals is shown in Table 2. Consistently low values were obtained from rats in the thyroparathyroidectomized groups as compared with the corresponding values from the intact rats. When the mean accretion rate

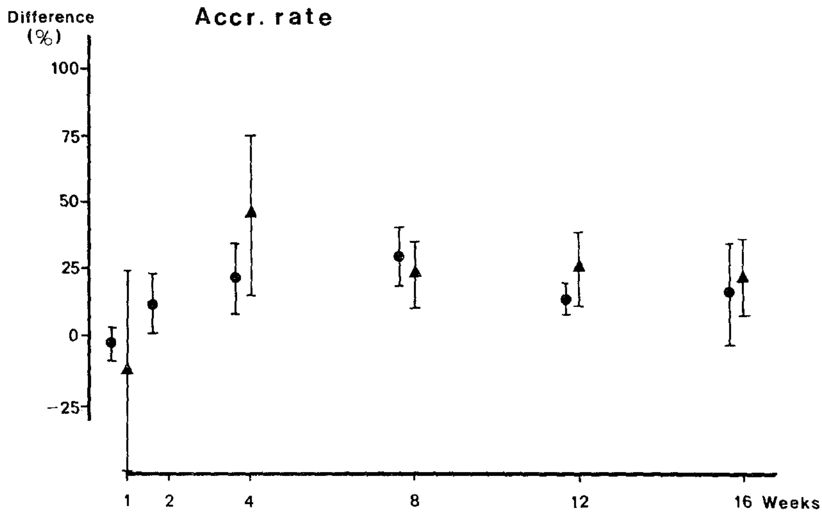

Fig. 1. Difference in accretion rate between right and left femur expressed as percentage of latter. Mean values ± SD, ⦶ Intact immobilized rats; ⊥ Thyroparathyroidectomized immobilized rats

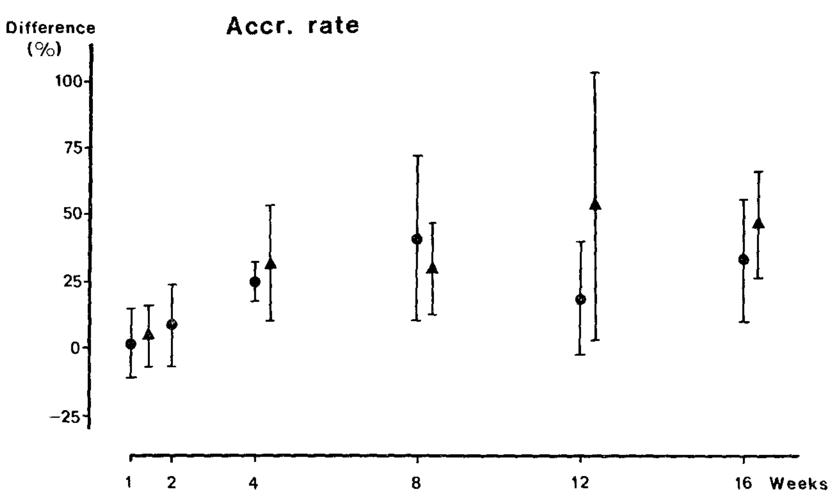

Fig. 2. Difference in accretion rate between right and left tibia expressed as percentage of latter. Mean values ± SD. ⦶ Intact immobilized rats; ⊥ Thyroparathyroidectomized immobilized rats

of the left femur and tibia together was compared between intact and thyroparathyroidectomized rats, the value was significantly lower in the latter group.

Increased accretion rate was noted in the right femur and tibia of the animals subjected to immobilization for 2 weeks or more. Compared with the corresponding bone of the non-immobilized extremity, the right femur in intact rats showed a significant ($p<0.01$) increase in accretion rate in the groups immobilized for 4–12 weeks, while the increase was almost significant ($0.01<p<0.05$) in the group immobilized for 2 weeks (Fig. 1). As for the right tibia, the increase was significant ($p<0.01$) in the 4 week group and almost significant ($0.01<p<0.05$) in the 8-week and 16-week groups (Fig. 2).

The same comparison in thyroparathyroidectomized rats showed, for the femur, a significant ($p<0.01$) difference in the group immobilized for 8 weeks; it was almost significant ($0.01<p<0.05$) in the groups immobilized for 4 and 12 weeks (Fig. 1). As for the tibia, the increase was significant ($p<0.01$) in the 8-week group and almost significant ($0.01<p<0.05$) in the 4-week group (Fig. 2).

Discussion

It is not certain what parathormone-producing tissue remains after thyroparathyroidectomy. It is known that, in rats, after the thyroid gland, ectopic parathyroid tissue is most often found in the thymus. However, this tissue is probably physiologically insufficient (Casewell and Fennel, 1969). Since parathyroid function and the serum calcium level are closely coupled, the hypocalcaemic state used as a criterion in the present investigation should indicate that only physiologically insufficient parathyroid tissue was left after the thyroparathyroidectomy.

The lower bone mass in the femur and tibia of the immobilized compared to the non-immobilized contralateral extremity is considered to indicate the presence and the severity of disuse osteoporosis (Lindgren, 1974). The finding that osteoporosis occurred in the thyroparathyroidectomized rats suggest that disuse osteoporosis and, hence, increased bone resorption can, although impaired, occur in the rat even in the absence of the parathyroids and the thyroid. Conaway et al. (1973) found similar results after parathyroidectomy.

Total bone formation may be measured by the uptake of radioactive calcium isotope by the bone, provided that resorption of the labelled bone and the so-called long-term exchange are negligible. Resorption of the isotope gives rise to falsely low values for bone formation. The long-term exchange, i.e. the exchange of calcium that takes place on preformed bone, excluding resorption, results in falsely high values for both formation and resorption (Harris and Heaney 1969). The error thus caused should be diminished by studies over a short period of time and by using closely related control material. Bauer and Carlsson (1954) reported that 72 h after the administration of a calcium tracer in rats, bone activity reaches a stable level.

Hypothyroidism and hypoparathyroidism have earlier been found to lead to lowered calcium accretion rates in bone (Heaney and Whedon, 1958; Dymling, 1966). This is regarded as a minifestation of the lower bone turnover in these conditions. The results of the present study are in agreement with these earlier observations.

A high calcium-accretion rate, ascribed to an increased bone formation, has earlier been observed during the development of dissue osteoporosis (Wendeberg, 1961; Heaney, 1962; Karmosh and Saville, 1965). The same condition was found both in the intact and the thyroparathyroidectomized immobilized groups of rats in the present investigation. Moreover, the percentage increase in the uptake of ^{45}Ca in the femur and tibia of the immobilized extremity and the corresponding bones of the non-immobilized extremity was not lower in the thyroparathyroidectomized than in the intact rats. It seems instead that, in this respect, proportionally the same reaction to immobilization occurred in both groups of rats. It is well known that bone resorption and formation are coupled mechanisms (Frost, 1963; Harris and Heaney, 1969). Increased resorption might consequently be assumed to be the primary cause of the increased accretion rate found after immobilization.

This evidence suggests the following sequence of events during the immobilization of an extremity in the adult rat. Disuse osteoporosis developes in the virtual absence of the parathyroid glands. Deficiency of parathyroid hormone and of thyroxine as well, suppresses the rate of skeletal turnover resulting in suppression of bone reaction to immobilization too. Accordingly the parathyroid glands play no permissive role in the development of the osteoporosis, but they rather moderate the rate of reactions of the bone.

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