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Periosteocyte Demineralization in Disuse Osteoporosis

The Effect of Calcitonin

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Summary. *We have investigated disuse osteoporosis in the calcaneum of the rabbit. Periosteocytic demineralization is an accurate indication of the degree of osteoporosis. It is influenced by the degree of skeletal maturity and the administration of calcitonin.*

Résumé. *L'étude des paramètres classiques de l'ostéoporose (ostéogénèse, résorption, volume absolu osseux et volume trabéculaire osseux), nous a permis dans un premier temps de nous assurer que nous disposions d'un modèle expérimental satisfaisant. Puis nous avons analysé la valeur de la mesure de la déminéralisation périostéocytaire dans l'étude de l'ostéoporose d'immobilisation. La déminéralisation périostéocytaire est différente suivant le degré de maturation squelettique et elle est freinée par l'administration de calcitonine.*

Key words: *Disuse osteoporosis, Periosteocytic demineralization, Calcitonin*

Prolonged immobilisation leads to a rapid onset of osteoporosis. The biological processes involved in this bone atrophy are not yet clear. The changes in intraosseous blood flow and in the balance between bone formation and bone resorption have been the subject of many previous experimental studies [6, 12, 19, 26, 27, 31, 34, 39, 40 41]. Little work has been done on the role of the osteocytes in osteoporosis, although Krempien has studied their activity during disuse atrophy using the electron microscope [28].

We have analysed the changes present in osteoporosis produced experimentally in the os calcis of the rabbit. Histological studies of the activity of the osteoblasts and osteoclasts were undertaken and microradiographs of undecalcified sections of bone were analysed to measure changes in the size of the periosteocyte lacunae. The affect of the administration of calcitonin on these measurements was assessed.

Material and Methods

Twenty "Fauves de Bourgogne" rabbits were used; thirteen were aged 18 months and skeletally mature and seven were aged 6 months and immature. Nine mature animals had no treatment and 4 were treated with calcitonin. Three immature rabbits were not treated and 4 received calcitonin. Two rabbits, one mature and one immature, belonging to the same litters as the other groups served as controls, and were not immobilized, treated or operated upon. The average weight of the young rabbits was 2,750 g and the mature 4,120 g.

The tendo Achilles was exposed through a longitudinal posteromedial skin incision. It was then divided at its calcaneal insertion and at the fibromuscular junction with resection of 15 mm of the tendon. The foot was immobilised in flexion with an adhesive bandage. The tendon was only divided on the left side with the right serving as a control. The animals were sacrificed at 1, 2, 4 or 6 weeks after operation (Table 1). All received an intravenous injection of 50 mg/Kg of Terramycin 48 h before they were killed to provide fluorescent marking of

Table 1. Material

		Controls N.T., N.O.	Delay between operation and sacrifice (weeks)			
			1	2	4	6
Immature	NT	1	–	1	1	1
	TCT	–	1	1	1	1
Mature	NT	1	2	2	3	2
	TCT	–	1	1	1	1
		Total	22 rabbits			

NT: untreated controls
TCT: calcitonin treated
NT, NO: untreated, non-operated controls

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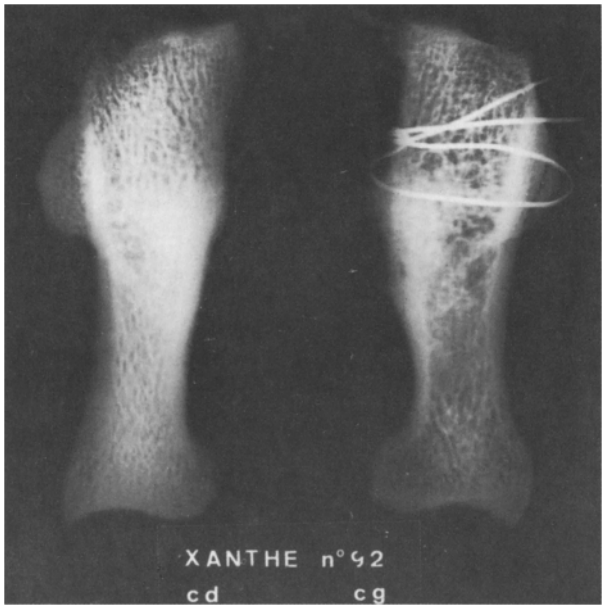

Fig. 1. Osteoporosis of left calcaneum. Radiograph 6 weeks after operation

the zones of osteogenesis [31, 39]. All animals treated with calcitonin received an injection of ½ unit MRC of porcine preparation daily from operation until death.

Qualitative Study [23, 25] (Fig. 1)

After sacrifice a radiograph of both calcanei was taken on the same plate. The control side was allocated a level of density of 5 and a comparative score assessed for the porotic side by two independent observers in double blind conditions.

Histological Study [18, 29, 30, 35]

Histological studies were carried out on undecalcified sections. After fixation in 80% ethanol the bone samples were set in methyl methacrylate, cut on a Bronwill saw and ground to a thickness of 50 μ (± 2). Microradiographs were then taken on Kodak Maximum Resolution plates using a Machlett AEG 50 tube with a berylium window. The exposure time was 15 min at 5 milliamps and 10 kV. The plates were developed automatically so that the duration of each stage of development and the temperature were strictly controlled. The surface area of the lacunae and the bone volume were then measured.

The same sections were examined in ultra-violet light to assess fluorescence and the degree of osteogenesis, and similar preparations were stained in order to estimate the degree of osteoclasis.

Measurements

Absolute Bone Volume (ABV) and Trabecular Bone Volume (TBV). The Absolute Bone Volume is the total percentage of both cortical and cancellous bone present in the specimen and the Trabecular Bone Volume is the percentage of cancellous bone only. The Microvideomat allows an instant, accurate measurement of these factors.

Osteogenesis

The sections were examined with a linear graticule which facilitated measurement of the width of the fluorescent bands produced by Tetracycline marking [31, 32].

Osteoclasis

The degree of osteoclasis was measured on stained sections by means of a linear test graticule.

Mean Lacunar Area (MLA)

The variation of the osteocyte lacunae seen on the radiographs of non decalcified bone sections is proportional to the functional activity of the osteocytes. A change in the surface area of the lacunae reflects either periosteocyte demineralization or mineral deposition [1, 3, 4, 5, 10, 17, 24, 38].

Under precise technical conditions [13, 17] the microradiographs of the non decalcified bone sections permit an accurate measurement of the dimensions of osteocyte lacunae, but the absence of homogeneity of the osteocyte population requires a large number of measurements to be carried out for each specimen.

The thickness of the sections was such that there were numerous intact osteocyte lacunae. The superficial lacunae, which were damaged by grinding the surface of the sections, gave a hazy radiographic appearance and were therefore excluded from the count. With the microvideomat 500 lacunae were measured in each group, and 5 sections were analysed for each calcaneum. The mean lacunar area and histogram gave the distribution of the lacunae according to their size based on 2,500 estimations for each calcaneum.

Results

1. Qualitative Study by Macroscopic Radiology (Fig. 2)

In untreated immature rabbits there was a progressive reduction in bone density which in-

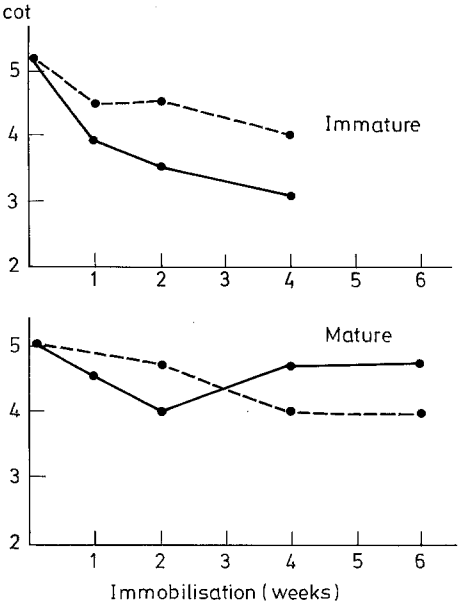

Fig. 2. Qualitative radiographic study. – Untreated rabbits; – – – Calcitonin treated rabbits

creased with prolonged immobilization, with final reduction of the initial bone density by half. In animals treated with calcitonin this decrease in density was less marked. In older rabbits the reduction in bone density was less pronounced and was not influenced by calcitonin.

2. Absolute Bone Volume and Trabecular Bone Volume

The ABV in the control rabbits which were not given calcitonin and did not undergo operation was 51.5% (Fig. 3). In untreated rabbits the ABV of the operated side fell rapidly during the first 2 weeks but then much more slowly until after 6 weeks of immobilization it measured 43%. However on the unoperated side the ABV increased progressively to 59%, probably because of compensatory overgrowth in relation to the increased function of the intact limb.

In rabbits treated with calcitonin the findings were similar but on the operated side the reduction of ABV was less marked, particularly after the second week.

The results of the calculation of TBV were parallel to the ABV indicating that bone rarifaction is generalized and affects both cortical and medullary bone alike.

3. Osteogenesis (Fig. 4)

There is a pronounced reduction in osteogenesis particularly in the first 2 weeks, with an estimation of about 30% below normal at the end of 6 weeks.

4. Osteoclasis (Fig. 5)

The rate of osteoclasis increased dramatically during the first two weeks to 13% greater than the controls. It then fell progressively although the rate still remained faster.

5. Mean lacunar area (MLA)

Figures 6 and 7 show the progressive increase in MLA with the duration of immobilization. Comparison between the calcanei of the operated and the control sides, together with the results from

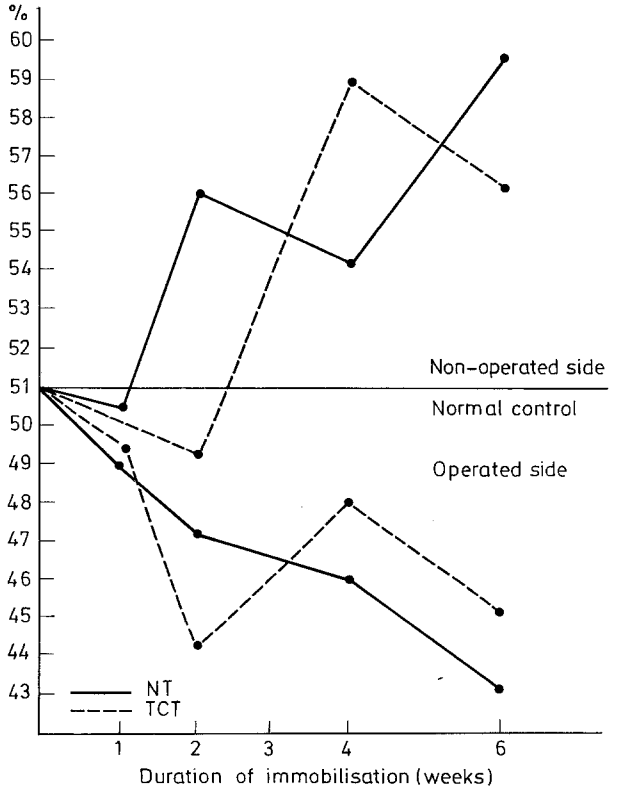

Fig. 3. Variation of A.B.V.

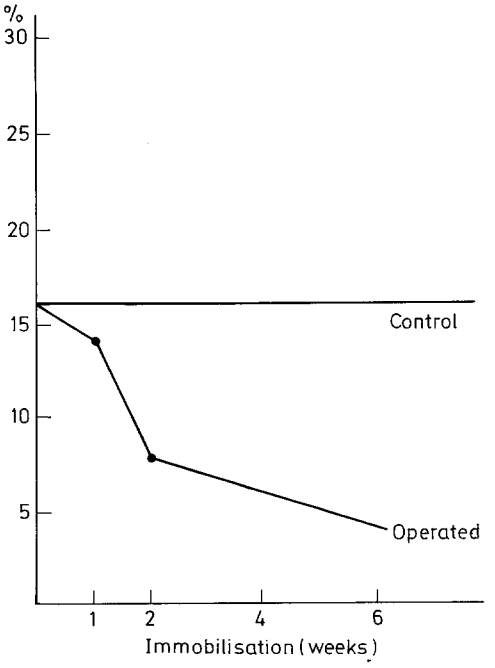

Fig. 4. Variation of osteogenesis in non-treated rabbits

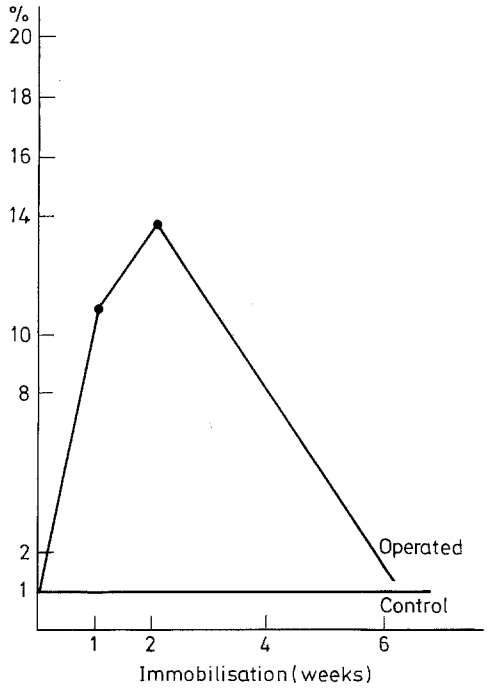

Fig. 5. Variation of osteoclasis in non-treated rabbits

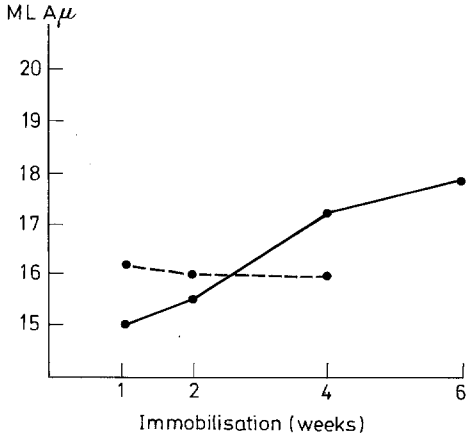

Fig. 6. MLA in the untreated immature rabbit. – Operated; – – – Control. Each point on the graph represents the mean of the measurements of between 5000 and 10000 osteocyte lacunae (500 lacunae per histological section, 5 sections per calcaneum)

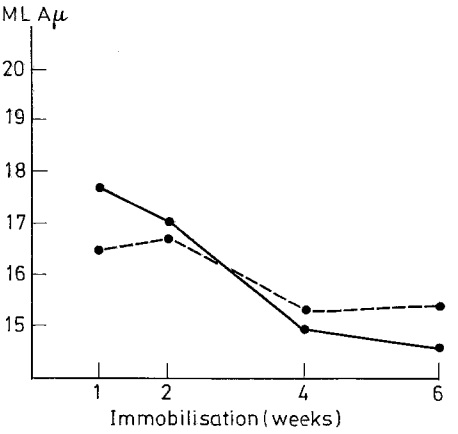

Fig. 7. MLA in the immature rabbit treated with calcitonin. – Operated; – – – non-operated. Each point on the graph represents the mean of the measurements from 5000 to 10000 osteocyte lacunae (500 lacunae per section, 5 sections per calcaneum)

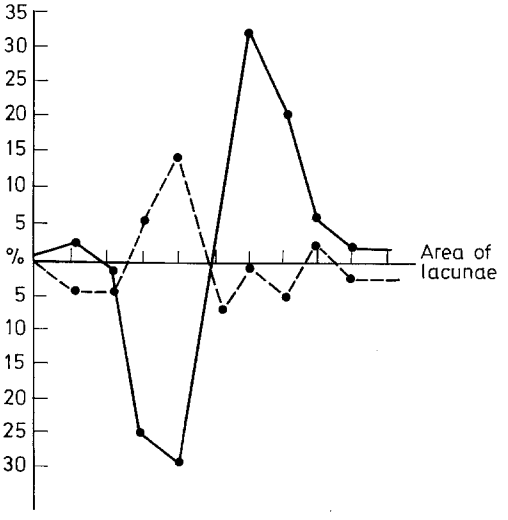

Fig. 8. Differential histogram comparing the percentages of lacunae of each size in the operated calcaneum and in the non-operated control side.

If the percentage of the lacunae of the same size were to be equal in the two calcaneal specimens, the differential histogram would coincide with the "x" axis. The black graph is beneath the "x" axis, for the lacunae between 5 u^2 and "y" u^2, shows that these lacunae are much less numerous on the operated side.

On the other hand, the slope of this same graph above the "x" axis shows that the surface is greater than "y" u^2; these lacunae are therefore more numerous on the operated side.

The same comparison in rabbits treated by calcitonin show that the lacunae of the same size are in equal proportion on both the operated and non-operated sides.

– NT: Non-treated rabbits

– – TCT: Calcitonin treated rabbits

the non-operated and non-treated controls, showed a clear variation in the distribution of the lacunae according to size, even for relatively small differences in MLA.

When the left calcaneum (operated side) and the right calcaneum (control) were compared in mature rabbits, untreated and treated with calcitonin (Fig. 8) the number of small lacunae was greatly reduced on the operated side compared with the contralateral side in those not treated while the large lacunae were increased in number. In the treated group the graph was distinctly flatter showing that the difference between the number of lacunae of the same size was reduced.

Comparison of the operated side in rabbits treated with calcitonin and those untreated (Fig. 9) showed a marked increase in the number of small lacunae with reduction in the large lacunae in the treated group. The effect was much more marked in mature rabbits.

The non-operated and non-treated control rabbits had far fewer small lacunae and more large lacunae than the control side of the operated rabbits. It appeared that in the latter group periosteocyte mineral deposition reduced the dimensions of the lacunae following the increased functional demands of the intact limb.

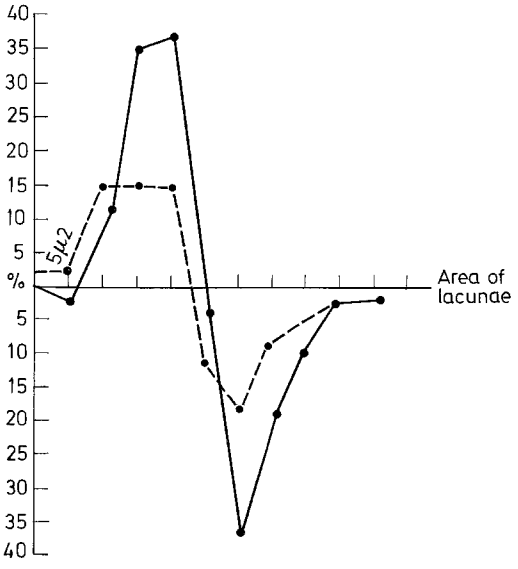

Fig. 9. Differential histograms comparing the percentage of lacunae of each size in the operated calcaneum of the treated rabbits and in the controls.

The shape of the graphs above the "x" axis give the size of the lacunae between 5 u^2 and "y" u^2 and shows that the small lacunae are more numerous in the treated rabbits than in the controls.

On the other hand, the shape of the graphs for lacunae with an area greater than "y" u^2 shows that large lacunae are less numerous in the treated animals.

These differences are more marked in immature rabbits *(black graph)* than in the mature rabbits *(red graph)*.

Mature – TCT/NT; Imature – – TCT/NT

Discussion

Previous investigation of disuse osteoporosis has included metabolic studies, the role of the thyroid and parathyroid during immobilization [2, 6, 8, 9, 22, 23, 25, 32, 39, 42], the demonstration of increased vascularity with increased metabolic activity and analysis of the imbalance between the deposition and resorption of bone [4, 12, 31, 34, 36]. The role of the osteocytes has recently become of interest and it has been shown that immobilization gives rise to fewer "small lacunae" and more "large lacunae", an increase in empty lacunae, and the appearance of periosteocyte lysis. Immobilization appears to increase osteocyte catabolic activity [4, 28].

The functional activity of osteocytes is well known [1, 3, 5, 15, 16, 24, 38] and the present study shows that they play an important role in the changes provoked by disuse osteoporosis. The measured variations of radiological density, the degree of osteogenesis and osteoclasis, and the measured ABV and TBV confirm that disuse osteoporosis has been achieved in the experimental model. The measurements made on microradiographs of undecalcified bone sections show an increase in the mean lacunar area during immobilization, with an increase in the numbers of large lacunae and a reduction in the small. These changes indicate periosteocyte demineralization, and are considerably modified by treatment with calcitonin.

The changes demonstrated on the non-operated calcaneum are secondary to increased functional demand and confirm the influence of physical activity on bone architecture.

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