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Using Frost's method for undecalcified bone sections, long bones of the lower extremities of ten rhesus monkeys were examined following two months' immobilization and compared with thirteen controls. A decrease in appositional rate and in the surface extent of the ossification process were noted in the immobilized animals. No typical change in resorption was noted. The immobilized animals showed a decreased cortical-total area ratio. These findings suggest that a decrease in activity affects bone by depressing functions mediated by the osteoblast without necessarily evoking an increased remodelling response.

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It has been demonstrated that loss of bony substance is associated with a decrease in physical activity.[1,2] Early studies regarding sudden decreases in musculoskeletal activity have revealed negative calcium balance and roentgenographic osteopenia, which were reversible with the resumption of activity. More recently, efforts have been directed toward defining the mechanism of these changes. The site of bone loss associated with inactivity was studied by a number of investigators. Roentgenographic studies pointed out the presence of a decreased cortical thickness[3,4] while other microscopically oriented studies pointed out the location of the loss as characteristically occurring adjacent to the periosteal and endosteal surfaces.[5,6] Other work attempted to further define the process of disuse osteopenia in terms of the balance between the resorptive and appositional processes. Some authors demonstrated a lag in the onset of calcium loss and roentgenographic osteopenia following decreased activity,[7,8] others found immediate effects.[9,10] To resolve some of these apparent paradoxes, the concept of a coupling of the resorptive and appositional phases of remodelling was advanced by Frost and coworkers,[11] based in part on the fact that virtually all remodelling units were bordered by a scalloped cement line, indicating an initial osteoclastic phase. Such a model allows one to consider effects which influence the birth rate of such remodelling units and, in addition, influence the rate and extent of completion of the formative phase.[12] Kazarian and Von Gierke introduced a model to investigate the effects of altered musculoskeletal activity in the

Table 1

Histologic Index Haversian	Mean S. D. S. E. M.	Tibia Control n = 9	Tibia Immob. n = 5	Femur Control n = 16	Femur Immob. n = 16	Fibula Control n = 10	Fibula Immob. n = 8
C/T, Ratio cortical/total area		0. 58 0. 11 0. 03	0. 49* 0. 04 0. 02	0. 43 0. 07 0. 02	0. 40 0. 05 0. 01	0. 89 0. 06 0. 02	0. 78** 0. 02 0. 01
A_f, No. osteoid seams/mm^2		1. 51 1. 04 0. 35	1. 26 0. 76 0. 34	2.98 1. 33 0. 33	1. 43** 0. 98 0. 24	1. 90 1. 18 0. 37	0. 95 1. 16 0.44
Ar, No. resorption spaces/mm^2		0. 26 0. 27 0. 09	0. 60 0. 43 0. 19	1. 28 0. 79 0. 20	0. 52** 0. 43 0. 11	0. 47 0. 40 0. 13	0. 67 0. 98 0. 49
M, Appositional rate, (microns/day)		1. 05 0. 26 0. 09	0. 96 0. 31 0. 14	1. 63 0. 32 0. 08	1.63 0. 30 0. 08	1. 14 0. 21 0. 07	0. 62 0. 58 0. 21
% L, of osteons labelled		64 18 6	49 21 9	86 12 3	82 13 3	91 7 2	60* 23 8

Remodelling parameters for the haversian system. One asterisk indicates a P value < .05 and two asterisks indicates a P value < .01. Standard deviation and standard error or the mean are shown below the mean.

Table 2

Histologic Index Endosteal	Mean S. D. S. E. M.	Tibia Control n = 7	Tibia Immob. n = 5	Femur Control n = 16	Femur Immob. n = 7	Fibula Control n = 10	Fibula Immob. n = 8
A_f, No. osteoid seams/mm		0. 43 0. 27 0. 10	0. 18* 0. 07 0. 03	0. 77 0.27 0. 07	0. 43** 0. 21 0. 06	0. 50 0. 27 0. 09	0. 30 0. 17 0. 06
M, Appositional rate (microns/day)		1. 07 0. 23 0. 09	0. 96 0. 35 0. 25	1. 54 0. 23 0. 06	0. 80** 0. 47 0. 13	0. 88 0. 32 0. 10	0. 67 0. 29 0. 10
% L, Percent perimeter labelled		53 35 13	13* 6 3	88 7 2	29** 23 6	59 34 11	32 24 9
% R, Percent perimeter resorbing		9 13 5	6 6 3	4 3 1	16** 9 2	11 7 2	7 6 3
% F, Percent perimeter forming		54 37 14	36 10 5	88 7 2	37** 26 7	59 34 11	56 36 13
Wall thickness (mm)		0. 119 0. 048 0. 020	0. 185 0. 017 0. 008	0. 072 0. 005 0. 001	0. 070 0. 009 0. 002	0. 079 0. 013 0. 004	0. 084 0. 012 0. 005

Remodelling parameters for cortical-endosteal surface. One asterisk indicates a P value < .05 and two asterisks indicates a P value < .01. Standard deviation and standard error of the mean are shown below the mean.

Table 3

Histologic Index Periosteal	Mean S. D. S. E. M.	Tibia		Femur		Fibula	
		Control n = 9	Immob. n = 5	Control n = 16	Immob. n = 16	Control n = 10	Immob. n = 8
A_f, No. osteoid seams/mm		0. 17 0. 21 0. 07	0. 04 0. 01 0. 01	0. 39 0. 11 0. 03	0. 25** 0. 15 0. 04	0. 20 0. 09 0. 03	0. 16 0. 11 0. 04
M, Appositional rate (microns/day)		0. 90 0. 27 0. 14	0. 0	0. 97 0. 35 0. 09	0. 44** 0. 36 0. 10	0. 80 0. 36 0. 12	0. 24** 0. 11 0. 04
% L, Percent perimeter labelled		23 20 7	0. 0	24 11 3	22 20 5	57 29 10	15** 14 5
% R, Percent perimeter resorbing		6 9 3	18 25 11	61 16 4	49* 15 4	25 21 8	36 19 7
% F, Percent perimeter forming		31 30 10	11 7 4	33 16 4	23 18 5	56 28 9	27* 18 6
Wall Thickness (mm)		0. 115 0. 059 0. 022	0. 117 0. 011 0. 008	0. 059 0. 009 0. 002	0. 058 0. 013 0. 003	0. 074 0. 033 0. 010	0. 064 0. 012 0. 004

Remodelling parameters for the periosteal surface. One asterisk indicates a P value < .05 and two asterisks indicates a P value < .01. Standard deviation and standard error of the mean are shown below the mean.

rhesus monkey,[13] and scaling factors have been utilized to relate monkey data to humans.[14] The similarity of the monkey skeleton to that of the human aids such correlation. The current study is undertaken as part of a larger effort in which biochemical, biomechanical and histological parameters of disuse osteoporosis are being investigated.

Experimental Design

The histological material was obtained for 23 male rhesus monkeys located at the primate laboratory at Wright-Patterson Air Force Base, Dayton, Ohio. All animals had been captured in their wild state. The time in captivity ranged from 22 to 35 weeks. The animals were all of a late adolescent age with closing long bone epiphyses. They were maintained in cages measuring 91 X 91 X 122 cm. The animals were examined frequently and were demonstrated to be disease free prior to the initiation of the study. They were fed commercial monkey chow and water in optimum amounts based on weight. Thirteen control animals were labelled with tetracycline and sacrificed. Ten monkeys were immobilized in a total body plaster cast in a sitting position with the extremities partially flexed. They evidenced minimal discomfort during the immobilization period and did not lose weight. The animals were labelled with tetracycline prior to the end of the eight-week immobilization and then sacrificed. Those selected for histologic analysis were designated on the basis of random, non-systematic factors including suitability of bone following biomechanical testing and effectiveness of staining techniques which in some instances precluded accurate measurement of histologic parameters. Eighty

per cent of the monkeys included in the study contributed bilateral specimens.

Technical Methods

Using a fine jeweler's saw or Bronwill's thin sectioning machine, bone sections were cut measuring 1 mm in thickness at a location 3 cm from the distal end of the bone. These slabs of bone were hand ground to a thickness of approximately 100 micra, stained with the Villanueva bone stain for 48 hours, reground to remove surface stain, differentiated, dehydrated, cleared and mounted permanently in Eukitt's mounting medium. Three sections per specimen were mounted on a slide, and all were utilized in determining the microscopic remodelling parameters.

Primary parameters for the haversian, cortical-endosteal, and periosteal surfaces were derived as follows:

A) Haversian surface

1. C/T, cortical-total area ratio. The cortical area is divided by the total area (cortical plus marrow cavity area).

2. A_f, number osteoid seams/mm² bone. The number of osteoid seams in the total section is divided by the cortical area.

3. A_r, number resorption spaces/mm² bone. The number of scalloped resorption spaces is divided by the cortical area.

4. M, appositional rate (microns per day). The average distance between tetracycline labels is divided by the number of days between labels.

5. % L, percent labelled systems. The number of osteoid seams bearing a tetracycline label is divided by the total number of osteoid seams.

B) Cortical-Endosteal and Periosteal Surface

1. A_f, number of osteoid seams/mm perimeter. The number of osteoid seams on the perimeter is divided by the length of the perimeter.

2. M, appositional rate (microns per day). The average distance between tetracycline labels is measured, and divided by the number of days between labels.

3. % L, percent perimeter labelled. Measured directly.

4. % R, percent perimeter resorbing. Measured directly.

5. % F, percent perimeter showing osteoid seam. Measured directly.

6. Wall thickness (mm). The average distance from the cement line to the periphery is measured.

Data

The values for C/T show a decrease for all three bones, significantly in two of the three bones (Figure 1). % F shows a consistent decrease for both the periosteal and cortical-endosteal surfaces. In two cases the decreases were statistically significant (Figure 1). The number of resorption spaces per mm² of haversian bone showed no characteristic alteration. (Figure 2) Likewise, percent perimeter resorbing on the cortical-endosteal and periosteal surfaces showed no characteristic alterations, with values in some instances increasing and in other instances decreasing (Figure 2). Percent labelling for all three surfaces showed a uniform decrease, significant in several instances. No label was deposited on the periosteal surface of the tibia in the experimental group (Figure 3). A_f showed a consistent decrease in the experimental group for all three surfaces and in several instances the decrease was statistically significant (Figure 4). The appositional rate for all three surfaces likewise showed an almost uniform decrease in the experimental group, most pronounced on the periosteal surface. Several of the comparisons were statistically significant (Figure 5). The periosteal and cortical-endosteal wall thickness did not show a characteristic change. The P values indicated are obtained by use of the "two tailed" t test.

Discussion

The value obtained for M, the appositional rate, is an example of an interesting overall trend. The most striking change after immobilization is seen in the periosteal envelope. Decreases to a lesser degree are seen in the cortical-endosteal and haversian

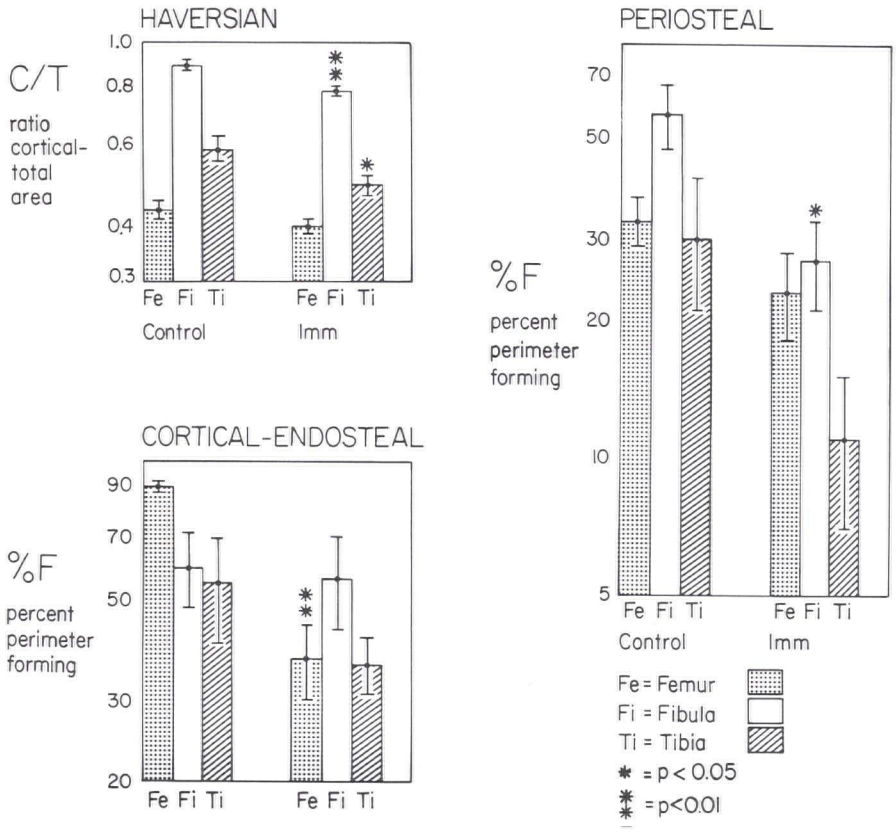

Figure 1
Semi-logarithmic histograms for the ratio of cortical/total area, percent forming perimeter for cortical-endosteal and periosteal surfaces in control and immobilized groups. Standard error of the mean and P values are indicated.

envelopes. We note in general that effects appearing throughout the bone cross sections are most pronounced on the periosteal surface. The parameter A_f shows a decrease with immobilization for all three surfaces. Given a decreased appositional rate, as seen above, and with other factors held constant, one would expect an increase in the number of osteoid seams due to their prolonged life span. The fact that the number appears to be decreasing causes us to speculate as to the possibility of a premature cessation of growth in some osteons associated with inactivity. Similarly, % L and % F show a decrease in bone formation on all three surfaces. The values for these four parameters seem to indicate a general decrease in osteoblastic function in the immobilized animals, manifest by a decreased rate of progression of the ossifying front, a possible premature cessation of osteoid deposition, and a decrease in the extent of mineralization as defined by sites of tetracycline uptake.

The parameters A_r and % R show no overall trend when comparing immobilized to control values. If the osteoporotic response to immobilization were one of increased generation of new remodelling units, one would expect an increase in the number of resorption centers provided the

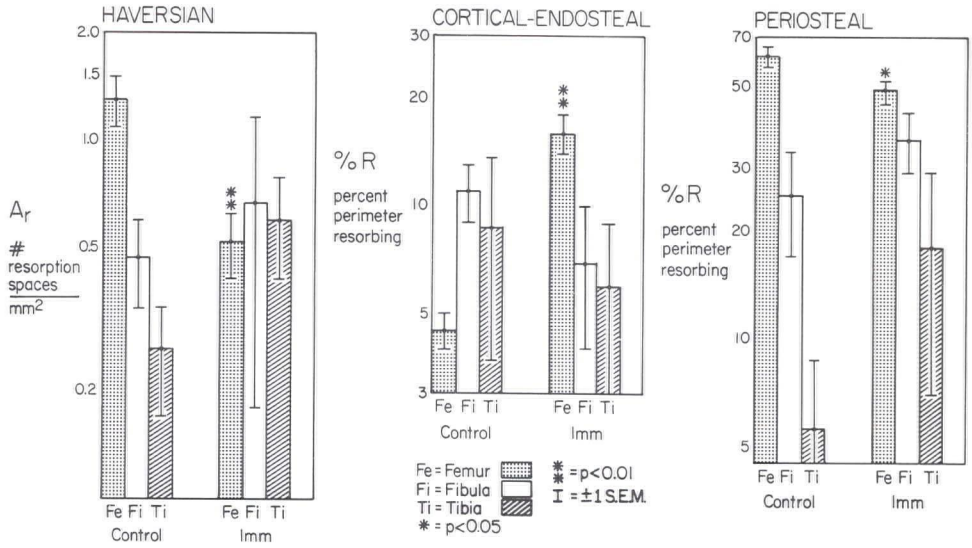

Figure 2
Semi-logarithmic histograms of values for A_r in the haversian system and % R for the cortical-endosteal and periosteal surfaces for control and immobilized groups. Standard error of the mean and P values are indicated.

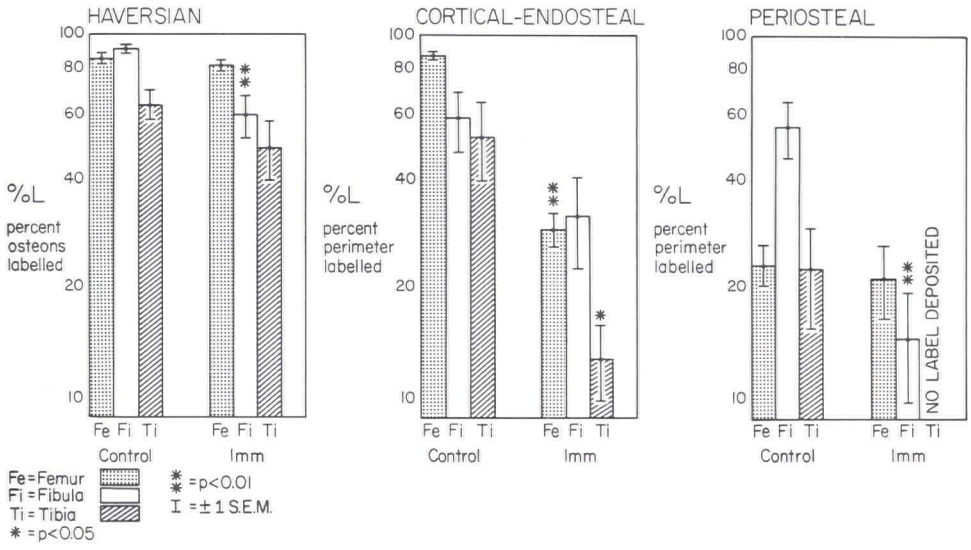

Figure 3
Semi-logarithmic histograms of values for % L in control and immobilized groups for all three surfaces. Standard error of the mean and P values are indicated.

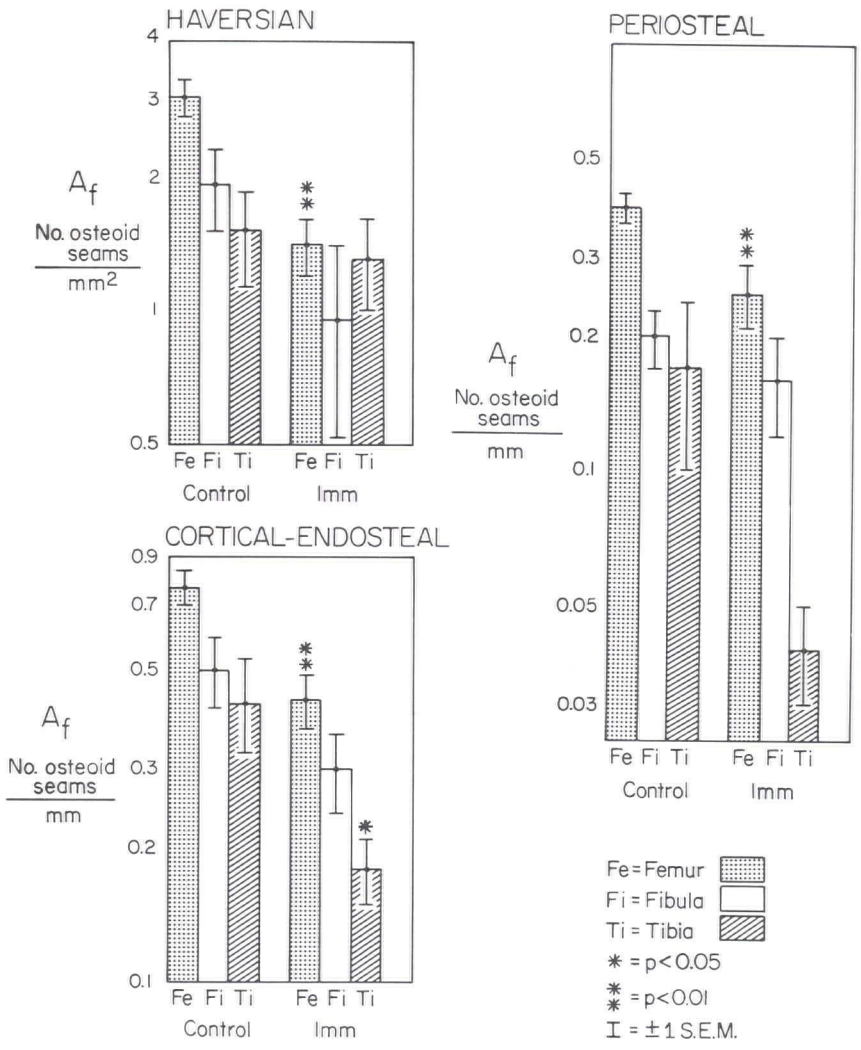

Figure 4
Semi-logarithmic histograms of values for A_f in control and immobilized groups for all three surfaces. Standard error of the mean and P values are indicated.

stimulus were to continue through the two-month study period. That the resorption parameters did not show any characteristic change suggests that the response to decreased activity is via an effect on osteoblastic function rather than through changes in the overall remodelling rate.

The cortical to total area ratio is decreased in the femur and fibula and to a lesser degree in the tibia. This may be partly accounted for by a decreased rate of completion of existing remodelling units, and a decreased percentage of total completion of these units on the periosteal and cortical-endosteal surfaces causing a net bone deficit if resorption is assumed to be constant. A change in duration and depth of the resorptive phase of remodelling might also contribute to this effect, but we have no data

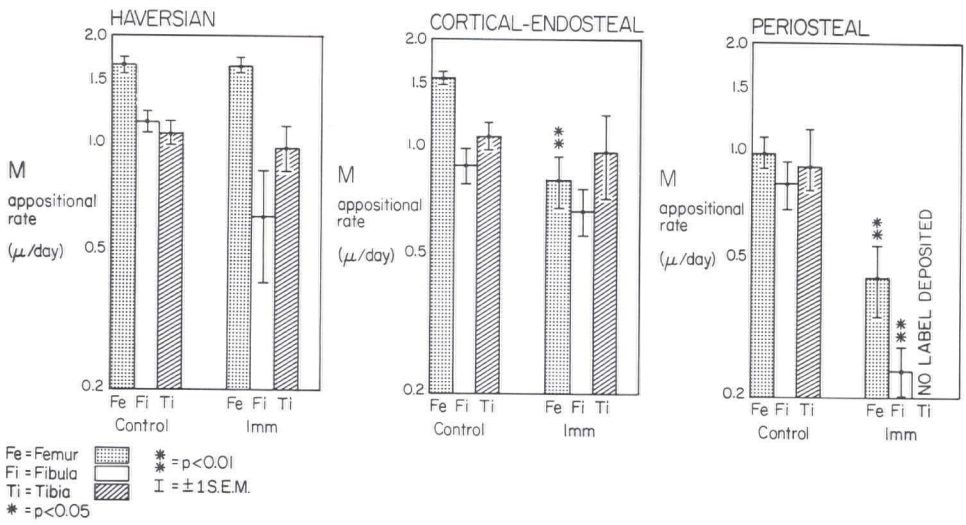

Figure 5
Semi-logarithmic histograms of appositional rate values for control and immobilized groups for all three surfaces. Standard error of the mean and P values are indicated.

regarding this. Measurement of wall thickness might ultimately give information concerning this issue, if measurements could be made in a situation in which transients have been eliminated. We saw no characteristic change in wall thickness. Others have demonstrated large resorption areas in the immediate sub-periosteal region of the bone.[5] These large resorption spaces could undermine the outer cortex and be represented as periosteal data by our method. Since there is not an increased percentage of resorbing surface, we conclude that a change in the overall tissue level remodelling rate is not primarily responsible for the changes observed.

Our interpretation is that the changes observed in conjunction with immobilization involve osteoblast-mediated activities and are most pronounced on the periosteal surface. Since the mechanical effects of torsion and bending are greatest in the subperiosteal bone, we are led to speculate regarding a direct influence of mechanical stress on the osteoblast. Such an effect is consistent with observed bone remodelling on a gross anatomic level as a response to mechanical demand.

Summary and Conclusions

Long bones of the lower extremities of rhesus monkeys were examined as controls or after two months of total body immobilization. Compared to control values, the immobilized animals showed a decrease in the appositional rate, a decrease in the prevalence of osteoid and of tetracycline labelling, and a decrease in cortical to total area ratio. We found no consistent change in the percentage of surface involved in

resorption. The changes were most pronounced in the periosteal envelope, slightly less prominent in the cortical-endosteal envelope and least prominent in the haversian envelope. These changes suggest the possibility that mechanical stress plays a role, as they are greatest in the sub-periosteal bone. We suggest that such an effect could be directly via the osteoblasts, manifested as a decreased appositional rate, a contracted osteoid surface and a diminished breadth of the mineralizing front as defined by tetracycline labelling. It is possible that this decreased cellular activity could contribute to the observed reduction in the C/T ratio, but it appears that other factors would have to contribute to explain the magnitude of this reduction. Our data shows no increase in the number of resorptive centers or in the percentage resorptive surface, and we therefore do not postulate an increased overall remodelling rate.

To delineate more completely the cellular response to inactivity, further studies with biopsies at various intervals may be helpful. If appositional rate and breadth of the ossification process are directly correlated with mechanical stress, it would be possible to explain bone remodelling by a simple mechanism. These studies also continue to emphasize the importance of utilizing methods which measure rate as well as percent of surface involvement in the remodelling processes.

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