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THE EFFECTS OF IMMOBILIZATION
AND RECONDITIONING ON REMODELLING
IN THE RHESUS MONKEY RIB

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Abstract

50 rhesus monkeys were experimental subjects in an effort to determine the effects of immobilization and reconditioning upon the quantitative remodelling dynamics of bone as measured by rib biopsies. Biopsies were obtained pre-immobilization, post-immobilization, and at three, six and twelve months post-release from immobilization. Sections of the rib were analyzed according to Frost's method following tetracycline labelling, coupled with Villanueva Bone stain. Mean values for all measured parameters in each of the experimental phases are contained in the body of the report. The following are those values which are considered to indicate significant trends in the data which have led to the conslusions indicated at the end of the report.

(I) Haversian system a) number of osteoid seams increased post-immobilization and during the reconditioning phase. b) appositional rate increased in the six and twelve month reconditioned groups. c) C/T ratio decreased maximally in the three month reconditioned group. d) number of resorption spaces and percent of seams labelled increased in the six month reconditioned group.

(II) Endosteal system a) percent perimeter resorbing showed maximal decrease three months post-immobilization and then returned to normal in the twelve month reconditioned group. b) appositional rate rose slightly in the six and twelve month reconditioned group. c) number of osteoid seams per mm, percent perimeter

forming, and per cent labelled perimeter all decreased post-immobilization but returned to normal values for the entire reconditioning period. (III) Periosteal system a) per cent perimeter resorbing progressively decreased to minimum values six month post-immobilization and then returned to normal twelve month post-immobilization. b) appositional rate rose slightly in the six month reconditioning group. c) number of osteoid seams per mm, per cent perimeter labelled, and per cent perimeter forming all showed a marked decrease immediately post-immobilization, returned to normal values in the three month reconditioned group and showed higher than normal values in the six month reconditioned group, finally returning to normal values in the twelve month reconditioning group.

We have previously concluded that all remodelling is composed of an initial resorptive phase followed by a formative phase. This current data indicates to us that the greatest immediate effect of immobilization is shown in the periosteal and endosteal parameter values. Periosteal and endosteal surface percentages of resorption and formation are both depressed immediately post immobilization suggesting a slowing in the rate of generation of surface remodelling units, and in the rate of transformation of these remodelling units from the resorptive to the formative phase. The formative phase may also be slowed or prematurely aborted with immobilization. The cortical to total ratio decreases slightly post-immobilization and shows a further decrease in the three month reconditioned group indicating the continuation of the resorptive phase in the remodelling unit. The fact that the bone forming parameters

are normal three months post-immobilization in the face of a further decrease in cortical to total ratio at that time suggests to us that the depressive effects of immobilization may have continued possibly two to three months into the reconditioning period. The fact that the percentage of resorbing surface is further decreased in the three month reconditioned group possibly indicates a shorter duration of the resorptive phase of the basic remodelling unit as a result of the increase in bone loading. The increase in the number of resorption spaces per mm^2 and the increase in the appositional rate seen in the haversian system in the six month reconditioned group may indicate a delayed remodelling response to remove fatigued areas of cortical bone which are present as a result of the superimposition of activity on an abnormally thinned cortex.

This study indicates that significant abnormalities in bone remodelling persist for at least six months into the reconditioning phase and that caution must be used before subjecting an organism to further stress during this period of time. Extrapolated to larger organisms such as man, this period of abnormality may extend for more than six months. The fact that an immobilization-like effect is observed in the bone two to three months into the remobilization phase indicate that further investigation may be in order to ascertain optimal methods of gradually increasing bone loading so as to speed transformation from a bone resorbing phase to a bone forming phase.

This data is to be correlated with mechanical and biochemical data obtained on the same monkeys, from which more specific and further readings overall conclusions are anticipated.

Final Report

The Effects of Immobilization and Reconditioning on Remodelling in the Rhesus Monkey Rib

Introduction

The overall purpose of this study has been to observe the histodynamics of the periosteal, endosteal and haversian surfaces of the monkey ribs before and after a period of two months immobilization in a plaster of paris cast. The first year of study had established complete histodynamic parameters of bone remodelling in rhesus monkeys maintained under specified conditions prior to any experimental procedures. These same monkeys were then subjected to 2 months of cast immobilization followed by a period of reconditioning. Data has been obtained from various times during the pre-immobilization and re-mobilization period. The data shows a rather interesting sequence of events in a number of the parameters at various times during the experimental sequence. These findings are now in the process of being correlated with changes in mechanical properties in the bones of these same monkeys with respect to time as determined in the Biomechanics Laboratory at Wright-Patterson Air Force Base, Dayton, Ohio. Scaling factors have been determined whereby the monkey data could be useful in predicting human response to similar conditions.

In the Calcified Tissue Laboratory, Henry Ford Hospital, Detroit, Michigan we have previously become experienced in interpreting quantitative and qualitative changes in bone which result from various metabolic bone diseases and

measured the remodelling parameters associated with these conditions. It is apparent that activity related factors as well as metabolic determinants are important in determining the remodelling condition of the bone at a particular time. The remodelling conditions in turn affect its mechanical behavior. We therefore possess a method which has been quite valuable in the elucidation of problems of bone metabolism, and plan to use this method in the possible explanation of observed biomechanical changes under various conditions of activity, mechanical stress and altered gravity.

Of interest is the fact that bone can be used as a tool, or a model system, to study various conditions. We believe that bone contains much useful information relating to diabetes, conjestive heart failure, hepatic cirrhosis, disuse osteoporosis and accompanying injury, malnutrition with respect to calcium and protein, and many more situations related to genetic factors. Our method is basic to the diagnosis of osteomalacia, and the effectiveness of various therapeutic regimens used in its treatment. Recent preliminary studies have demonstrated the use of our method in the quantitation of bone changes due to an altered state of musculoskeletal activity. We have been involved in such studies of bone remodelling in human and canine ribs and more recently in the rhesus monkey. Basic to our attempts to understand the remodelling behavior of the skeletal system is comparison of remodelling of one part of a given bone to another part. Difference between various bones also exists. We have found that there is no

general rule that can be applied to every bone site. We have included the evaluation of the endosteal and periosteal surfaces of the rib in our study even though the technique and interpretation of the results are new compared to those of the haversian surface. With increasing experience with the endosteal and periosteal surface parameters we have become more confident with our interpretation of this data. With some minor modifications, these methods may be used to solve problems of human significance.

In examining multiple bones including the ribs we know that the remodelling level may vary from human to human and monkey to monkey. However the relationship of these values of various anatomical sites to one another appear to be relatively constant, despite individual differences and absolute value. The rib is used here as a model because it is most active and it has all the surfaces involved in the process of remodelling. Other bones such as metatarsals, humerus, fibula, femur and tibia have been studied under a separate project. We also note some variation in the remodelling activity according to age in the human and in the monkey. There is a systematic change with age in the structure of bone at any given level so that establishing age specific parameter normals of these 3 surfaces will also be of great importance.

The first year of study in this project involving the establishment of periosteal, endosteal, and haversian parameters for 50 monkeys have established a backdrop against which the subsequent remodelling patterns

of the rib and the experimental situation may be evaluated. At the end of the second year of the project the parameters for the post-immobilization group of monkeys had been completed, and currently, at the end of the third and final year of the projects, values are available for the periods of three, six and twelve months post-release from immobilization.

Experimental Design

50 rhesus monkeys in the primate facilities of Wright-Patterson Air Force Base, Dayton, Ohio were selected for use in the experimental sequence. Following tetracycline labelling, rib biopsies were obtained from these monkeys prior to immobilization. The results of the histologic examination were represented in the first year report of this study, which included remodelling parameters for the haversian and endosteal and periosteal surfaces of the bone as determined by Frost's method. The monkeys were then immobilized in a quadruple body spica for a period of 2 months. During the immobilization period, complete biochemical data have also been obtained from these monkeys under the direction of Dr. Frank Noyes at Wright-Patterson Air Force Base, Dayton, Ohio. Shortly after removal of the cast repeat rib biopsies were obtained on all monkeys. The monkeys were then further subdivided into groups, three of which were sacrificed immediately following this post-immobilization biopsy. Other groups were retained for periods of approximately 3, 6, and 12 months post-immobilization and sacrificed at the specified time. A complete set of haversian, endosteal and periosteal parameters was made available for each biopsy time. The

data was used to determine the rate at which the musculoskeletal system gradually reverses the changes observed during immobilization and the immediate post-immobilization period. Since our method separates and evaluates individual multiple phases of the bone remodelling process, we were able to delineate more specifically the exact method by which the bone gradually returns to approximately control status following the phase of osteopenia. Computer facilities at Wright-Patterson Air Force Base, Dayton, Ohio are being used to correlate the various phases of data with each other and with the biochemical and biomechanical analysis of the monkeys and their various calcified tissues during the phases of immobilization and remobilization.

The age of each monkey was taken into account in this correlation, using data from our previous control group study establishing age specific parameters. Such a correlation of data on the same monkey has lead to some clues of relevance to the methods useful in the treatment and prevention of such bony changes in humans involved in situations of prolonged immobilization, altered activity or weightlessness. Conclusions may also be drawn regarding the ability of humans in similar situations to tolerate acceleration, impact loading, and a sudden burst of intense musculoskeletal activity. Since there appears to be a reduction in bone mass and its mechanical strength of bone following periods of immobilization, our data is very useful in determining the means by which this is accomplished. We are presenting data concerning the number of formative centers, number of

resorption centers, the rate at which such osteons are generated and the speed at which they are completed. Overall bone turnover for a given period of time is also evaluated. Since the anatomic, and hence mechanical status of the bone at any given time is a product of the processes which produced it, we feel that our data has important predictive value and in terms of anticipating the musculoskeletal system's ability to withstand loading conditions following periods of change in musculoskeletal activity. Because we have established meaningful scaling factors between the rhesus monkey and the human, these studies have direct bearing on the human ability to withstand a host of stressful conditions such as impact loading or acute aceleration following weightlessness or enforced inactivity.

Data will be submitted in tables showing mean values, standard deviations, and "n", the number of monkeys studied in each group. In addition, we have constructed graphs of the five primary parameters for the pre-immobilization, post-immobilization, and three, six, and twelve months reconditioning groups. Periosteal, endosteal, and haversian systems are graphed separately. While this data summarizing method shows what we feel to be interesting trends, it is not meant to replace the detailed computer analysis to which these figures are being subjected at Wright-Patterson Air Force Base as well as their correlation with the biochemical and biomechanical parameters. We do anticipate, however, that the production of this data will help in increasing our understanding of the body's response to altered activity - a very promising venture in as much as many

different parameters from the same monkey are being studied and correlated. We anticipate that publication of this data will be able to advance the understanding of the response to the musculoskeletal system to the types of altered activity described in this report.

Technical Methods

The specimens were received at the Calcified Tissue Laboratory, Henry Ford Hospital in 70% alcohol, having been obtained in the primate facilities at Wright-Patterson Air Force Base.

Following this fixation in 70% alcohol prior to sectioning, bone specimens were cut in Bronwill's thin sectioning machine to approximately 150-200 microns, and then hand ground according to Frost's method to a thickness of 75-100 microns. All sections were then stained with the Villanueva Bone stain for 48 hours. Upon completion sections were reground to remove surface staining, differentiated, dehydrated, cleared and then mounted permanently in Eukitt's mounting media. In the evaluation of each rib specimen an average of four sections were used for quantitative measurements. The following is the rational for the measured parameters:

A) Haversian surface

1. Cortical area, mm^2 (A_c) - This involves the histological quantitation of the area of the cortex using the square grid of Frost. In conjunction with this, the marrow area (A_m) and total area (A_t) of the rib cross section are also calculated. These parameters are important in this study because we have accordingly determined the

number of osteoid seams per mm^2 and the number of resorption spaces per mm^2. We have found that in our previous studies on humans these values have direct correlation upon the age of the individual.

2. The circumference of an osteoid seam (S_f) in mm has been determined. This measurement is important because of the size variability of osteoid seams found in certain metabolic bone disorders. For example, in osteogenesis imperfecta, the circumference of osteoid seams is much larger when compared with the equivalent of normal of the same age.

3. The appositional rate in microns per day (M) - This is an area of study to determine the cellular level activity. Measurement of the rate is ascertained by the presence of two known tetracycline markers which appear like growth rings in trees. Knowing the width between two tetracycline markers, measured at 4 equidistant points and knowing the number of days between labels, the appositional rate (M) in microns per day and radial closure rate, (M_f) in microns per day or mm per year is calculated. We found that the rates in osteomalacia and renal osteodystrophy have slowed down considerably.

4. The mean osteon formation time (O_f) in years - This is another important part of the investigation which is essential because in some metabolic bone disorders the length of time required to complete lamellar bone deposition in an average osteon takes much longer than the normal formation time calculated from the standard of normals. This value serves as an index of bone production at the level of the osteoblasts.

5. The activation frequency foci (μ_f) - The activation frequency foci is simply a notation of the number of new osteons introduced per year.

6. The bone formation rate (V_f) in mm^2 per mm^2 per year - The bone formation rate is a measure of the tissue level formation. This means that a fraction of bone cortex at the level of the osteon is replaced each year by new bone.

B) Cortical-Endosteal and Periosteal Remodelling

A group of measurements were made using Frost's bone remodelling dynamics analysis for the periosteal and cortical-endosteal envelopes. Parameters were derived from the same sections used in the measurements of the haversian remodelling dynamics. The following is a rationale of the measurements:

1. Thickness between tetracycline bands (Appositional rate M) This is measured with a Zeiss eyepiece micrometer calibrated with a stage micrometer using the Zeiss photofluorescence microscope. The distance between a series of double labels is measured at both periosteal and cortical endosteal envelopes. The width between the labels divided by the number of days between the administration of the labels yields the mean thickness of a layer of new osteoid added per day at bone forming foci. In cases where only one tetracycline label was observed, measurement was made from the middle of the tetracycline band to the zone of demarcation.

2. Thickness of the periosteal and cortical-endosteal wall (W.T.) This is measured by phase contrast microscopy using the method

described by Frost. Approximately 25 to 50 measurements of wall thickness were made on each rib section for both the periosteal and endosteal surfaces. This thickness, divided by the microns of new matrix added daily, (M_f), gives the number of days required to make a new periosteal envelope and/or endosteal envelope.

3. Number of osteoid seams (A_f) - On each surface of remodelling osteoid seams are counted separately because of the nature of remodelling at the periosteal and/or endosteal surfaces. Some of these seams cover almost a third of the perimeter of these envelopes. These long seams are still counted individually so that the number of seams in one section may total only two or three of these structures. Other seams appear smaller and shorter but these are also considered and counted accordingly. Dividing the number of osteoid seams by the total perimeter of a given surface gives the average number of forming foci per unit perimeter of either endosteal or periosteal envelope.

4. Percent tetracycline labelling (%L) - The number of osteoid seams which contain recently deposited tetracycline label is counted for each surface. When divided by the total number of osteoid seam accepting the label is obtained. Multiplying the percent of labelled osteoid seams by the rate of new matrix added per day, (M), and again multiplying by $\frac{365}{1000}$ gives the radial closure rate, (M_f), in mm per year.

5. Circumference osteoid seams (S_f) - The length of an osteoid

seam is determined by using the Zeiss integrating eyepiece II. Measurements are made at 128X with a 0.40 N.A. The eyepiece contains six parallel equidistant lines which are optically superimposed upon the surface containing osteoid: every line intersecting with osteoid is recorded as a "hit". Since the distance between these lines is a known value "a", the total length of osteoid can be calculated by multiplying the number of hits recorded with the constant "a". The total length of osteoid measured for each surface is divided by the total number of individual osteoid seams for that surface to obtain the average circumference of a seam.

6. Bone formation time ($\mathcal{O}_f$) - The bone formation time is the average length of time required to complete lamellar bone deposition at the endosteal and/or periosteal surface; it is determined by dividing the mean wall thickness (W.T.) by the mean appositional rate (M_f) (appositional rate, it is recalled, is the measured distance between two tetracycline labels, divided by the number of days elapsed between administration of the two labels). The result, divided by 365 days, gives the fractional part of a year necessary to complete lamellar bone formation.

7. Bone formation rate, surfaced based (V_f) - Bone formation rate at the endosteal and/or periosteal surface is the product of the average number of osteoid seams per perimeter times the average length of osteoid seam times the average rate of radial closure.

Mathematically, this is expressed by the equation:

$$^{E}A_{f} \times {}^{E}S_{f} \times {}^{E}M_{f} = V_{f} \text{ (surface based)}$$

or

$$^{P}A_{f} \times {}^{P}S_{f} \times {}^{P}M_{f} = V_{f} \text{ (surface based)}$$

where $^{E}A_{f}$ = average number of osteoid seams per mm perimeter of endosteal surface

$^{E}S_{f}$ = average length of osteoid seam, endosteal

$^{E}M_{f}$ = average rate of radial closure, endosteal surface

$^{P}A_{f}$, $^{P}S_{f}$ and $^{P}M_{f}$ are the periosteal surface equivalents

8. Mean cortical thickness (M.C.T.) - The mean cortical thickness is a measure of the average thickness of cortex. This is determined by using a calibrated eyepiece micrometer superimposed on the section at 8 equidistant points, and obtaining a mean value of the eight separate measurements.

9. Bone formation rate, volume based (V_{f}) - This is determined by dividing the bone formation rate, surface based, by the mean cortical thickness. The latter serves as the correction factor for the third dimension of the surface-based value.

10. Periosteal, Cortical-Endosteal perimeter - The total length of periosteal or endosteal surface perimeter is determined by the grid method of Frost. Briefly, this method involves superimposing a square grid usually consisting of 10 equally spaced lines, both horizontally and vertically, upon the rib section whose cortical-endosteal and periosteal perimeter are of irregular dimensions. Knowing the parallel spacing "a" between the lines, one can approximate the total length of surface. Each intersection of both horizontal and vertical lines with the perimeter being measured is

recorded as a "hit". Lines tangent to the surface being measured are recorded as "½ hit". By repositioning the grid several times upon the perimeter being measured, an adequate number of hits is accumulated to obtain a significant value for such determinations. The number of hits "h" is then substituted in the following formula to calculate the length of perimeter in question:

$$\text{Perimeter} = \frac{\pi}{2} \cdot \frac{h}{T} \cdot a$$

where $\pi = 3.1416$

h = hits, or intersections between surface and grid lines

T = number of repositionings, or throws

a = distance between parallel lines in the grid

11. Per Cent: Formation, Resorption, No-Activity, and Tetracycline Label - The Zeiss integrating eyepiece II is used for these percentages for each type of surface, formative, resorptive, inactivity, and tetracycline labelled, can be calculated simply by dividing hits of each surface by the total hits of all surfaces.

Data

Mean values of all parameters for the Haversian, Endosteal and Periosteal surfaces are presented in tables I, II and III, respectively. In addition, graphs 1-15 indicate the relationship between the mean values for groups at various times during the experimental sequence. For simplicity, those biopsies taken three or four months post release from immobilization were designated as the three month group, those biopsies taken between five to seven months post-release from immobilization were designated as the six month group, and those biopsies taken 11 or 12 months post-release were designated as the 12 month group. We have graphed what we considered to be the primary observed parameters of remodelling from which other secondary parameters

were calculated. We feel that an understanding of the trends of these primary parameters will convey the meaning of this experiment in terms of the trends occurring through the pre-immobilization, post-immobilization, and reconditioning phases. One will note that the variations observed for the periosteal and endosteal regions for the various time periods are almost identical and hence one can combine these in referring to trends in what could be called the "surface parameters". One may further note that for these surface parameters, three catagories having to do with bone formation (% label, A_f and % formation) all are comparable with one another and hence can be considered together. These bone forming parameters for the two "surface" areas show significant depression in the post-immobilization determinations. There is likewise a significant increase in the values for these parameters in the three month group, in some cases leading to supra-normal readings. Values continue at high levels, or increase even further, in the six month group. These bone forming oriented parameters on the surfaces appear to level off to roughly control values in the 12 month group. Despite the fact that the surface bone forming parameters appear to have recovered to at least normal levels by three to four months post-immobilization, there is to be noted a significant <u>decrease</u> in the cortical to total ratio. There is also continued depression of the percent surface occupied by resorption in the three to six month post-immobilization period, returning finally to control levels at 12 months. There is a slight variation in the appositional rate as determined by tetracycline labelling in the post-immobilization and re-mobilization periods but these do not appear to be of magnitude to be considered significant.

In the haversian system all changes appear to be of a less significant level than those observed on the periosteal and endosteal surfaces. There is a seemingly significant elevation in appositional rate at the six month period in the haversian system, but this is not mirrored by changes in other parameters and its significance is not clear at this time. Aside from this, the haversian system appears to show no other significant changes with immobilization and remobilization.

Discussion

We have analyzed monkey rib specimens for the purpose of determining quantitative histodynamic bone remodelling parameters. The monkeys were the subject of the study under the coordination of Dr. Frank Noyes at Wright-Patterson Air Force Base in which correlation is being made between the bone remodelling dynamics and systemic biochemical changes in bone biomechanics in monkeys subjected to various states of immobilization and remobilization. The first year of the study involved the establishment of normal control bone remodelling profiles in the rhesus monkey rib by use of the Frost method. 50 monkeys were included in the project. Biopsies of the 6th, 10th and 11th ribs were performed on these animals in various stages of their immobilization-remobilization sequence. The ribs were received by our laboratory in 70% alcohol and processed by the methods described above.

Inasmuch as the mechanical properties of a bone are very dependent upon its cross sectional geometry, it is not surprising to note from our data that the most active response to immobilization and remobilization in the monkey rib are seen in the surface parameters (periosteal and endosteal data) Tables II and III. The ribs participate in the loading produced by muscular contraction associated with the regular activities of an organism. This loading may occur by two

general mechanisms, first, motion of the torso and upper extremities are accomplished in part by muscles with direct insertion on the ribs, particularly the mid lateral aspect which was the area utilized for our biopsies. Secondly, the ribs are cyclically loaded by respiration which may be carried on at an increased or decreased level of intensity as dictated by the overall activity and oxygen demands of the organism. For these reasons, total body plaster immobilization would be expected to produce a distinct decrease in the rate and level of loading of the ribs. Similarly, total body remobilization following release from the cast would be anticipated to have its effect upon rib remodelling parameters by an appropriate increase in mechanical activity.

Following the two month immobilization period, the surface parameters showed a dramatic decrease in those values associated with bone formation. Our data indicates that this profound depression was nullified at a time three to four months post-immobilization, where values similar to controls are found. Surface bone forming parameters in the six month post-immobilization group show values in some cases higher than those in the controls, and the values again returned to roughly control levels in the 12 month group.

Despite the fact that bone forming activity appears to have returned to normal levels by three to four months post-immobilization the decrease in the C/T ratio becomes more prominent at this time. A slight decrease in cortical to total ratio was apparent in the post-immobilization group and a further decrease is noted in the three to four month group followed

by a mild increase in the six month group and finally returned to control values in the 12 month group. Inasmuch as a decreased C/T ratio indicates thinning of the cortex in cross section, we would predict a decreased mechanical competence and decreased ability to sustain high levels of loading to be particularly apparent at a time three to four months post-immobilization. While the bone forming parameters have returned to normal at this time, we must draw attention to the difference between the rate of recovery at a given time and the amount of recovery that has actually been accomplished and manifest in a restoration of normal cortical anatomy.

The cortical thinning observed in the post-immobilization and three to four month reconditioned groups appears to have been accomplished without an increase in the percentage surface involved in resorption. In fact, the percentage resorption is slightly decreased in the post-immobilization group and is further decreased in the three to four month reconditioned group. We initially considered this an unexpected finding in light of the fact that all remodelling activity, whether ultimately resulting in an increase or decrease of cortical thickness and mechanical competence, must be initiated by osteoclastic resorption, according to the sequence of activity now accepted by the majority of bone physiologists. There is, however, a possible explanation - normally, the resorptive phase of remodelling generally requires approximately 50% of the time it takes for the formative or osteoblastic phase to be completed. The data presented here, and other data, induce us to postulate that the normal physiological loading of bone (manifest by stress and strain) may influence the duration

of the resorptive and formative phases of remodelling, and in addition may directly influence the surface extent and in some cases the rate of progression of the ossifying front. Secondly, it is possible that the birth rate of such surface remodelling units is influenced by activity. One may further hypothesize that during immobilization the rate of generation of new osteons (as first manifest by resorption centers) and the transformation of resorptive remodelling units to formative remodelling units, may be significantly retarded. In the three to four month reconditioned group, formative activity has increased to normal loads, while resorbing surface has further decreased. This relative paucity of resorbing surfaces might represent rapidity of their transformation into forming centers rather than a low level of remodelling as a whole. If just prior to the three to four month reconditioned biopsies, activity had increased to a point where the extent and duration of the forming phase of the remodelling unit were stimulated by such activity, it is plausible that such recently increased bone formation would not yet have had a chance to bring about a reversal of the decrease in C/T ratio. Our data therefore seems to suggest that both the genesis of new remodelling units, and the surface extent and the duration of the formative phase of the remodelling unit may well be dependent on the level of activity. Furthermore, it would appear that the factors leading to loss of cortical substance continue to operate for a period approximating two to three months following the release from immobilization. Inasmuch as we have no observations during the first two to

three months of post-immobilization, such a hypothesis would, of course, need to be confirmed by further direct observation. It is possible that exercise oriented factors in the first few months post release from immobilization may be identified as important in determining when the bone removing phase changes into the bone forming phase.

This data has been forwarded to Dr. Frank Noyes at Wright-Patterson Air Force Base where a more detailed computer analysis of the results will be carried out and the significance of the variations observed determined.

Correlation with the biochemical and biomechanical properties will allow final conclusions to be reached as to the method by which bone undergoes its observed diminution in mechanical and physical properties following immobilization. The correlation of observations in all 3 of these areas (histologic, biochemical, and biomechanical) in the same animals under well defined experimental conditions afford a unique opportunity to observe the methods by which the bone responds to conditions of reduced activity, weightlessness, and other specific forms of alterations of musculoskeletal activity.

Conclusion

The effects of immobilization and reconditioning on bone remodelling have been interpreted in light of a basic sequence involving initial bone resorption followed by bone formation. The basic remodelling unit on any one of the three bone surfaces may be affected both in the rate of genesis and in the method of evolution. Alterations in both categories are suggested by our data. Assuming rate of bone loading to be proportional to the predominance of the osteoblastic phase of the remodelling unit

and to the rate of overall genesis of such units, we have interpreted our data to indicate that the depressive effects of immobilization appear to persist for roughly two months into the reconditioning phase. This may be associated with joint and musculotendinous stiffness, and symptoms related to the attempted loading of bone with sub-optimal mechanical properties. Many of the remodelling abnormalities persist in the six month reconditioned group, but are largely eliminated by twelve months. Inasmuch as scaling factors have already been established indicating that these processes progress more slowly in a large organism such as man, we would expect more than the six month period of remodelling abnormality to be present in the human. More far-reaching correlations may be reached when this data is viewed in light of mechanical and biochemical observations made on the same monkeys.

These conclusions represent our best effort to interpret data obtained between the three and 12 months post-immobilization, but would be considerably strengthened by additional observations made at one or two month periods post-release from immobilization. Nevertheless, we feel that the existing findings will be of considerable value in our attempts to predict human tolerance to acceleration, impact loading, and other forms of musculoskeletal stress following periods of weightlessness or immobilization associated with scientific or military activity.

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Table I
Haversian Surface Comparative Parameters
Monkey Rib

Histologic Index Haversian	n = 13 Control	n = 42 Pre	n = 29 Post	n = 11 Exercise	Recon 3 months	Recon 6 months	Recon 12 mo
A_c, Cortical area mm^2	5.40 ± 0.95	5.66 ± 1.04	6.03 ± 1.07	5.65 ± 0.59	7.27 ± 1.55	7.50 ± 1.35	7.70 ± 1.7
C/T Ratio cort-total area	0.80 ± 0.08	0.80 ± 0.03	0.77 ± 0.04	0.82 ± 0.05	0.70 ± 0.08	0.75 ± 0.05	0.80 ± 0.0
A_f, No. osteoid seams/mm^2	4.03 ± 1.64	3.10 ± 0.92	3.81 ± 1.11	3.58 ± 1.47	3.45 ± 1.42	3.12 ± 0.90	3.63 ± 1.5
A_r, No. resorp. spaces/mm^2	1.96 ± 1.15	1.11 ± 0.41	1.11 ± 0.34	1.03 ± 0.66	0.66 ± 0.36	1.78 ± 0.90	0.81 ± 0.4
S_f, Circumference osteoid seams	0.20 ±0.03	0.24 ±0.01	0.22 ± 0.03	0.25 ± 0.07	0.24 ± 0.04	0.26 ± 0.04	0.21 ± 0.0
M Appositional rate, microns/day	1.33 ± 0.23	1.22 ± 0.13	1.18 ± 0.14	1.08 ± 0.32	1.03 ± 0.26	1.45 ± 0.11	1.20 ± 0.3
$M_f^{\bullet}$ Radial closure rate, mm/yr	0.42 ± 0.08	0.36 ± 0.06	0.33 ± 0.06	0.33 ± 0.11	0.28 ± 0.10	0.48 ± 0.03	0.34 ± 0.1
U_f Activation eq. foci/year	27.9 ± 13	19.2 ± 8.8	19.9 ± 6.1	20.4 ± 11.7	15.1 ± 9.9	19.5 ± 10.4	17.8 ± 7.4
O_f Osteon form. time, years	0.15 ± 0.03	0.21 ± 0.06	0.25 ± 0.11	0.22 ± 0.08	0.28 ± 0.19	0.13 ± 0.01	0.21 ± 0.0
A_r/A_f Ratio, resorp./form.	0.51 ±0.24	0.38 ± 0.07	0.38 ± 0.20	0.27 ± 0.13	0.23 ± 0.16	0.57 ± 0.21	0.27 ± 0.1
V_f Bone form. rate $mm^2/mm^2/yr$	0.359 ±0.186	0.3110 ± 0.1358	0.2748 ± 0.0585	0.3463 ± 0.2188	0.2358 ±0.1548	0.3929 ± 0.1353	0.242 ± 0.0
W.O.S. Width osteoid seams/M	NA	7.9 ± 0.9	6.3 ± 0.5	7.5 ± 1.3	7.4 ± 1.2	8.9 ± 1.4	7.6 ± 1.4
% Percent labelled system	86 ± 8	80 ± 9	75 ± 10	83 ± 8	72 ± 18	91 ± 6	77 ± 14

Table II

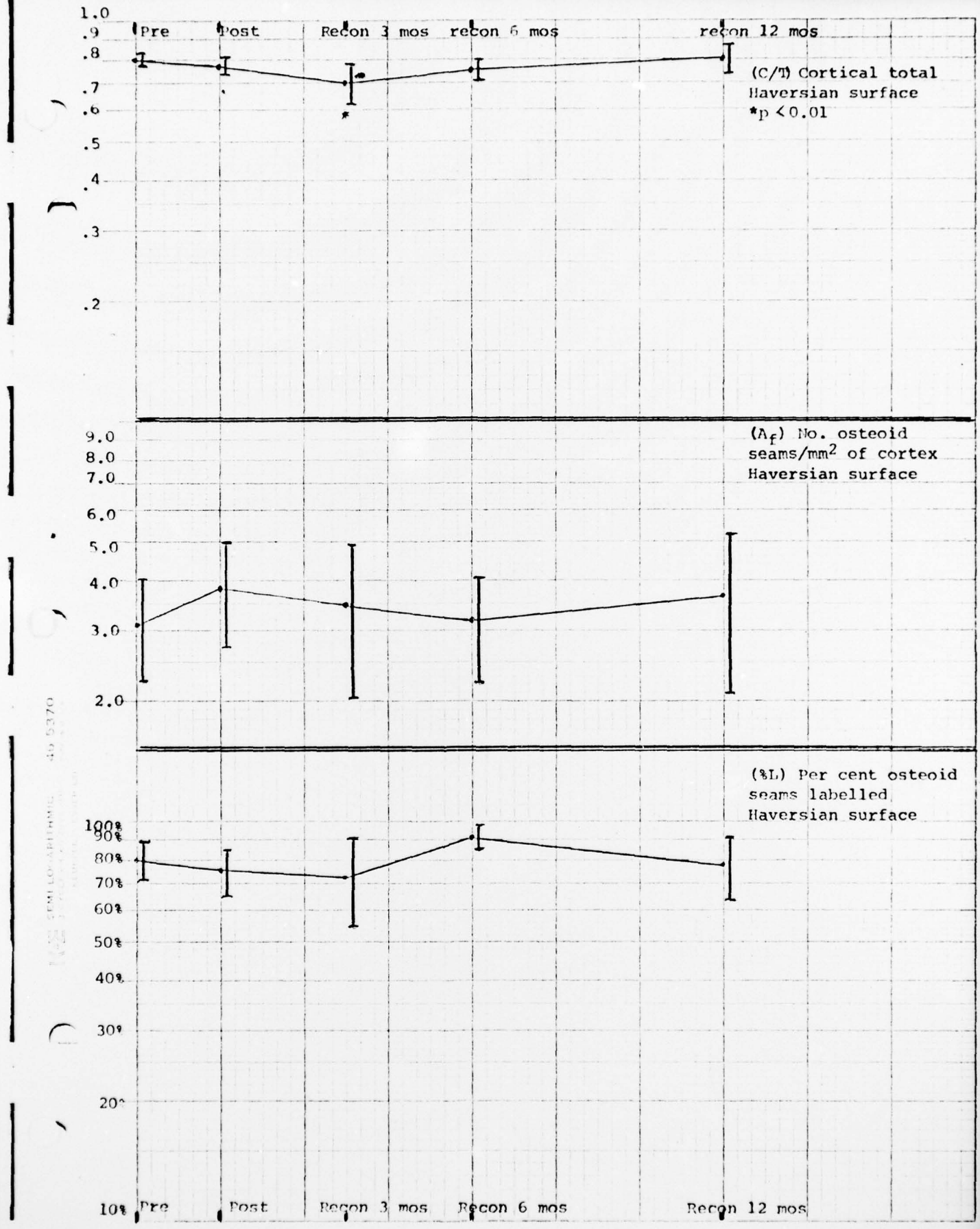
Endosteal Surface Comparative Parameters

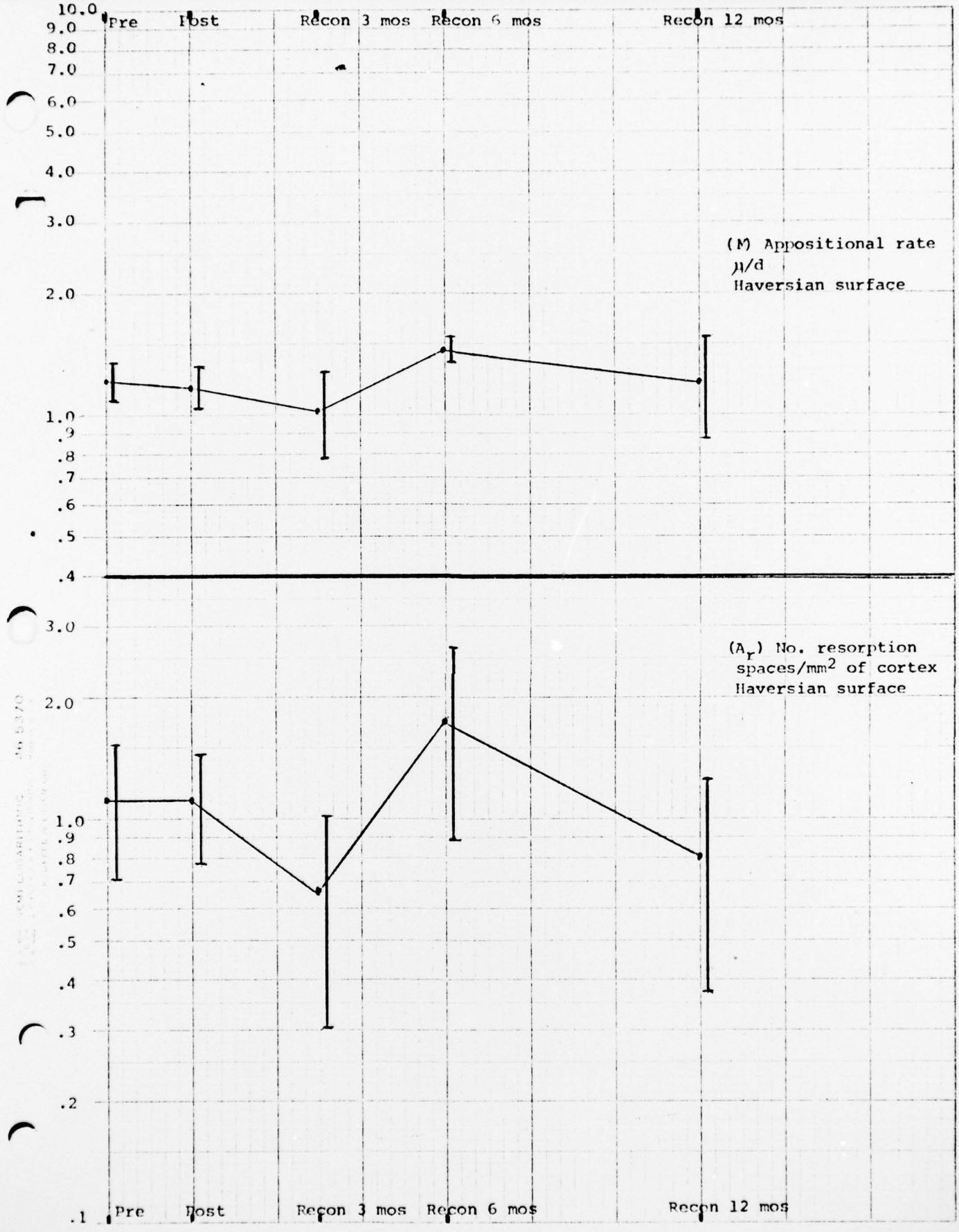
Monkey Rib

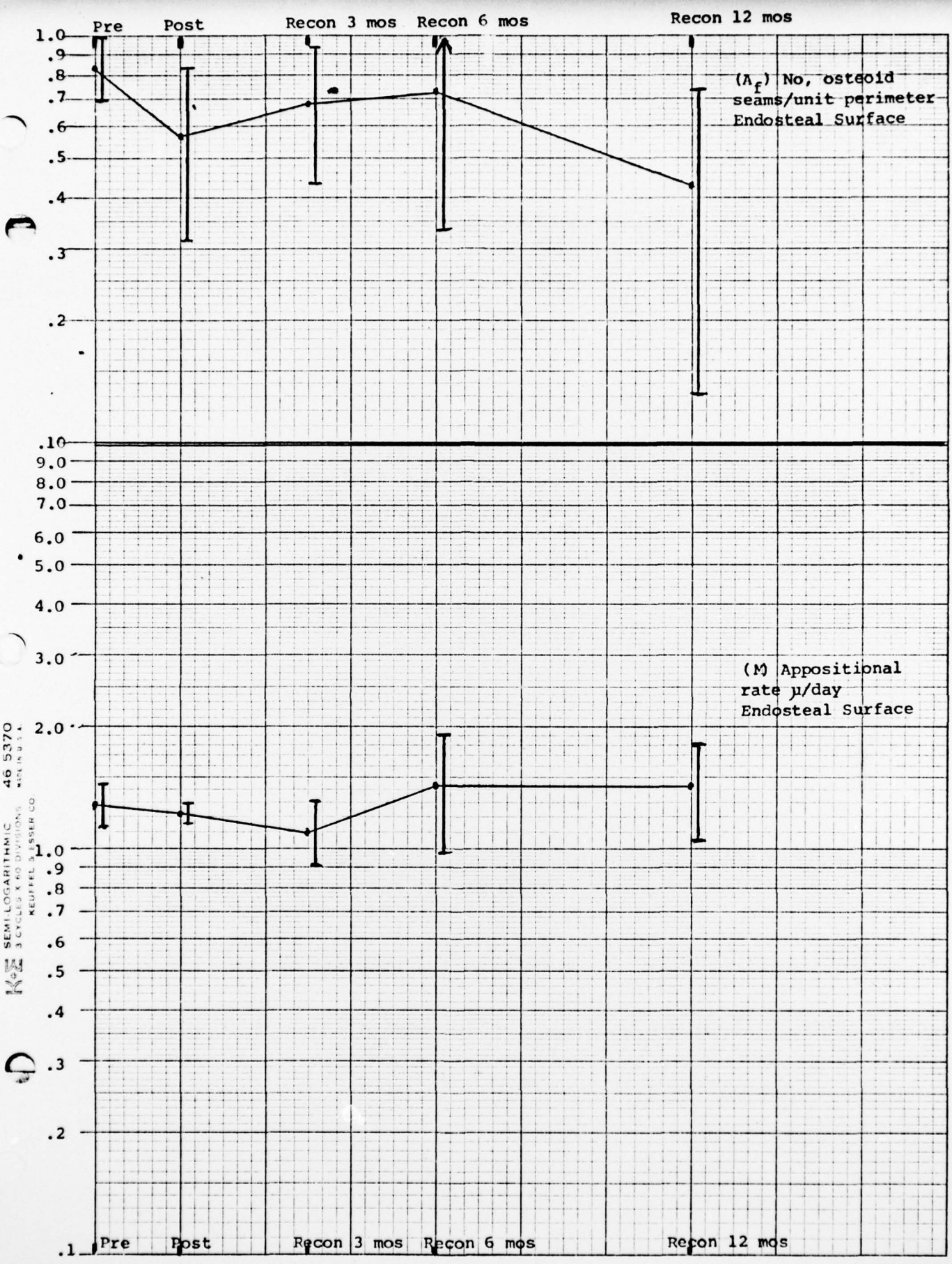
stologic Index Endosteal	n = 13 Control	n = 12 Pre	n = 29 Post	n = 11 Exercise	3 months Recon	6 months Recon	12 month Recon
Perimeter P, mm	6.10 ±1.87	5.28 ±1.03	5.89 ± 1.01	6.73 ±1.22	8.58 ±2.07	7.17 ±0.89	6.20 ±1.48
#O.S./mm A_f	0.84 ±0.26	0.84 ±0.15	0.57 ±0.26	0.42 ±0.29	0.68 ±0.25	0.73 ±0.39	0.43 ±0.30
Circumf. O.S. Sf, mm	0.69 ±0.29	0.83 ±0.13	0.69 ±0.22	0.48 ±0.22	0.63 ±0.18	0.51 ±0.12	0.67 ±0.44
Wall thickness W.T. mm	0.060 ±0.009	0.079 ±0.027	0.092 ±0.026	0.058 ±0.006	0.063 ±0.009	0.064 ±0.006	0.060 ±0.007
Appositional Rate M, microns/day	1.25 ±0.24	1.28 ±0.16	1.22 ±0.07	1.06 ±0.43	1.10 ±0.20	1.43 ±0.46	1.42 ±0.39
Radiol closure rate, mm M_f, yrs.	0.44 ±0.11	0.45 ±0.07	0.42 ±0.03	0.33 ±0.19	0.39 ±0.08	0.49 ±0.16	0.52 ±0.20
Formation time σ_f yrs.	0.15 ±0.08	0.20 ±0.09	0.24 ±0.09	0.39 ±0.66	0.18 ±0.06	0.16 ±0.05	0.13 ±0.03
tivation sites, U_f	7.24 ±5.54	5.7 ±3.4	3.1 ± 2.7	2.51 ±2.26	4.1 ± 1.2	3.7 ± 2.0	3.1 ± 2.
% labelled system	39 ± 28	45 ± 8	26 ± 8	26 ± 20	41 ± 21	36 ± 20	35 ± 23
% No-activity	32 ± 15	40 ± 13	60 ± 8	62 ± 30	48 ± 16	52 ± 13	66 ± 27
% Resorption	15 ± 10	16 ± 6	13 ± 7	23 ± 19	9 ± 9	10 ± 9	17 ± 14
% Formation	54 ± 18	47 ± 10	29 ± 8	23 ± 17	44 ± 20	38 ± 22	26 ± 19
Bone formation rate surface based V_f, $mm^2/mm^2/yr.$	0.2528 ±0.1226	0.3064 ±0.0529	0.1666 ±0.0627	0.0671 ±0.0571	0.1801 ±0.0605	0.1775 ± 0.1120	0.1807 ± 0.1824
M.C.T. Mean cortical thickness	0.706 ±0.158	0.620 ±0.077	0.681 ±0.058	0.6514 ± 0.1140	0.6151 ± 0.1163	0.6874 ± 0.073	0.775 ± 0.174
Bone formation rate volume based V_f $mm^2/mm^2/yr.$	0.4044 ±0.2779	0.5232 ±0.1455	0.2536 ±0.1208	0.1000 ± 0.0784	0.3080 0.1446	0.2657 ± 0.1905	0.2766 ± 0.293

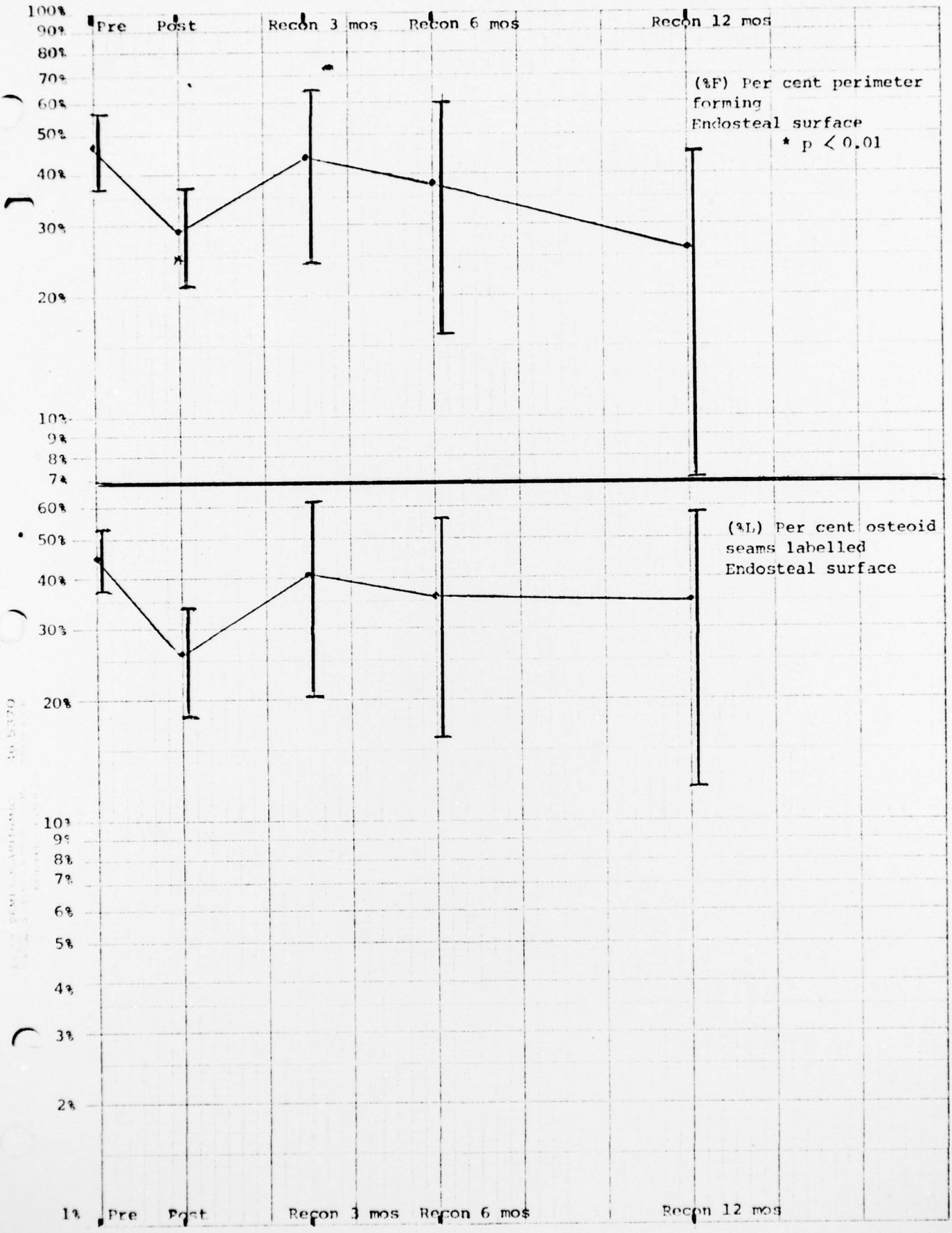
Table III
Periosteal Surface Comparative Parameters
Monkey Rib

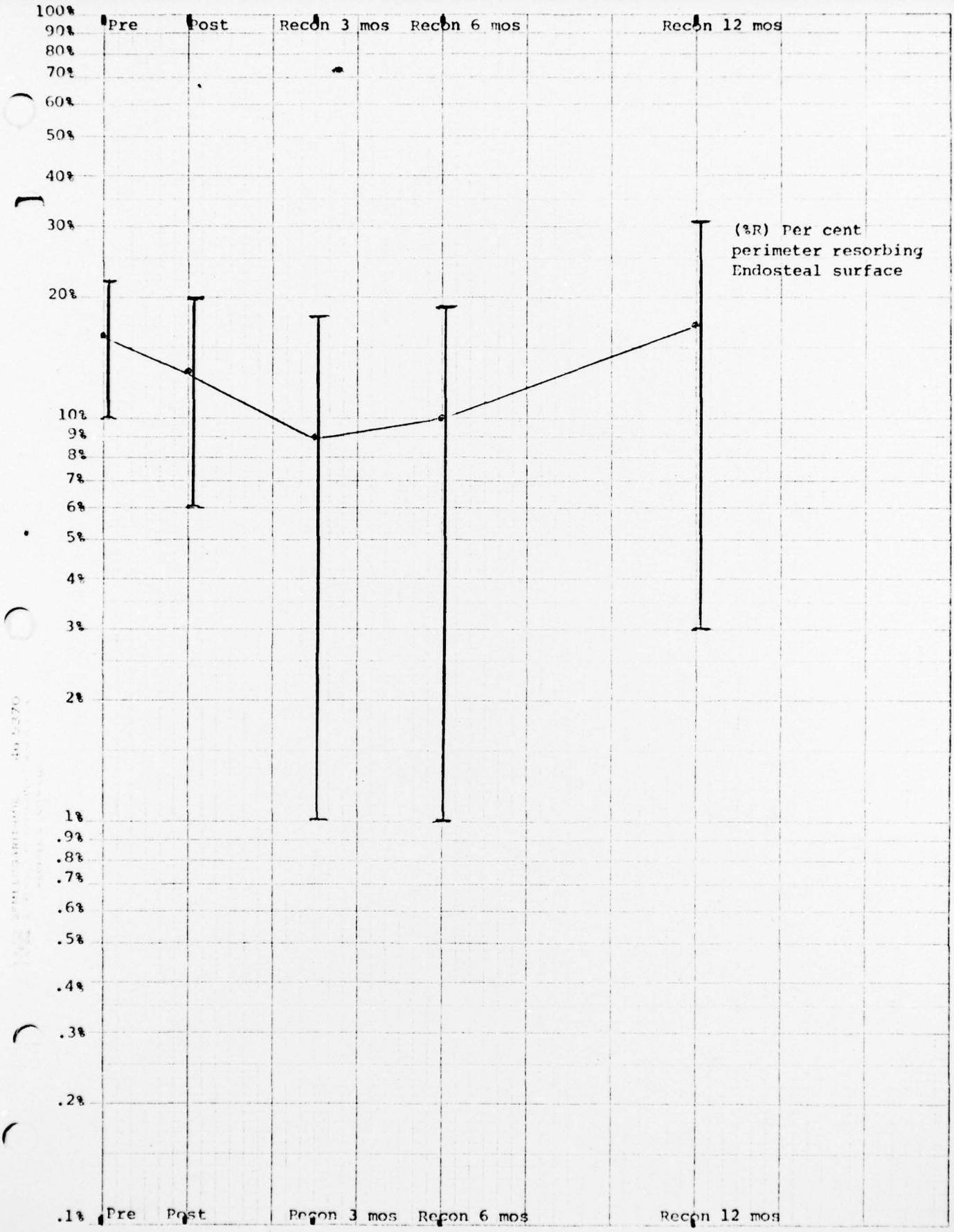
Histologic Index Periosteal	Control n = 13	Pre n = 42	Post n = 29	Exercise n = 11	3 months Recon	6 months Recon	12 months Recon
Perimeter P, mm	12.99 ± 1.44	8.86 ± 1.67	10.70 ± 1.01	10.93 ± 1.48	12.21 ± 1.87	12.15 ± 1.39	12.49 ± 1.75
#O.S./mm A_f	0.29 ± 0.08	0.28 ± 0.08	0.26 ± 0.16	0.33 ± 0.22	0.24 ± 0.08	0.30 ± 0.07	0.19 ± 0.04
Circumf. O.S. S_f, mm	2.01 ± 0.67	2.25 ± 0.96	1.20 ± 0.51	0.85 ± 0.66	1.57 ± 0.83	1.99 ± 0.49	1.07 ± 0.40
Wall thickness W.T. mm	0.077	0.095 ± 0.047	0.095 ± 0.041	0.052 ± 0.015	0.068 ± 0.016	0.066 ± 0.001	0.065 ± 0.010
Appositional rate M, microns/day	1.01 ± 0.23	0.92 ± 0.13	0.85 ± 0.14	0.64 ± 0.35	1.00 ± 0.26	1.14 ± 0.22	0.92 ± 0.46
Radial closure rate, mm M_f, yrs.	0.34 ± 0.09	0.30 ± 0.04	0.24 ± 0.04	0.18 ± 0.13	0.31 ± 0.12	0.37 ± 0.12	0.27 ± 0.20
Formation time δ_f yrs.	0.22	0.33 ± 0.16	0.49 ± 0.22	0.80 ± 1.18	0.27 ± 0.13	0.25 ± 0.08	0.69 ± 0.57
Activation sites, N_f	0.82	1.4 ± 1.1	1.1 ± 1.4	1.35 ± 1.63	1.2 ± 0.9	1.3 ± 0.6	0.35 ± 0.29
% labelled system	55 ± 13	35 ± 11	14 ± 4	21 ± 16	35 ± 22	± 56 ± 19	18 ± 12
% No-activity	28 ± 16	40 ± 10	57 ± 18	53 ± 19	57 ± 23	39 ± 18	74 ± 11
% Resorption	18 ± 10	20 ± 14	24 ± 23	25 ± 15	5 ± 5	3 ± 2	9 ± 6
% Formation	54 ± 15	40 ± 15	19 ± 10	24 ± 10	36 ± 21	59 ± 18	20 ± 7
Bone formation rate surface based V_f, $mm^2/mm^2/yr.$	0.1929 ± 0.0903	0.1730 ± 0.0608	0.069 ± 0.0237	0.0492 ± 0.0402	0.1269 ± 0.1431	0.2068 ± 0.0766	0.0730 ± 0.0704
M.C.T. Mean cortical thickness	0.706 ± 0.158	0.6513 ± 0.0573	0.6805 ± 0.0578	0.651 ± 0.114	0.615 ± 0.116	0.687 ± 0.073	0.775 ± 0.174
Bone formation rate volume based V_f $mm^2/mm^2/yr.$	0.2898 ± 0.1363	0.2898 ± 0.1235	± 1014 ± 0.0422	0.0775 ± 0.0686	0.1861 ± 0.1572	0.3007 ± 0.1059	0.0992 ± 0.1013

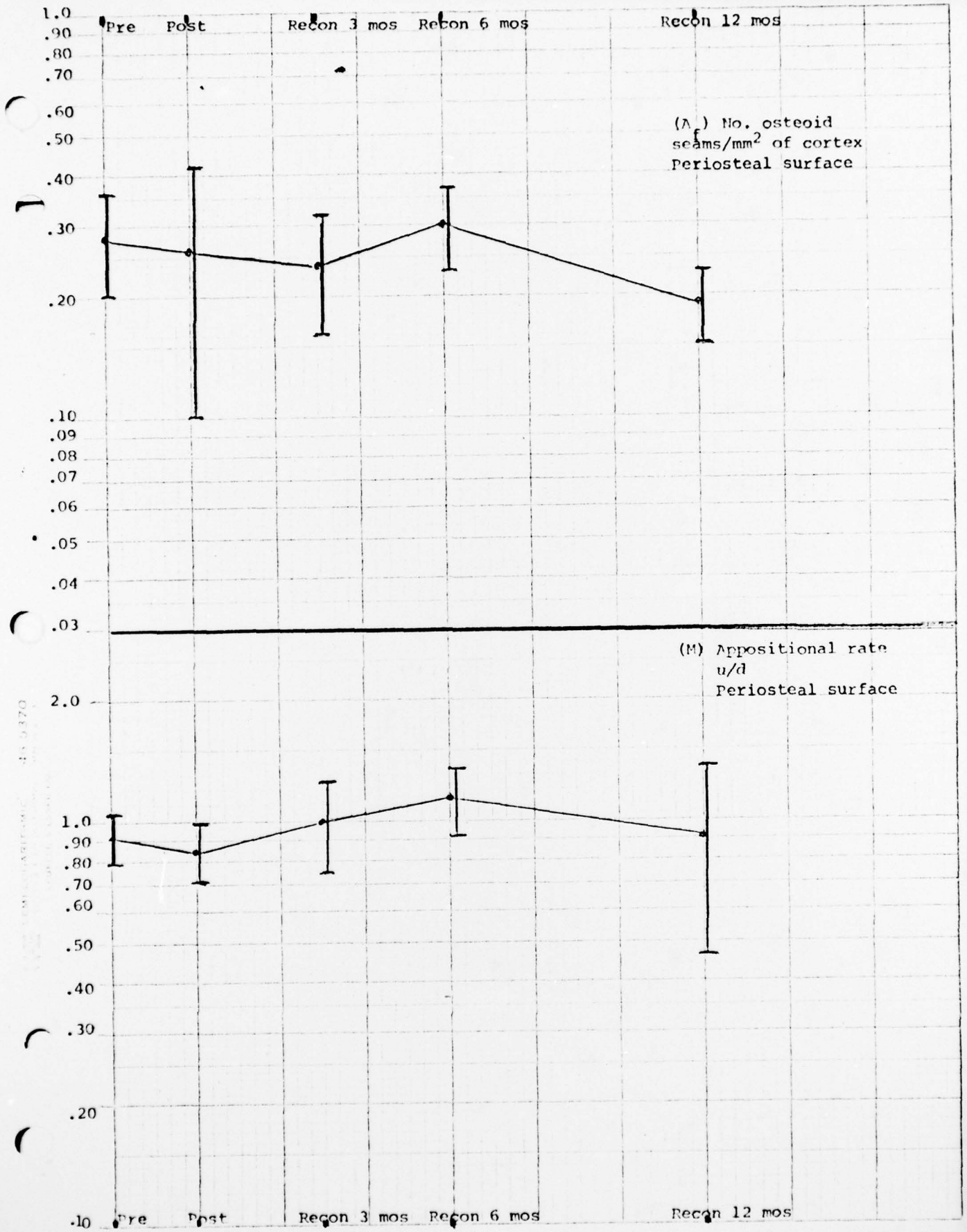

1.0
.9
.8
.7
.6
.5
.4
.3
.2
Pre
Post
Recon 3 mos
recon 6 mos
recon 12 mos
(C/T) Cortical total Haversian surface
*p < 0.01
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
(Af) No. osteoid seams/mm2 of cortex Haversian surface
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
(%L) Per cent osteoid seams labelled Haversian surface
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos

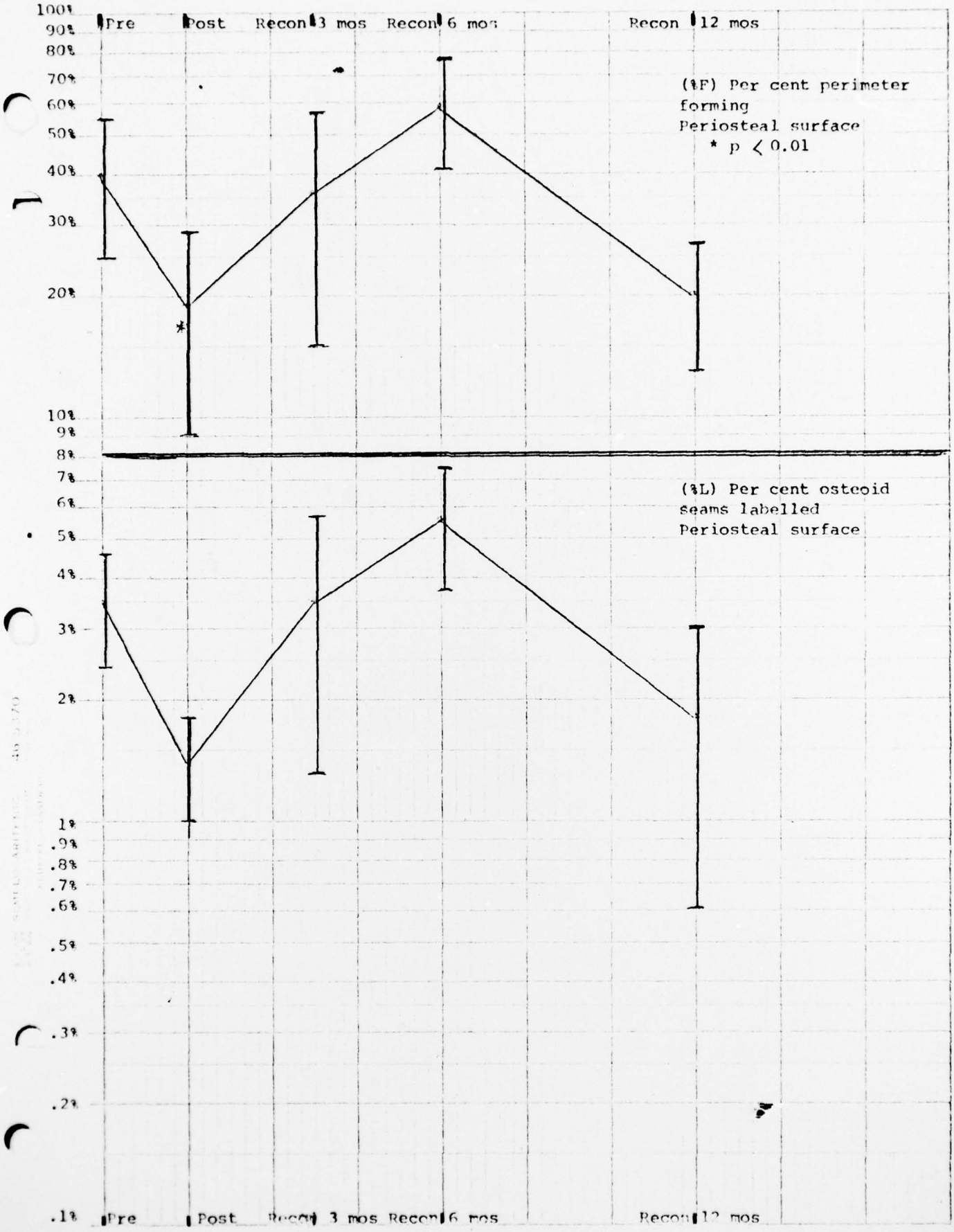

Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
.9
.8
.7
.6
.5
.4
(M) Appositional rate
μ/d
Haversian surface
3.0
2.0
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
(A_r) No. resorption spaces/mm² of cortex
Haversian surface
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos

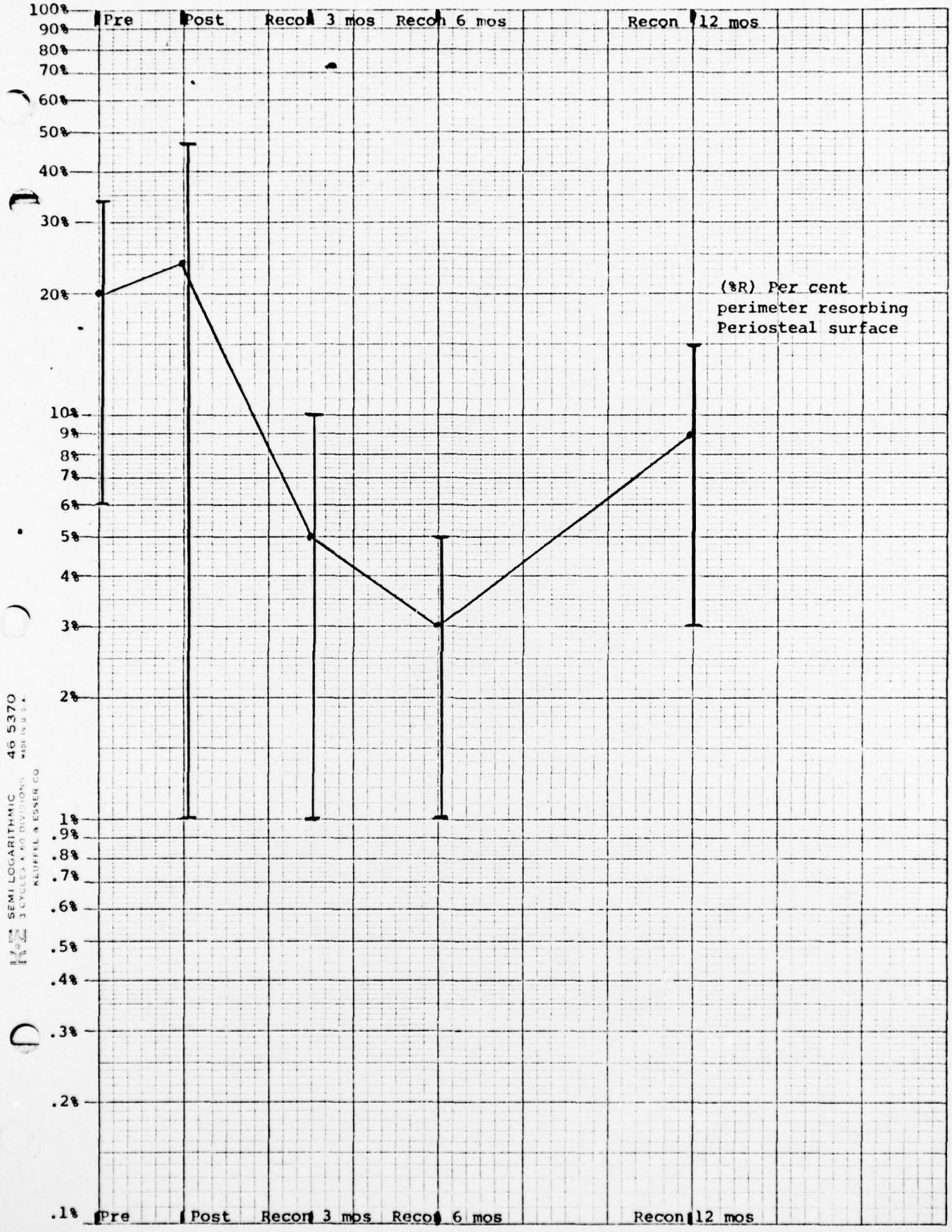

Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.10
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
(Λf) No, osteoid seams/unit perimeter Endosteal Surface
(M) Appositional rate μ/day Endosteal Surface
SEMI-LOGARITHMIC
3 CYCLES X 50 DIVISIONS
KEUFFEL & ESSER CO
46 5370


Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
9%
8%
7%
(%F) Per cent perimeter forming Endosteal surface
* p < 0.01
60%
50%
40%
30%
20%
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
(%L) Per cent osteoid seams labelled Endosteal surface
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos


100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
.9%
.8%
.7%
.6%
.5%
.4%
.3%
.2%
.1%
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
(%R) Per cent perimeter resorbing Endosteal surface
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos


Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
(A_f) No. osteoid seams/mm² of cortex Periosteal surface
1.0
.90
.80
.70
.60
.50
.40
.30
.20
.10
.09
.08
.07
.06
.05
.04
.03
(M) Appositional rate u/d Periosteal surface
2.0
1.0
.90
.80
.70
.60
.50
.40
.30
.20
.10
Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos


Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
(%F) Per cent perimeter forming Periosteal surface
* p < 0.01
9%
8%
7%
6%
5%
4%
3%
2%
1%
.9%
.8%
.7%
.6%
.5%
.4%
.3%
.2%
.1%
(%L) Per cent osteoid seams labelled Periosteal surface


Pre
Post
Recon 3 mos
Recon 6 mos
Recon 12 mos
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
9%
8%
7%
6%
5%
4%
3%
2%
1%
.9%
.8%
.7%
.6%
.5%
.4%
.3%
.2%
.1%
(%R) Per cent
perimeter resorbing
Periosteal surface
SEMI LOGARITHMIC
3 CYCLES X 60 DIVISIONS
KEUFFEL & ESSER CO
46 5370

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The effects of immobilization and reconditioning on bone remodelling have been interpreted in light of a basic sequence involving initial bone resorption followed by bone formation. Collected data indicate that the depressive effects of immobilization persist two months into reconditioning and may be associated with joint and musculotendinous stiffness. Many of the remodelling abnormalities persist in the six month group but are largely eliminated by twelve months. Based on established scaling factors, it is expected that the six month period of remodelling abnormality to be exceeded in the human

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These and other collected data should be of considerable value in predicting human tolerance to acceleration, impact loading and other forms of musculoskeletal stress.