Regulation of Bone Formation by Applied Dynamic Loads*

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ABSTRACT: **In studies on a functionally isolated avian-bone preparation to which external loads could be applied *in vivo*, we determined the following information.**

1. Removal of load-bearing resulted in substantial remodeling endosteally, intracortically, and, to a lesser extent, periosteally. Since the balance of this remodeling was negative, bone mass declined. It therefore appears that functional load-bearing prevents a remodeling process that would otherwise lead to disuse osteoporosis.

2. Four consecutive cycles a day of an externally applied loading regimen that engendered physiological strain magnitudes but an altered strain distribution prevented remodeling and was thus associated with no change in bone mass. A small exposure to, or the first effect of, a suitable dynamic strain regimen appears to be sufficient to prevent the negatively balanced remodeling that is responsible for disuse osteoporosis.

3. Thirty-six 0.5-hertz cycles per day of the same load regimen also prevented intracortical resorption but was associated with substantial periosteal and endosteal new-bone formation. Over a six-week period, bone-mineral content increased to between 133 and 143 per cent of the original value. Physiological levels of strain imposed with an abnormal strain distribution can produce an osteogenic stimulus that is capable of increasing bone mass. Neither the size nor the character of the bone changes that we observed were affected by any additional increase in the number of load cycles from thirty-six to 1800.

CLINICAL RELEVANCE: **The results of this experiment must be considered in relation to the type and duration of the non-physiological loads that were imposed. The sensitivity of bone-remodeling in this model to prevailing mechanical circumstances is evident. Functional levels of bone mass in patients may only be maintained under the effects of continued load-bearing. The osteogenic effect of an unusual strain distribution suggests that a diverse exercise regimen may engender a greater hypertrophic response than an exercise program that is restricted. A substantial osteogenic response may be achieved after remarkably few cycles of loading.**

In previous investigations, a number of approaches have been used to study the relationship between the mechanical environment of bones and the remodeling that contributes to the development of their mass and architecture. These approaches have included exercise[10,18], disuse[16], stress protection adjacent to implants of different stiffnesses[17], overload caused by osteotomy[6,12], and externally applied loading[2,7,8,13]. In these experimental models the new loading regimen was superimposed on effects arising from normal load-bearing, and it was therefore difficult to accurately associate a particular remodeling response with a specific parameter of the newly applied mechanical situation.

The model employed in this investigation enabled us

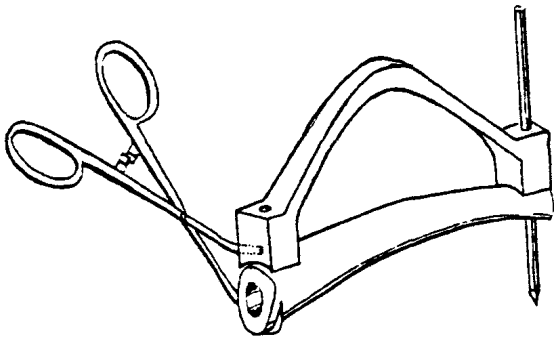

FIG. 1-A

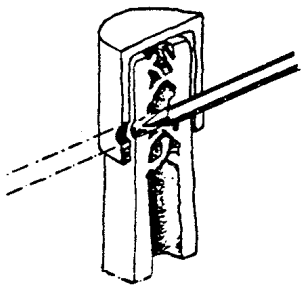

FIG. 1-B

Fig. 1-A: The template used for parallel osteotomies and pilot-hole placement. The clamping device is shown on only the proximal end and pin placement, on the distal end. This surgical procedure, performed on the left ulna, allowed cap placement and pin insertion to be completed through two small incisions, leaving the entire mid-part of the shaft undisturbed. The contralateral ulna was left undisturbed and served as a control.

Fig. 1-B: Diagram of a longitudinal section of the ulna through the proximal cap preparation, showing the insertion of the transfixing pin through the pre-drilled cap and bone. The cap limits the potential migration of the loading pin and allows for loading of the bone immediately postoperatively. The space between the cap and the bone is filled with viscous polymethylmethacrylate to ensure uniform load distribution.

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to apply known intermittent loads *in vivo* over a period of six weeks to a long-bone diaphysis that retained its normal muscular and vascular attachments but was otherwise isolated from any functional input[14]. The strains experienced within this bone preparation were predominantly those engendered by the external loading apparatus. The features of this strain environment were measured from the bone surface and could be related directly to the remodeling that was produced. An identical load waveform was applied for each animal and the experimental variable was the number of consecutive 0.5-hertz load-cycles that were applied during a single period each day.

Materials and Methods

Surgical Procedure

The ulna in the wing of skeletally mature roosters is a curved bone that is eighty-five millimeters long and ten millimeters in diameter. Under halothane anesthesia, the middle of the shaft of the left ulna was functionally isolated by removal of the bone's articular extremities, leaving seventy millimeters of the diaphysis undisturbed. This was achieved by making incisions over each epiphysis and using a template to make two parallel transverse osteotomies (Fig. 1-A). The cut ends of the bone were covered with stainless-steel caps which were held in place by 5/32-inch (3.97-millimeter) Steinmann pins piercing the bone and fitting snugly into holes previously drilled through the caps. A small amount of viscous methylmethacrylate bone cement was placed in each cap prior to placement, to ensure uniform load distribution (Fig. 1-B). The ends of these pins emerged through the skin on the dorsal and ventral surfaces of the wing and were held together by external fixation clamps. Once a day these clamps were removed and the pins were connected to a pneumatically operated loading device that was capable of imposing a 0.5-hertz intermittent compressive load through the pin-cap implant to the functionally isolated bone shaft.

Assessment of Strain

The strain at the middle of the ulnar shaft in two animals was assessed at three experimental stages.

Strain environment of the intact ulna: The normal strain at the mid-shaft of two intact ulnae was determined during wing-flapping. Under general anesthesia, three three-element rosette strain-gauges were attached around each bone's circumference using standard procedures[11]. One and two days after surgery, strain data from the nine channels were collected as the animals vigorously flapped their wings. Using simple beam theory and data from the three rosette strain-gauges, a mathematical model[15] was used to calculate a first-order approximation of the strain distribution acting across the bone section.

Strain induced by muscle activity in the isolated ulna with the external fixators attached: Once the natural strain situation of the intact bone had been determined, the birds were anesthetized again, the ends of the bone were osteotomized, capped, and pinned (as already described) and the external fixators were applied. The strains induced at the mid-shaft of the bone were again recorded from the bone-bonded strain-gauges during wing movement.

Strains induced in the isolated ulna by external loading: The fixators were removed from the prepared ulna, the Steinmann pins were engaged in the loading device, and strains at the mid-shaft were recorded during external loading. Strains were also recorded from these bones post mortem, during the external loading procedure. To ensure that the difficulties of attaching gauges *in vivo* had not resulted in defective gauge attachment, two additional ulnae were similarly instrumented *in vitro* and were loaded in the pneumatic device. This experiment was based on the assumption that the loads imposed on the "calibration" ulnae would elicit the same strain in the ulnae of the experimental group. The cockerels used were all of similar size and breeding, and their bones were similar enough in length and cross-sectional area to make this a justifiable assumption.

Assessment of Remodeling

The attachment of strain-gauges involves periosteal stripping, and this precludes any subsequent measurement of remodeling. The quantification of strains was therefore done in a group of "calibration" animals. Remodeling was investigated in a separate group of "experimental" animals. Two methods were used.

In vivo photon-absorption densitometry: The pins that were used for loading the mid-shaft were also used for accurate relocation of the ulna in a photon-absorption densitometer using a 200-millicurie ^{125}I gamma-emitting radioactive source. For this procedure, the wing was surrounded by two water-filled condoms that were pressed together between parallel Plexiglas plates to provide a constant tissue equivalent around the wing. The radioactive source and scintillation tube scanned transversely across the mid-shaft section of the bone. The calculation of bone-mineral content was made from the number of photon counts across the wing according to the method published by Cameron and Sorenson: the mineral content of the bone is approximately proportional to $\Sigma_i \ln (I_o/I_i)$, where I_o = the average of counts in the soft tissue and I_i = the count at each point (i) within the bone. Photon-absorption scans of each ulnar preparation were made at weekly intervals. Daily *in vitro* calibration of the bone-scanner was done by scanning a prepared ulna that had been isolated in a fluid-filled Plexiglas box. Over a six-week period, comparative assessments of the bone-mineral content of this standard calibration phantom showed that no value was more than 1.6 per cent different from the mean.

Postmortem histology and microradiography: Single-pulse doses of a fluorescent label were given intravenously to each bird at weekly intervals. The distribution of these labels in 100-micrometer undecalcified sections taken post mortem from the mid-shaft of both experimental and contralateral intact ulnae were used to determine the course of remodeling. The ulnar preparation was capable of lasting for only six weeks, and failure (generally associated with the pin-cap fixation) meant that several birds were killed earlier than this. The histological data from these birds was useful in determining the course of the remodeling process. Microradiographs of each section were also made.

Load Regimen

The load waveform (ramped square wave) was identical for each animal. The frequency was 0.5 hertz and dwell times on and off were of equal duration (0.95 second). Ideally, the double-action capability of the pneumatic piston should have enabled us to maintain the strain rate during load imposition at a level similar to that on load release. Unfortunately, the apparatus could not be controlled well enough for this to be achieved, and the strain rate during load release was faster than that during imposition.

Experimental Groups

The experimental variable in this study was the number of consecutive 0.5-hertz load-cycles that were imposed during a single period each day. Groups of six birds were used in which the isolated ulna either was not loaded or was subjected to four, thirty-six, 360, or 1800 consecutive cycles (occupying eight seconds, seventy-two seconds, twelve minutes, and one hour, respectively) on each day of the load regimen just described. All of the birds were male and were fifty weeks old at the beginning of the experiment, and therefore they were skeletally mature. They were from the same genetic stock, and were similar enough in weight and conformation for us to assume that the single load regimen that we employed would produce levels and distributions of strain in the ulnae of the experimental group that were similar to those recorded from the ulnae instrumented with strain-gauges in the group used for calibration.

Results

Strain Data from the Middle of the Ulnar Shaft

Data from three rosette strain-gauges attached around the circumference of the mid-shaft of the ulna allowed us to calculate the magnitude and location of the peak longitudinal strains; the position and orientation of the neutral axis; and the maximum strain rates engendered during normal wing-flapping in the intact bone, wing movement in the functionally isolated bone with external fixators attached, and external loading of the isolated bone preparation.

In the birds used for calibration, the peak longitudinal strain in the intact bones during wing-flapping (six flaps, normalized at each gauge for each of the two birds) was -1900 ± 83 (standard deviation) microstrain (Fig. 2). Less than 150 microstrain was recorded in the bone preparation during wing movement with fixators attached, while the peak strain magnitude during externally applied loading was -2050 ± 7 microstrain. Although the peak strain magnitude was similar in the natural and artificial loading situations, the position of the neutral axis during artificial loading was rotated approximately 90 degrees from its position at peak strain during wing-flapping in the intact bone. Thus, although the peak strains that were engendered artificially were never hyperphysiological, the distribution of strain was quite different from normal. The maximum strain rates engendered by the external loading apparatus, 10,000 microstrain on loading and 12,000 microstrain on unloading, were well below the maximum rates recorded during

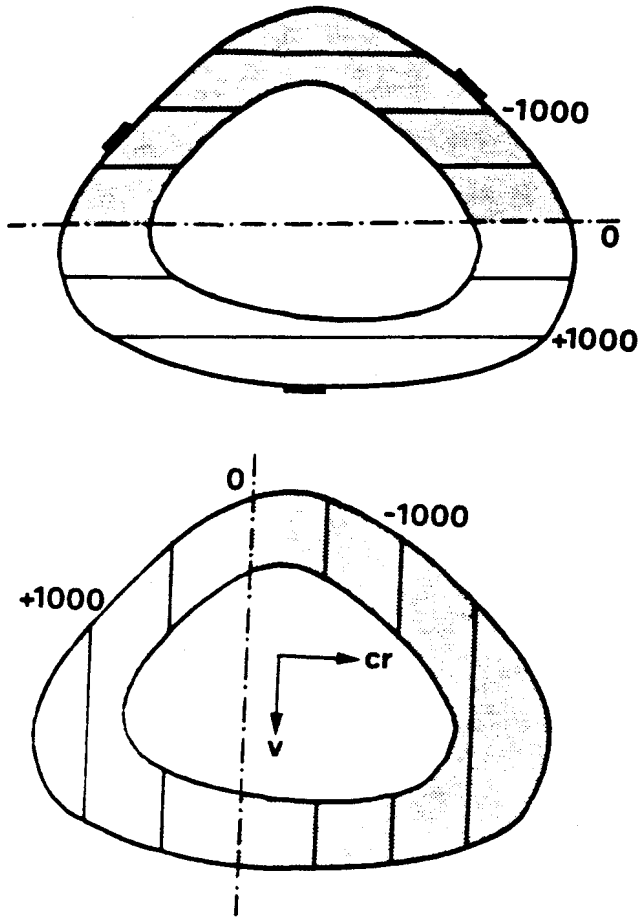

FIG. 2

Transverse sections of the mid-shaft of the ulna show the strain distribution at peak strain caused by (1) natural loading of the intact bone during wing-flapping, and (2) external loading of the isolated ulna preparation. In both cases almost half of the bone section is in tension and the remainder is in compression (shaded area). Although the peak strains produced by the two methods of loading are similar, the proportion of bone under compression, the orientation of the neutral axis, and thus the strain distribution are different (units are microstrain and rectangles depict gauge locations).

flapping in the intact bone (30,000/sec. and 36,000/sec., respectively). The strain situation at the mid-shaft during *in vivo* loading was identical to that produced post mortem.

Remodeling

The remodeling response was assessed in five groups of birds in which the ulna was prepared with pins and caps in the manner already described but in which the mid-shaft was left undisturbed. Therefore no strain data were available for these bones, but the same loads were applied to them as in the calibration group to which strain-gauges were attached. In bones that were placed in the apparatus daily but to which no loads were applied, photon-absorption densitometry indicated that there was a steady decline in bone-mineral content to 88 per cent (± standard deviation of 2 per cent; n = 6) of the original postoperative value by the end of the six-week experimental period (Fig. 3). Histological and microradiographic examination of these "no-load" birds showed cortical thinning due to endosteal resorption, intracortical porosis due to the formation of secondary osteons that were only partially in-filled, and some subperiosteal new-bone formation (Fig. 4-A). Observation of labeled secondary osteons in the transverse sections allowed an estimate to be made of the rate of erosion and infilling. Comparison of these estimates with the published data for human and canine bone[4,5,9] indicated that the osteonal remodeling rate in this preparation was between two and three times faster than the rate that has been reported to occur in those species.

Ulnae that were subjected to four loading cycles a day showed practically no remodeling at any location and were difficult to distinguish from their normal untouched contralateral ulnae (Fig. 4-B). The bone-mineral content remained constant (103 ± 4 per cent of the postoperative value; n = 6).

Ulnae that were subjected to thirty-six cycles per day of an identical strain regimen showed extensive subperiosteal and endosteal new-bone formation. The values for bone-mineral content reached a maximum (143 ± 10 per cent; n = 8) at twenty-eight days, and stabilized at a somewhat lower level (134 ± 8 per cent; n = 6) at five weeks (Fig. 3). Histological study of the bones from birds that were killed at intervals throughout the experimental period showed the phases of this remodeling process. The first changes involved subperiosteal fronds of osteoid developing vertically from the periosteal surface. The calcification of these periosteal fronds coincided with the twenty-eight-day

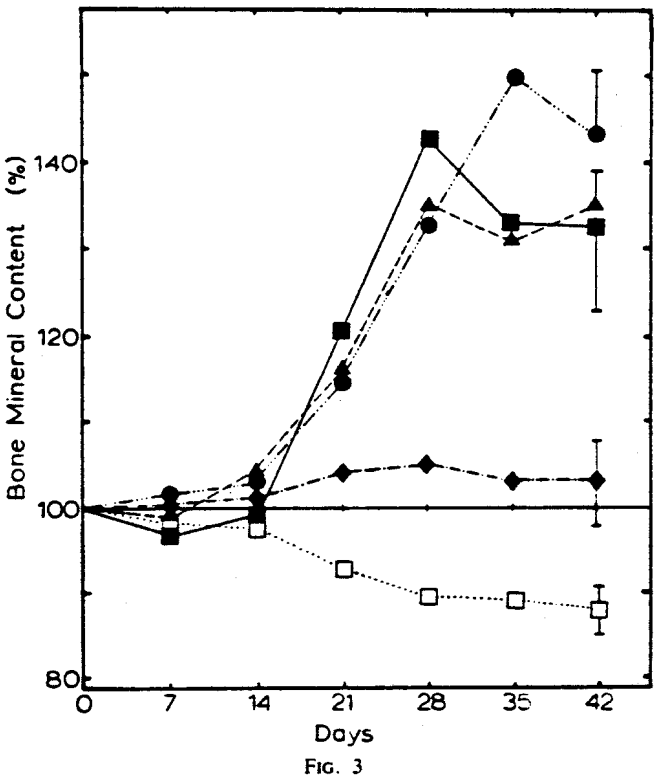

FIG. 3

Percentage change in bone-mineral content at the mid-shaft of the ulna preparation over the six-week experimental period in bones subjected to zero (□), 4 (◆), 36 (■), 360 (▲), or 1800 (●) consecutive cycles a day of an identical load regimen. The vertical lines for six-week values indicate standard deviations. The transverse scans were made using a ^{125}I source.

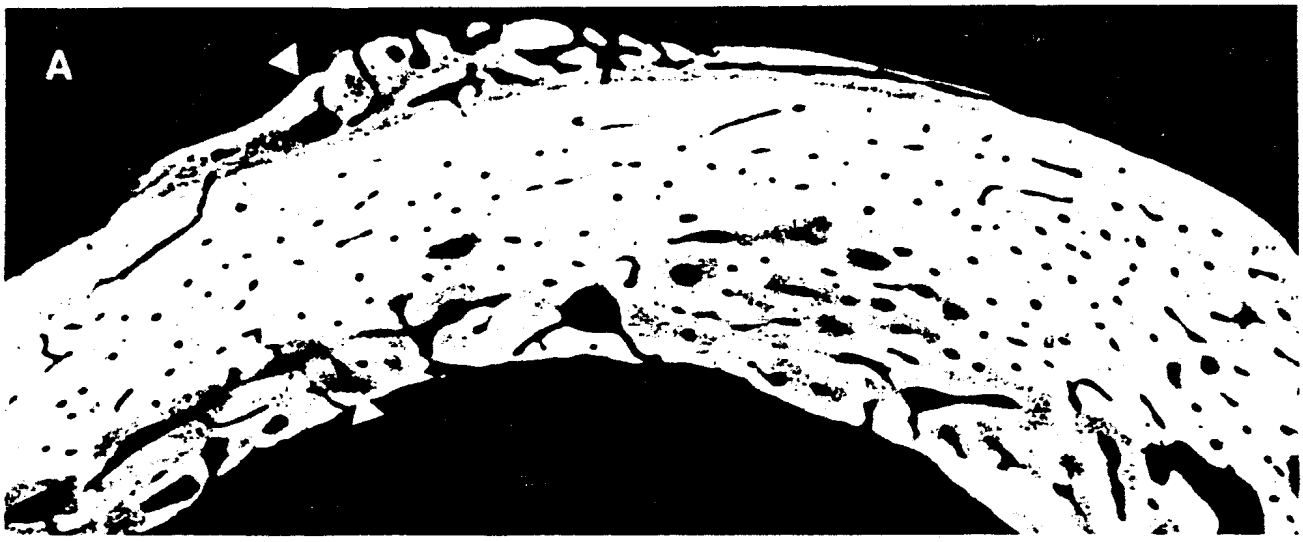

FIG. 4-A

Transverse microradiograph of the functionally isolated and unloaded mid-shaft of the ulna, showing partially in-filled haversian systems (lower arrow) and a cortex that was thinned primarily by endosteal resorption. Some subperiosteal new-bone formation is also evident (upper arrow). The presence of labeled secondary osteons which were formed and subsequently remodeled within six weeks indicates that the remodeling rate in these birds was at least twice as fast as that in humans[5].

peak of bone-mineral content (Fig. 4-C). By five weeks, this transient system of fronds was disintegrating and was being replaced by consolidating bone that was firmly attached to the periosteal surface. At six weeks, the increase in bone-mineral content (133 ± 11 per cent; n = 6) was accounted for by consolidating periosteal new-bone formation being added circumferentially (Fig. 4-D). These proliferative events on the bone's surface were not accompanied by any substantial intracortical remodeling.

The data on histology and bone-mineral content in ulnae subjected to 360 or 1800 strain cycles showed no significant difference in either the arrangement or mass of tissue deposited when compared with the ulnae that were subjected to thirty-six cycles. The final bone-mineral content was 134 ± 3 per cent (n = 6) and 143 ± 6 per cent (n = 5) of postoperative values, respectively (Fig. 3).

Discussion

To be certain that the substantial remodeling events that we observed were primarily the result of the externally applied mechanical input, it is necessary to be sure that the structural changes were not a response to trauma, vascular disturbance, or any other factor that was unrelated to the specific characteristics of the applied loading regimen. Since traumatic disturbance of live bone almost invariably leads to resorption, the virtual absence of any remodeling activity in the bones that were loaded at four cycles per day clearly suggests that neither surgical damage nor the procedures

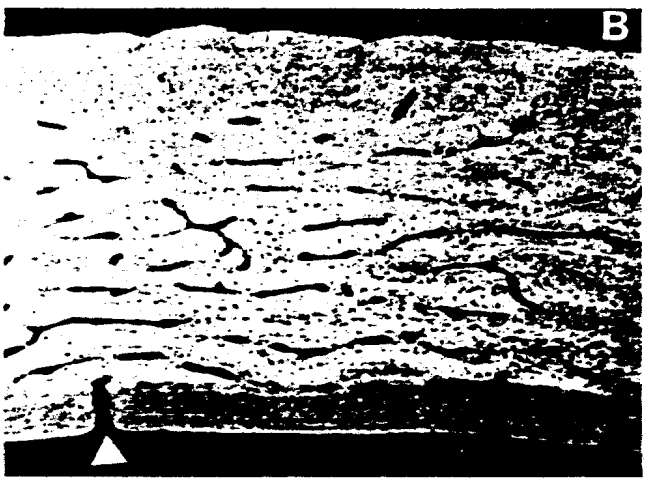

FIG. 4-B

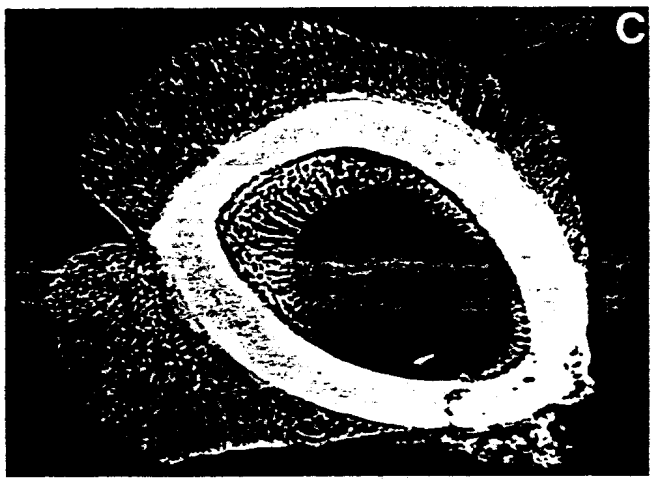

FIG. 4-C

Fig. 4-B: A mid-shaft that was loaded for four cycles a day. The experimental ulnae from this group were difficult to distinguish from their untouched contralateral pairs. The arrow shows a Volkmann's canal traversing the normal band of less well mineralized bone of the endosteal surface. This layer of bone was completely removed in the non-loaded ulnae (see Fig. 4-A).

Fig. 4-C: A section of the mid-shaft of an ulna that was loaded for thirty-six cycles a day for twenty-four days, immediately after calcification of the osteoid fronds. This explosive hyperplastic reaction is remodeled by six weeks to a more consolidated new-bone formation, seen here.

FIG. 4-D

The cortex of an ulna that was subjected to thirty-six cycles a day for six weeks. The newly deposited bone is seen in the entire thickness of the periosteal band (P). Note that there is no remodeling within the pre-existing cortex. E indicates the same endosteal area seen in Fig. 4-B.

associated with external loading would, by themselves, produce aberrant changes in remodeling. We conclude, therefore, that the increase in bone mass seen with thirty-six cycles per day or more was indeed the response to a strain-related osteogenic stimulus rather than an artefact stimulated by surgical interference and subsequent trauma. This view is further supported by the fact that the remodeling engendered by thirty-six cycles per day or more involved little or no resorption and resulted primarily in layers of consolidating new bone firmly attached to the pre-existing cortical surfaces.

If it were to be assumed that the objective of functional adaptation in bone tissue is to regulate peak strain magnitude alone, then in this experiment there would have been no requirement for an increase in bone mass, since the peak strains during external loading were similar to those produced in the intact bone during wing-flapping. Previous experiments using artificial loading in sheep have shown that the rate of change of strain[13] has a profound effect on bone-remodeling, faster strain rates inducing far more new-bone formation than slower ones. For this reason we were careful to ensure that the strain rates that were engendered by external loading in this experiment were less than those recorded during wing-flapping. Since neither the peak strain magnitude, the customary strain rate, nor the number of loading cycles was hyperphysiological, we concluded that the stimulus for the osteogenic response observed in this study originated from the altered strain distribution. This conclusion is consistent with that from long-term remodeling data following selective osteotomy in sheep[12]. In clinical situations, a large number of unusual loading cycles producing such an altered strain distribution not uncommonly leads to stress fracture. The absence of anything resembling a stress fracture in this series may be related to the small number of strain reversals that were employed.

To control against the possibility that the remodeling changes seen in this series were due to some disturbance other than the altered strain situation, it would be necessary to show an absence of adaptive change in bones prepared in a manner similar to those in this study that were externally loaded in such a way as to reproduce the natural strain distribution. Unfortunately, the complexity of the natural loading situation is such that this cannot be achieved by a simple loading device. Thus, while the precise origin of the osteogenic stimulus cannot be identified in this study, we have been able to demonstrate that such a stimulus does exist and can be engendered reproducibly by manipulation of a bone's strain situation.

The resorptive remodeling and decrease in bone mass seen in bone preparations that were not subjected to any external loading is the predictable response to disuse. It can most reasonably be attributed to the removal of a pre-existing stimulus that is engendered by load-bearing in the intact wing. In the absence of this stimulus, bone resorption occurs. That this resorption should be accompanied by bone formation is entirely consistent with the existence of a coupling factor contained within the bone matrix and released by osteoclastic activity[3].

The inferences from this experiment are consistent with the hypothesis that, in bone tissue, some aspect of imposed deformation is capable of producing an osteogenic stimulus, and that possibly this stimulus is responsible for the achievement and maintenance of normal bone mass. It seems that removal of this stimulus permits negatively balanced remodeling, while an increase can produce an adaptive response in which bone formation occurs practically unaccompanied by resorption. In this model, the maximum osteogenic response that we observed was produced by so few strain reversals, occupying such a short time (thirty-six cycles in seventy-two seconds a day), that it seems likely that

the cell population is triggered by a specific stimulus rather than responding to a non-specific (such as a reparative) effect. A small exposure to, or the first result of, this osteogenic stimulus (in this experiment generated within the first four strain cycles and occupying only eight seconds a day) appears to be sufficient to prevent the negatively balanced remodeling that occurs in the absence of any imposed strain regimen.

That this degree of control of remodeling can be achieved by such a short period of applied loading each day is remarkable. If it is applicable to the clinical situation, it suggests that the adaptive processes necessary to ensure structural competence within the skeleton can be engendered by comparatively infrequent loading events rather than requiring long periods of repetitive activity.

NOTE: This study would not have been possible without the active help and advice of colleagues and the participation of the technical staff of the Department of Anatomy, University of Bristol.

References

1. CAMERON, J. R., and SORENSON, JAMES: Measurement of Bone Mineral in Vivo: An Improved Method. Science, **142:** 230-232, 1963.
2. CHURCHES, A. E., and HOWLETT, C. R.: The Response of Mature Cortical Bone to Controlled Time Varying Loading. *In* Mechanical Properties of Bone, edited by S. C. Cowin. AMD ser., vol. 45, pp. 69-80. American Society of Mechanical Engineers, 1981.
3. DRIVDAHL, R. H.; HOWARD, G. A.; and BAYLINK, D. J.: Extracts of Bone Contain a Potent Regulator of Bone Formation. Biochim. Biophys. Acta, **714:** 26-33, 1982.
4. FROST, H. M.: Mean Formation Time of Human Osteons. Canadian J. Biochem. and Physiol., **41:** 1307-1310, 1963.
5. FROST, H. M.: Bone Remodeling and Its Relationship to Metabolic Bone Diseases. Springfield, Illinois, Charles C Thomas, 1973.
6. GOODSHIP, A. E.; LANYON, L. E.; and MCFIE, H.: Functional Adaptation of Bone to Increased Stress. An Experimental Study. J. Bone and Joint Surg., **61-A:** 539-546, June 1979.
7. HEŘT J.; LIŠKOVÁ, M.; and LANDA, J.: Reaction of Bone to Mechanical Stimuli. Part 1. Continuous and Intermittent Loading of Tibia in Rabbit. Folia Morphol., **19:** 290-300, 1971.
8. HEŘT, J.; SKLENSKÁ, A.; and LIŠKOVÁ, M.: Reaction of Bone to Mechanical Stimuli. Part 5. Effect of Intermittent Stress on the Rabbit Tibia after Resection of the Peripheral Nerves. Folia Morphol., **19:** 378-387, 1971.
9. JAWORSKI, Z. F., and LOK, ERIC: The Rate of Osteoclastic Bone Erosion in Haversian Remodeling Sites of Adult Dog's Rib. Calcif. Tissue Res., **10:** 103-112, 1972.
10. JONES, H. H.; PRIEST, J. D.; HAYES, W. C.; TICHENOR, C. C.; and NAGEL, D. A.: Humeral Hypertrophy in Response to Exercise. J. Bone and Joint Surg., **59-A:** 204-208, March 1977.
11. LANYON, L. E.: The Measurement of Bone Strain *in Vivo*. Acta Orthop. Belgica, **42** (Supplementum 1): 98-108, 1976.
12. LANYON, L. E.; GOODSHIP, A. E.; PYE, C. J.; and MACFIE, J. H.: Mechanically Adaptive Bone Remodelling. J. Biomech., **15:** 141-154, 1982.
13. O'CONNOR, J. A.; LANYON, L. E.; and MACFIE, H.: The Influence of Strain Rate on Adaptive Bone Remodelling. J. Biomech., **15:** 767-781, 1982.
14. RUBIN, C. T., and LANYON, L. E.: Bone Remodelling in Response to Applied Dynamic Loads. Orthop. Trans., **5:** 237-238, 1981.
15. RUBIN, C. T.; DELAURA, R.; and LANYON, L. E.: A Mathematical Model to Determine Skeletal Loads from *in Vivo* Bone-Bonded Strain Gauges. Unpublished data, 1983.
16. UHTHOFF, H. K., and JAWORSKI, Z. F. G.: Bone Loss in Response to Long-Term Immobilisation. J. Bone and Joint Surg., **60-B(3):** 420-429, 1978.
17. WOO, S. L.-Y.; AKESON, W. H.; COUTTS, R. D.; RUTHERFORD, LADD; DOTY, DAVID; JEMMOTT, G. F.; and AMIEL, DAVID: A Comparison of Cortical Bone Atrophy Secondary to Fixation with Plates with Large Differences in Bending Stiffness. J. Bone and Joint Surg., **58-A:** 190-195, March 1976.
18. WOO, S. L.-Y.; KUEI, S. C.; AMIEL, DAVID; GOMEZ, M. A.; HAYES, W. C.; WHITE, F. C.; and AKESON, W. H.: The Effect of Prolonged Physical Training on the Properties of Long Bone: A Study of Wolff's Law. J. Bone and Joint Surg., **63-A:** 780-786, June 1981.