Functional Adaptation of Bone in Response to Sinusoidally Varying Controlled Compressive Loading of the Ovine Metacarpus

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Functional adaptation of bone, *i.e.*, the response of bone to conditions of changed mechanical loading, has been recognized for many years.[1,7,18] Wermel,[18] Chamay and Tschantz,[1] and Goodship *et al.*[7] used bone excision techniques (removal of either radius or ulna) to produce gross overload in the remaining bone, and to evaluate from the massive bone proliferation which followed. However, Heřt *et al.*[9,10] and Lišková and Heřt[13] demonstrated a method of applying external loading to a bone which allows the loading to be quantified, and at the same time, preserves relatively physiologic loading conditions. These authors applied controlled bending loads to rabbit tibiae via pins in the proximal and distal metaphyses. Both static and intermittent loading were used, and the resulting bone changes were measured at mid-diaphysis, a site which was as far removed as possible from surgical trauma and the point of load application. Among others, these authors[9,10,13] concluded that, in mature bones, long-term static bending caused no appreciable change in bone shape or structure, whereas intermittent bending loads resulted in bone proliferation in highly stressed regions of both young and mature bones. This work established the importance of time-varying, as distinct from static, loading in promoting bone growth.

Lišková and Heřt[13] remarked that, although their work had established a basic relationship between mechanical loading and bone growth, several factors remained to be investigated, *e.g.*, the threshold value of stress (Throughout this paper the word "stress" is used strictly in its engineering sense of force per unit area.) required to promote osteogenesis, the amount of bone formation at different stress levels, and the rate of bone formation as a function of time. Goodship *et al.*[7] suggested that, in addition, relationships between magnitude of bone strain, strain rate, and strain duration may be important variables in the load-growth phenomenon. In the article of Churches *et al.*,[2] the authors pointed out that quantitative data were needed, not only for different stress levels, but also for different types of stress fields (*e.g.*, pure compression, tension and torsion, as well as bending) if a full understanding of the load-growth relationship was to be obtained. The authors also suggested the use of sinusoidally-varying loading as a means of obtaining simple and repeatable, yet realistic, test conditions. The

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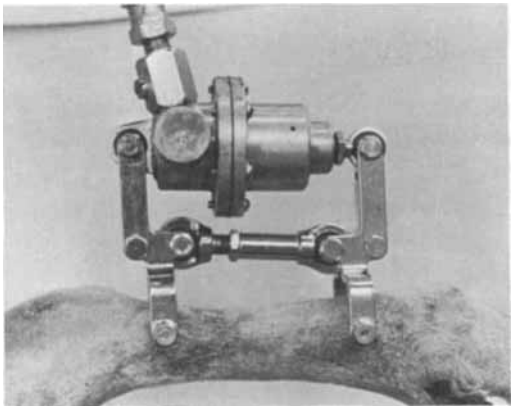

FIG. 1. An assembly of pins, levers and hydraulic slave cylinder on the leg of a test animal.

article described a method of obtaining some of the data, by applying controlled *in vivo* axial compressive loading to the ovine metacarpus, and presented some preliminary results that showed that functional adaptation occurred as a result of the applied loading. A more detailed investigation of the response of the ovine metacarpus to various magnitudes of sinusoidally-varying axial compressive loading is now reported.

METHOD

MECHANICAL LOADING

The basic method, equipment, and surgical procedures have previously been described in detail;[2] hence, these aspects will be only briefly summarized with details of modifications.

Young adult sheep aged between one and two years were used for all tests. Most of the animals were castrated males; females were used when males were unobtainable. The load was applied to the right metacarpus of the test animal via two Steinmann's pins inserted transversely through the proximal and distal metaphyses, in a lateromedial orientation.[2,4] The pin positions chosen were such that the applied loading imposed a compressive stress, as free as possible from extraneous bending or stress concentration effects at the middiaphyseal cross-section.[2,17] A small, light hydraulic slave cylinder was connected by forked levers to the ends of the bone pins. Application of a controlled hydraulic pressure to the slave cylinder thus produced known loads in the bone. An assembly of pins, levers, and hydraulic slave cylinder on the leg of a test animal is demonstrated in Figure 1.

The required hydraulic pressure was applied to the slave cylinder by a specially designed hydraulic pressure generator, which was connected to the slave cylinder via a long flexible hydraulic hose. For all tests, constant amplitude sinusoidally-varying loading was applied at a cyclic rate of 24 cycles/minute for two 1-hour sessions per day, with a test period of 28 days. The load was cycled between zero and some preset maximum value, and at the end of each loading session, the equipment was switched off in the zero load position.

The maximum stress[1] on the bone, σ_{max}, was calculated from the peak value of the applied sinusoidal load and the original cross-sectional area of the bone, assuming that the load applied to the pins produced a spatially uniform stress at middiaphyseal level[2] and that the force on the pins was the only load acting on the bone.

MANAGEMENT OF TEST ANIMALS

Throughout the 28-day test period, the sheep were confined individually in pens (1.0 m × 1.5 m) with raised slatted wooden flooring and were provided *ad libitum* with water and a 1:1 mixture by weight of lucerne (alfalfa) chaff and oats.

The management procedures varied slightly during the series. Immediately after operation, Streptopen (Procaine penicllen *B.P.* 250 mg/ml, Dihydro-streptomycin hydrochloride 250 mg/ml) was administered intramuscularly at 25 mg/kg to the first six sheep tested, but not to subsequent animals. On commencement of loading (24 hours after surgery), Terramycin (Pfizer Laboratories, New York, New York) Oxytetracycline hydrochloride, 50 mg/ml was administered to all sheep tested at 8 mg/kg/24 hours for three days. A similar 3-day tetracycline marking regimen was performed at the end of the loading period, the last injection given 48 hours before killing the animals.

CONTROLS

For all animals tested, pins were inserted only in the right metacarpus, the left bone remaining free from surgical intervention. The left metacarpus was compared directly to the contralateral bone and thus, functioned as a control in each test. However, for convenience, the right and left metacarpi have been designated the *experimental* (E) and *normal* (N) bones, respectively. The term *control* has been reserved for a separate series of tests to determine the effects of the surgical procedure and the presence of the loading apparatus.[4]

As an additional check on possible left *versus* right variations in metacarpal bone areas of crossbred sheep, pairs of metacarpi from ten lambs, 19 mature sheep, and ten aged sheep were ob-

tained from freshly slaughtered animals at an abattoir. The only examination performed on these bones was a simple measurement of the mid-diaphyseal cross-sectional area, using complete bone sections, prepared as described (see Undecalcified Sections).

Bone Examination Methods

Radiographic. Radiographs of both metacarpi from each test animal, showing lateromedial and dorsovolar views, were obtained before surgery, and immediately after death.

Decalcified Sections. Sections were prepared using tissue obtained from around the bone pins in all of the experimental bones and from corresponding sites in the normal bones. Complete transverse cross-sections were also prepared from the mid-diaphysis of all experimental and normal bones. The tissue was fixed in 10% buffered formalin, decalcified in 5% formic acid, and embedded in hydroxyethylmethacrylate before being cut to 6 μm thickness and stained in Toluidine blue and eosin.

Undecalcified Sections. Using unfixed tissue from the mid-diaphysis of each bone, 50 μm sections of the complete bone cross-section, cut at right angles to the long axis of the bone, were prepared by manual grinding.[6]

The sections were examined, photographed, and measured, as previously described,[2] to determine the *original* (O) and *final* (F) cross-sectional areas (A) and changes in area (ΔA) during the test period. In these measurements the *original* area was defined as the area of cortex not labelled with tetracycline. No account was taken of any isolated resorption area or labelled osteon that lay within the corticalis, but the measuring method was such that any cortical remodelling areas lying immediately beneath regions of active bone proliferation were probably assessed as part of the new bone formation.

Microbiology. After death, swabs were obtained from pin tracts of all sheep, placed in Stewart's Transport Medium and refrigerated at 4° until cultured, routinely within 24 hours of sampling. Material from the swabs was plated onto sheep blood and McKonkey's agar as well as placed in cooked heart medium, and then incubated aerobically and anaerobically at 37° for 24 hours.

Statistical Methods

The significance of intracortical osteon counts and the left *versus* right bone area differences were determined by the Student's *t* test. The relationship between increases in the net corrected area and applied stress was obtained by linear regression analysis, followed by the calculation of multiple confidence intervals.[5]

RESULTS

General

All of the sheep recovered quickly from the effects of surgery. Some animals tended to favor the experimental leg for 24–48 hours, but all were judged to be walking normally after 48 hours, except that the experimental limb was generally not used for weight-bearing during the two 1-hour loading periods.

Macroscopic Examination

The preoperative radiographs were used to ensure that the metacarpal growth plates had closed and that there were no gross bone abnormalities.

In general, several macroscopic changes were observed on the experimental bones at the end of the test period. All bones exhibited some periosteal proliferation in the region surrounding the pins, the amount usually increasing with increasing load. This was most readily visible radiographically (Fig. 2). In addition, new tissue also extended at least part way across the metaphyses (Fig. 2). No attempt was made to measure the bone proliferation quantitatively from the radiographs, either in the metaphysis or the diaphysis. Figure 3 shows the newly formed osseous tissue occurring in the proximal metaphyseal region of the bones demonstrated radiographically in Figure 2. In some of the more heavily loaded bones, the periosteal growth had funnel-like osteophytic protrusions surrounding the lateral and medial ends of the pins, particularly those placed proximally. Also, new osseous tissue extended without a break across the metaphysis, thereby encasing the pin in a bone cylinder.

On the loaded bones, there was generally a detectable loss of the initial interference fit of the pins in their drilled holes, although no significant slackness developed. However, in some sheep the pins moved measurably

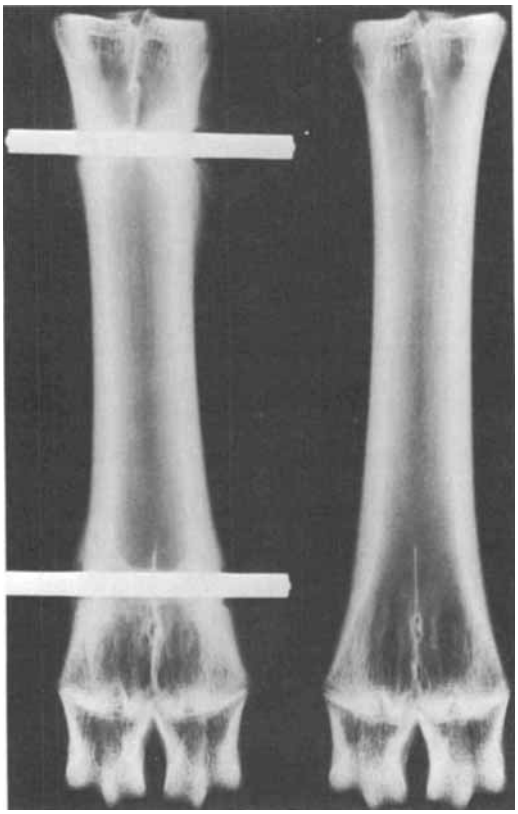

FIG. 2. Dorsovolar view radiographs of a pair of metacarpi at the end of the 28-day test period. The experimental bone had been subjected to a cyclic load of 600N imposed via the bone pins for two 1-hour periods/day throughout the test period. The corresponding mid-diaphyseal stress was $\sigma_{max} = 5.4\ N/mm^2$.

closer together, leaving the hole noticeably enlarged and oval in cross-section. These latter sheep were subsequently found by bacterial culture to have a mixed heavy growth of commensal or pathogenic organisms in the pin tracts; data from these animals are not included in the results. Most often, movement of the pins occurred in the proximal metaphysis. In the mid-diaphyseal region, there was little macroscopic difference between the experimental and normal bone.

MICROSCOPIC OBSERVATIONS

Microscopically, all periosteal proliferation in the immediate vicinity of the pins, including any funnel-like protrusions laid down around the pins, consisted of an open interlacing trabecular network of lamellar bone interspersed by small areas of woven bone. These proliferations were firmly adherent to the compact periosteal bone of the metaphyses. Within the metaphyses the pins were surrounded by a thin layer of dense undifferentiated mesenchymal tissue composed of new thin-walled blood vessels, spindle cells and a light sprinkling of neutrophils and macrophages. Around this layer were trabeculae of osteoid and new bone, which in turn merged into a layer of mature lamellar bone arranged in condensed cancellous fashion, the whole forming a continuous "cylinder" of bone (Figs. 4 and 5). The overall thickness of this "cylinder" wall within the metaphysis was roughly 1 mm. However, on those pins which had been subjected to heavier loads, the undifferentiated mesenchymal layer surrounding the pin was generally thicker (up to 2 mm in the most extreme cases) and this tissue layer was surrounded by less differentiated tissue.

Ground sections prepared from the mid-diaphysis of experimental and normal metacarpi revealed that the bones subjected to external loading responded by periosteal

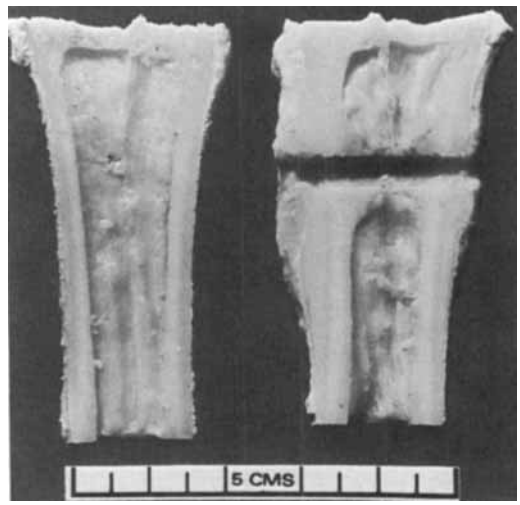

FIG. 3. Longitudinal sections of the proximal ends of the metacarpi shown radiographically in Fig. 2.

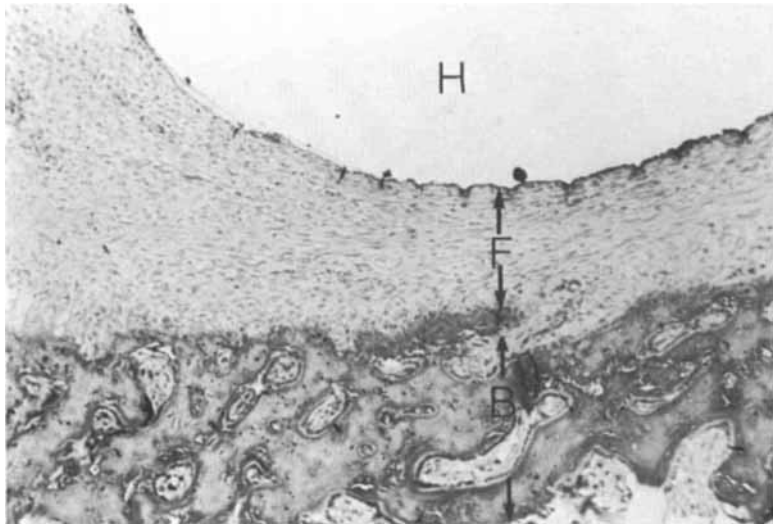

FIG. 4. Proximal pin, Sheep No. 108, σ_{max} = 8.3 N/mm^2. Adjacent to the pin hole (H) is a layer of fibrous tissue (F). Circumferential to the fibrous layer, a dense trabecular layer of bone (B) has formed (Toluidine blue and eosin, original magnification ×78).

and, occasionally, endosteal proliferation at what appeared to be preferential sites. In only two cases of 13 was there any measurable growth on the normal bone, and in both cases, this growth was small compared with the changes in the corresponding experimental bone.

Some relationships were observed between the magnitude of the applied loading, the sites of bone formation, and the structure of the newly formed bone. Where low loads were applied, regions of proliferation appeared on the ground sections as two narrow crescent areas of approximately equal extent on the medio- and laterovolar borders. A typical bone cross-section in this category is shown in Figures 6A and 6B. In bones subjected to moderate loads, these crescent areas became wider and tended to extend further toward the dorsal and volar aspects of the bone. At higher loads, the crescent areas were even wider and sometimes merged into a thin rim of new bone that extended over the remainder of the periosteal surface (Figs. 7A and 7B). On the experimental bones, maximum proliferation generally occurred in approximately equal amounts at the medio- and laterovolar periosteal borders, although on three bones, the proliferation was largely unilateral. At the medio- and laterovolar aspects, there was very tight

FIG. 5. A higher power view of Fig. 4 displays the layered orientation of the fibrocytes (arrow), which become less orderly closer to the osseous tissue (B). Many thin-walled blood vessels (V) occur. Osteoblasts (arrow head) line the osseous trabeculae (Toluidine blue and Eosin, original magnification ×198).

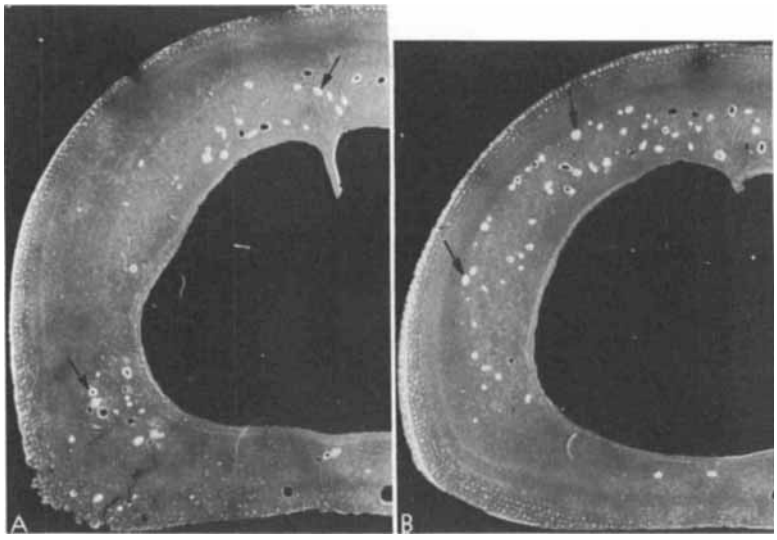

FIG. 6A AND 6B. (A) Normal bone, and (B) Experimental bone. Mid-diaphyseal cross-sections of a lightly loaded experimental metacarpus (σ_{max} = 2.2 N/mm^2) and the normal metacarpus from the same sheep (No. 121). In this animal, tetracycline labelled bone is visible as the light-colored areas on the periosteal surfaces of the experimental bone (ΔA_E = 3. 1 mm^2) and the normal bone (ΔA_N = 0.4 mm^2). Intracortical active sites (arrow) were not included in the area measurements (Unstained 50 μm ground section, high pressure mercury vapour lamp with UG1 excitation filter and K520 barrier filter, original magnification ×7.5).

adherence of the periosteum and its thick overlying layers of dense connective tissue. By contrast, only thin layers of soft tissue were present on the dorsal cortex, and these could be stripped away quite easily. Within the corticalis, the surface layers at the mediovolar, laterovolar, and volar surfaces were composed mainly of remodelling haversian bone, whereas lamellar bone was frequently found on portions of the dorsal surface.

Thin layers of new periosteal bone, which occurred in lightly stressed bones and toward the ends of the crescent areas of all loaded bones, were typically laid down in the form of primary osteons (Fig. 8). As the layer thickness increased, the structure changed

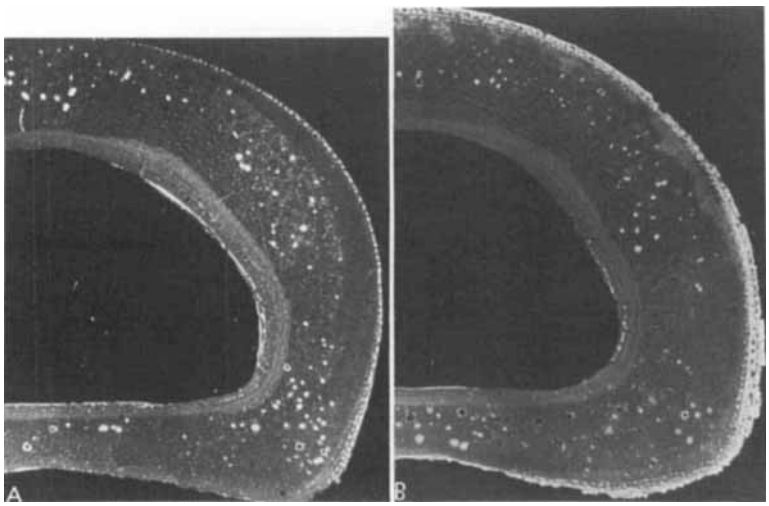

FIG. 7A AND 7B. (A) Normal bone, and (B) Experimental bone. As for Fig. 6, with a more heavily loaded experimental bone (Sheep No. 113, σ_{max} = 7.1 N/mm^2). In this case, there is no measurable labelled area on the normal bone, while the experimental bone has ΔA_E = 10.3 mm^2 (Unstained 50 μm ground section, high pressure mercury vapor lamp with UG1 excitation filter and K520 barrier filter, original magnification ×7.5).

to a trabecular pattern (Fig. 9). For moderately thick layers, the trabeculae often appeared to be wrapped around the pre-existing bone surface while, for the thickest layers observed, the trabeculae were angulated to the surface, almost perpendicular in very thick layers (Fig. 10). In high loading situations, some woven bone was observed immediately subperiosteally in addition to new trabecular bone (Fig. 11). Examination of the superficial haversian systems of the pre-existent bone beneath areas of periosteal osseous proliferation revealed enlarged central haversian canals containing plump basophilic osteoblasts and occasional osteoclasts. In all bones in which periosteal proliferation had occurred as a result of loading, deep cellular troughs extended from the pre-existing periosteal surface into the cortex, but no multinucleated osteoclasts were observed in these troughs. In the metacarpi subjected to higher loads, these apparent troughs were filled with new bone, forming alternate bands of pre-existing bone and new bone, which resulted in a very firm adherence. Microscopic examination of undecalcified sections showed that the thin, almost continuous tetracycline line marking the beginning of the test period was not visible in areas where the bone had subsequently undergone extensive periosteal apposition. Although the assessment is somewhat subjective because of the thickness of the sections (50 μm), apparently, at certain points,

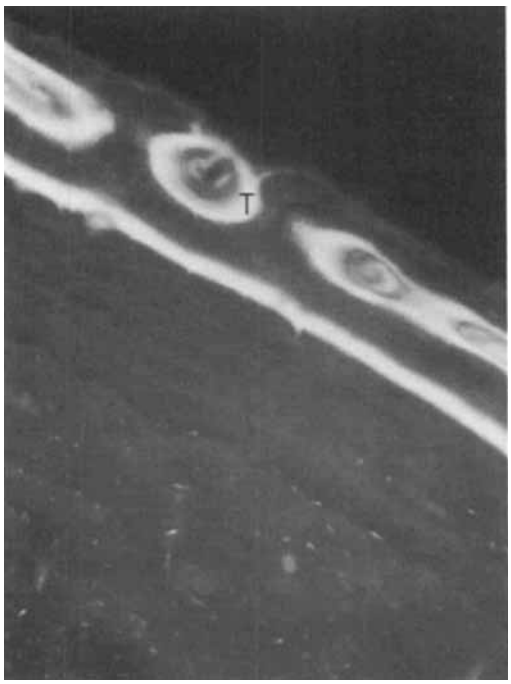

FIG. 8. New periosteal bone, formed in response to low stress and at the ends of the crescent areas, is organized as haversian systems. Tetracycline label (T). Sheep No. 105, $\sigma_{max} = 6.2$ N/mm^2 (Unstained 50 μm ground section, high pressure mercury vapor lamp with UG1 excitation filter and K520 barrier filter, original magnification ×300).

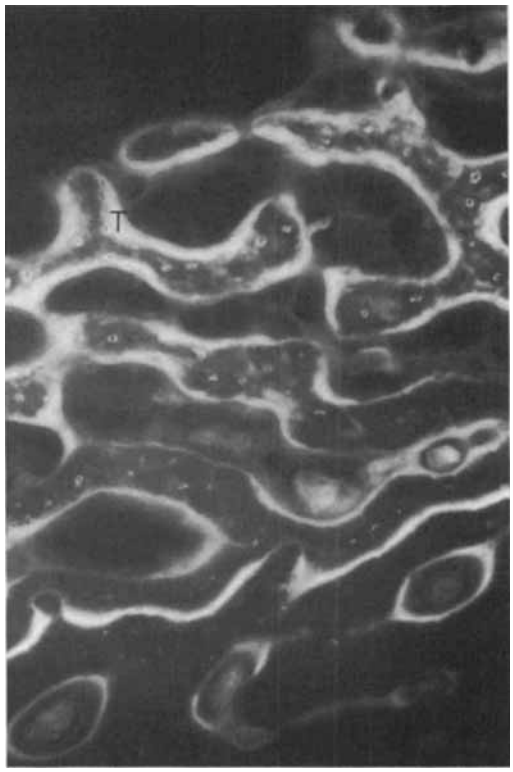

FIG. 9. New periosteal bone, formed in response to moderate stress, is organized as trabeculae. Tetracycline label (T). Sheep No. 117, $\sigma_{max} = 5.5$ N/mm^2 (Unstained 50 μm ground section, high pressure mercury vapor lamp with UG1 excitation filter and K520 barrier filter, original magnification ×300).

FIG. 10. New periosteal bone (B), formed on the mid-diaphyseal border of a test metacarpus, is arranged in an angulated trabecular pattern. This metacarpus had been subjected to a stress of $\sigma_{max} = 5.5\ N/mm^2$ (Sheep No. 109). The newly formed bone is fused tightly to the pre-existing compact bone (C) and the junction is highlighted by arrows. A cuff of preosteogenic cells (P) covers this recently laid-down bone beneath the surrounding soft tissue (T) (Toluidine blue and Eosin, original magnification ×54).

the second tetracycline label extended further into the cortex than the first label. Accompanying the periosteal cellular activity at the various nominated sites was a mild concomitant proliferation and dilation of the small periosteal blood vessels.

In contrast with the activities observed in the periosteal region, there was little cellular activity on the endosteal surface. Generally, measurable areas of endosteal apposition were found on only a few of the more highly loaded bones. Such areas were always of very limited extent and occurred almost entirely on the medio- and laterovolar aspects of the medullary cavity.

In one sheep, which had been subjected to a relatively high load (No. 107), the observed areas of bone apposition were much smaller than might have been expected from the magnitude of the loading. Microscopic examination showed that this sheep had a low level of cellular activity on both the periosteal and endosteal surfaces.

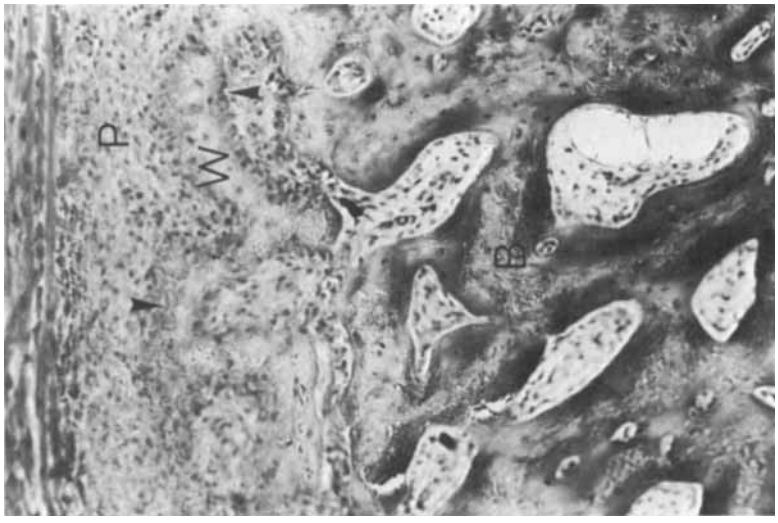

FIG. 11. A higher power view of the outer portion of newly formed trabecular bone (B) in Fig. 10. Plump osteoblasts (arrow head) line the recently differentiated woven bone (W). Preosteogenic cells (P) (Toluidine blue and Eosin, original magnification ×120).

TABLE 1. Number of Reforming Osteons and Resorption Areas in Experimental and Normal Bones*

	Experimental Bone (E)			*Normal Bone (N)*			
Sheep No.	*Osteons*	*Resorption Areas*	*Total Active Areas*	*Osteons*	*Resorp-tion Areas*	*Total active Areas*	*Difference in Total Active Areas, E-N*
105	33	18	51	8	3	11	+40
106	56	38	94	16	6	22	+72
107	18	5	23	24	3	27	−4
108	98	26	124	25	2	27	+97
109	32	23	55	4	4	8	+47
110	8	58	66	10	1	11	+55
111	3	5	8	0	2	2	+6
112	375	43	418	298	55	353	+65
113	196	24	220	143	7	150	+70
117	6	4	10	3	12	15	−5
118	23	4	27	38	9	47	−20
121	77	25	102	50	21	71	+31
134	3	3	6	4	1	5	+1
138	17	36	53	14	3	17	+36
Mean ± SEM**	67.5 ± 27.5	22.3 ± 4.6	89.8 ± 29.6	45.5 ± 21.8	9.2 ± 3.8	54.7 ± 25.2	35.1 ± 9.4

* The number of osteons includes all single labeled and double labeled sites. Resorption areas include all areas with "ragged" edges, regardless of tetracycline labelling on any portion of the rim. The difference in total activity between experimental and normal bones is significant ($p < 0.005$).

** SEM = Standard error of the mean.

The total cortical activity in the experimental and normal bones was estimated by counting the active sites visible on the ground sections. The number of active sites on each bone, *i.e.*, the sum of all of the labelled osteons plus all the resorption areas, is given in Table 1. These figures indicate a large variation in cortical bone activity, from highly active to almost completely quiescent, for the different pairs of bones. Smaller variations in activity occur within each bone pair. There is a significant increase in the number of active sites in the experimental bones when compared with their contralateral normal counterparts (Student's *t* test, $p < 0.005$; Table 1). A similar statistical result was obtained by counting the osteons alone, but the result for resorption areas alone was less significant ($p < 0.025$).

Large resorption areas were frequently found in the corticalis adjacent to regions of maximum periosteal proliferation. Notwithstanding their somewhat ragged appearance, permanently fixed tetracycline, indicative of osseous formation, was usually present on portions of the rim of the resorption areas.

Microbiology

In all of the animals, the microbiologic growth found in the cultures varied from nil to moderate, most having very few colonies. This growth usually consisted of a mixed flora suggestive of sampling contamination by the following species (in order of frequency of occurrence): *Escherichia coli, Streptococcus faecalis, S. epidermidis, Staphylococcus albus,* and other coagulase negative strains as well as other untyped nonhemolytic streptococci and micrococci. Three animals were rejected from the series because of bacterial osteomyelitis. Two of these animals were rejected on clinical and microbiologic evidence, one with a very heavy growth of *E. coli*, the other with moderate growths of *E. coli* and *Staph-*

TABLE 2. Data for Experimental and Normal Bones from Animals Subjected to Applied Loading*

			Bone Cross-sectional areas $(mm)^2$								
			Experimental Bone (E)			Normal Bone (N)					
Sheep No.	Sheep Mass (kg)	Applied Loading (N)	Original Area (A_{EO})	Final Area (A_{EF})	Area Increase (ΔA_E)	Original Area (A_{NO})	Final Area (A_{NF})	Area Increase (ΔA_N)	Difference in Original Areas ($A_{EO} - A_{NO}$) (mm^2)	Corrected Area Increase (ΔA_C) (%)	Applied Stress σ_{max} (N/mm^2)
105	63.0	690	112.0	116.2	4.3	110.4	110.4	0.0	+1.6	3.3	6.2
106	43.5	493	104.6	110.0	5.3	93.5	94.6	1.1	+11.1	2.4	4.7
107	52.0	801	98.4	99.7	1.3	102.7	102.7	0.0	−4.3	0.8	8.1
108	63.5	890	107.7	114.1	6.4	111.4	111.4	0.0	−3.7	5.4	8.3
109	63.5	645	117.9	126.5	8.6	119.0	119.0	0.0	−1.1	6.8	5.5
110	54.5	458	116.0	116.9	0.9	116.3	116.3	0.0	−0.3	0.3	4.0
111	53.5	356	102.1	106.2	4.0	106.7	106.7	0.0	−4.6	3.5	3.5
112	44.0	587	117.1	119.3	2.2	124.1	124.1	0.0	−7.0	1.5	5.0
113	47.5	823	115.6	125.8	10.3	115.1	115.1	0.0	+0.5	8.4	7.1
117	36.0	476	86.8	90.8	4.0	98.2	98.2	0.0	−11.4	4.1	5.5
118	47.5	476	100.4	106.7	6.3	108.4	108.4	0.0	−8.0	5.8	4.7
121	43.5	227	101.6	104.7	3.1	106.2	106.6	0.4	−5.4	1.9	2.2
134	56.5	414	101.7	104.9	3.2	105.8	105.8	0.0	−4.1	2.7	4.1
138	52.0	761	104.8	111.2	6.4	104.9	104.9	0.0	−0.1	5.6	7.3
Σ/n = SEM			108.8 ± 2.2			106.2 ± 2.4			−2.6 ± 1.5		

* All bone areas were measured at mid-diaphysis. The applied stress, σ_{max}, has been calculated on the assumption of uniformly distributed stress from the peak value of the applied loading and the original cross-sectional area of the experimental bone, A_{EO}.

O = original.

F = Final.

A = Cross-sectional areas.

ΔA = Changes in area.

C = Corrected.

ylococcus aureus. The third sheep had no clinical symptoms but was rejected on microbiologic and histopathologic grounds when evidence of low grade osteomyelitis was found in the proximal pin tract.

Assessment of Bone Area Changes

Data relating to the test animals and the measured changes in bone cross-sectional areas at mid-diaphysis are given in Table 2.

In a previous nonloaded control series,[4] the expected reaction of the experimental metacarpus to the surgical procedure was established. For most animals, no evidence of bone proliferation was found on the normal metacarpus at the end of the test period and only a small area of proliferation on the experimental metacarpus. However, some surgical control animals had small areas of proliferation on the normal metacarpus, always with significantly increased proliferation on the experimental metacarpus.

The results of the nonloaded surgical control series are summarized in Figure 12, as a plot of the measured areas of experimental bone proliferation, $\Delta A'_E$, *versus* normal bone proliferation, ΔA_N, for individual bone pairs. The linear regression line may then be used to determine the expected value of proliferation on an experimental bone, due to the surgical procedure alone, from an observation of the proliferation on the contralateral normal bone. For example, where there is no area increase on the normal bone, an area increase of 0.5% is expected on the experimental bone; where a 1.0% area increase is observed on the normal bone, the expected reaction on the experimental bone is 2.5%. Subtractive corrections obtained from Figure 12 have been used to determine the corrected area response, ΔA_C, of the experimental bones resulting from the applied loading.

The corrections applied to the compressive loading test data in this paper were generally quite small, since in 11 of the 13 tests there were no measurable area increases in the normal bone, and in the remaining two animals, this change did not exceed 1.2%.

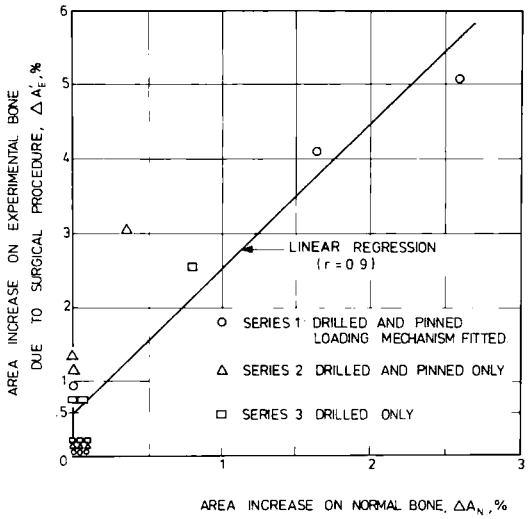

FIG. 12. Data from the surgical control series (Churches *et al*, 1980), showing the mid-diaphyseal reaction of the experimental metacarpus to the surgical procedure. The corrected area increase, ΔA_C, for bones subjected to compressive loading may be obtained by subtracting from the observed area increase, ΔA_E (Table 2), an area $\Delta A'_E$, which depends on the observed activity of the normal bone. Reproduced, by permission, from Journal of Biomechanics.

Values of ΔA_C are shown in Table 2 and have been plotted against the applied stress, σ_{max}, in Figure 13. These data points show a general trend toward increasing bone proliferation with increasing applied stress. The straight line represents the linear regression line ($r = 0.75$), while the curved lines are the 90% multiple confidence intervals for the mean regression line.[5] The result for sheep No. 107 was not included in the statistical analysis (see Discussion).

The original cross-sectional areas, A_{EO} and A_{NO}, of pairs of experimental and normal bones shown in Table 2 vary by up to 11 mm^2 (approximately 11%) within individual pairs. The mean area difference within pairs, defined as $\Sigma (A_{EO}-A_{NO})/n$ (where n is the number of animals), has a value of -2.6 mm^2 (approximately -2.5%), with a

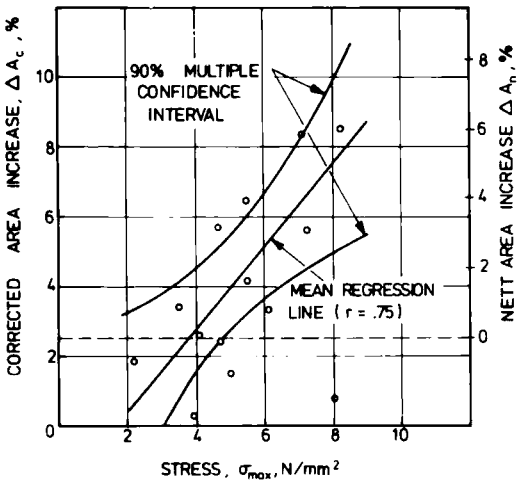

FIG. 13. Corrected area increase, ΔA_C, on the experimental metacarpi as a function of applied stress, σ_{max}. The figure shows the mean regression line and the 90% multiple confidence interval for the mean regression line. The auxiliary (right) ordinate shows the *net* area increase, ΔA_n, which accounts for the initial resorption on the experimental bone.

standard error of 1.5 mm.^{2} The nonzero mean is significant ($p < 0.05$).

The results of the mid-diaphyseal cross-sectional area measurements on the bone pairs obtained from the abattoir are summarized in Table 3. For the three groups of bones (from lambs, mature sheep, and aged sheep), none of which had been subject *in vivo* to any surgery or external loading, the mean area differences within pairs are small and are not significantly different from zero.

DISCUSSION

For the work reported in this paper, it was important that the applied loading should be controllable and quantifiable, yet similar in its essential characteristics to the natural environmental load experienced by the bone. Data on bone strain obtained from *in vivo* tests with strain gauges bonded to the osseous surface, are available for several mammalian bones, including the ovine radius[11] and tibia,[12] porcine radius,[7] and equine metacarpus, radius, tibia, and metatarsus.[16] These data demonstrate the cyclic nature of strain and hence, loading, to which such bones are subjected during locomotion. The data indicate wide variations in the type and magnitude of loading for the various bones. While a compressive loading component is common to all of the bones tested, large bending moments may be superimposed, particularly where the bone has significant curvature. Hence, a bone such as the radius may actually be subjected to tensile strains on one aspect.[7,11,16] While there are no published data for the ovine metacarpus, the anatomy of the ovine forelimb suggests that the natural loading on the metacarpus will be predominantly compressive with, perhaps, minor bending loads due to forces in the suspensory ligament. The sinusoidally varying compressive loading used in these tests may, therefore, be regarded as a reasonably natural type of loading condition, yet one that is readily generated and allows standard, repeatable loading.

The change most clearly visible on the experimental bones after the test period was osseous proliferation surrounding the Steinmann's pins. The bone stresses close to the pins are expected to be high and the subsequent proliferation, both within the metaphyses and on their periosteal surfaces, primarily a response to those stresses. This is demonstrated by the fact that there was very little proliferation around the bone pins on the nonloaded surgical control series.[4] Recognizing the complexity of the stress field in this region, and the probability of a mild foreign body reaction, no conclusions have been made related to stress induced growth in this zone. Instead, concentration has been on the mid-diaphysis, a cross-section as far removed as possible from the sites of surgery and on which a reasonably uniformly distributed compressive stress is expected to exist.

There are two requirements for the assumption of uniformly distributed stress to be valid: (1) The position of the pins must be such that the plane containing the two

TABLE 3. Summary of Cross-Sectional Areas, Area Differences within Pairs and Statistical Analysis for Bone Pairs from Lambs, Mature Sheep and Aged Sheep not Subjected to Surgery*

	Lambs	*Mature Sheep*	*Aged Sheep*
Number of bone pairs	10	19	10
Mean cross-sectional area ± SEM** (mm^2)	92.5 ± 4.1	99.9 ± 4.8	94.8 ± 4.2
Mean area difference (right-left) ± SEM (mm^2)	−1.1 ± 1.1	−0.2 ± 0.7	−0.9 ± 0.8
p	>0.1	>0.3	>0.1

* All cross-sectional areas were measured at mid-diaphysis. Values of p refer to the significance of the area differences.

** SEM = Standard error of the mean.

pins passes through the centroid of the mid-diaphyseal cross-section.[2] This ensures that no bending stress is applied to the bone. (2) The pins must be far enough from the mid-diaphysis for stress concentration effects to be negligible. Waldron[17] has shown theoretically that in an ovine metacarpus with pins in the metaphyses, stress concentration effects are expected to be negligible over the mid-third of the diaphysis. The fact that test metacarpi are free from any significant stress concentration at mid-diaphysis, using strain gauges on bones *in vitro*, was also verified experimentally.[2]

Since the reaction of the metacarpus to the surgical procedure has already been documented[4] and can be taken into account when assessing the bone proliferation at mid-diaphysis, it may be argued that the experiments indicate quantitatively the changes to be expected when a bone is subjected to an increased level of natural environmental stress. Such a claim is difficult to make, even in qualitative terms, when bone growth has occurred following major surgical intervention, *e.g.*, osteotomy of the radius or ulna, leaving the remaining bone to support the load due to locomotion.[1,7,18] Furthermore, the basic method is applicable to a systematic investigation of the effects of strain, strain rate, and strain duration[7] and, with some modification to the loading mechanism, could be used to investigate the effects of different types of stress field on a particular bone.[2]

The authors' experiments have shown that the experimental metacarpi have a markedly nonuniform distribution of periosteal apposition on the mid-diaphyseal cross-section. To demonstrate that the areas of maximum apposition on the medio- and laterovolar borders did not arise from and were not merely an extension of the osseous fillets buttressing the pins, tests were conducted on an additional four sheep, with pins inserted in the metacarpus in the dorsovolar direction rather than lateromedial.[3] These metacarpi were subjected to sinusoidally applied loading within the range reported in this paper. Distinct swelling of the limb below the pinned carpus developed in all four sheep, apparently because of damaged venous return on the volar surface. In one sheep which had only minor swelling, the amount of mid-diaphyseal apposition was consistent with the results reported for the sheep with lateromedial pins. In a second sheep which had gross edema of the lower limb, although no evidence of infection was found, periosteal apposition was much greater than might have been expected from the level of applied stress. The remaining two sheep developed clinical signs of cellulitis and possible osteomyelitis, which were controlled by high levels of systemic antibiotics. However, in both

of these animals, *post-mortem* examination revealed bacteriologic and histopathologic evidence of osteomyelitis and massive periosteal proliferation on the mid-diaphyseal cross-section. Nevertheless, in all four sheep pinned in the dorsovolar direction, the distribution of new bone on the mid-diaphysis was similar to that found in sheep with lateromedial pins, the areas of maximum apposition again appearing on the medio- and laterovolar borders.

By contrast, the areas of mid-diaphyseal proliferation on our nonloaded surgical control series[4] were somewhat more randomly distributed, although still generally favoring the medio- and laterovolar sites. Of the 18 control sheep which were subjected to either drilling or drilling and pinning of the metacarpus, measurable proliferation occurred in nine animals. Of these, maximum proliferation was found on the dorsal aspect of two sheep, equally on the medio- and laterovolar borders of three sheep and unilaterally on either the medio- or laterovolar aspect of four sheep. Although the assessment was subjective, there was a recognizable difference in the distribution of new bone between the controls and the test animals. Thus, while there appears to be some evidence supporting the concept of biologically preferred periosteal growth sites, it would seem likely that the presence of additional loading is also a factor in determining the location of the periosteal response.

No clear-cut reason was found for the almost complete absence of endosteal proliferation. There were always more osteoblasts on the periosteal surface than on the endosteum. Many of the endosteal osteoblasts appeared flattened and lacking cytoplasm, as compared with the plump periosteal osteoplasts. Thus, the endosteal cells may have been functionally quiescent, needing longer or stronger stimuli to induce recognizable osteogenic activity.

The wide range of cortical activity for the various bone cross-sections (Table 1) is consistent with the findings of Harris *et al.*,[8] who found dramatic temporal and spatial variations in cortical remodelling activity in adult dogs. However, they observed that when a large number of bone sections from the same animal were analyzed, equal cortical bone formation rates occurred overall on both skeletal sides. Similarly, with a large enough sample of animals, statistically equal amounts of cortical activity might be expected from an assessment of two homotypic sites in each animal. By contrast, in the data for pairs of homotypic metacarpal sites present in Table 1, the level of cortical activity in the experimental bones is significantly greater ($p < 0.005$) than in the normal bones. Since the nonloaded control series[4] showed no significant difference in the level of cortical activity between experimental and normal metacarpi, the increased cortical activity for the test sheep must be due to the increased loading, rather than to the surgical procedure.

While some of the scatter in the increased area shown in Figure 13 is certainly due to biologic factors, at least part of the variation may be attributed to uncertainties in the magnitude of the applied loading. The animals were confined in pens throughout the test and their limited ambulatory efforts were certainly not expected to cause functional adaptation on any of the limb bones. On the other hand, while the possibility of some disuse atrophy can not be completely eliminated, no microscopic evidence of atrophy was found on any of the normal bones. The experimental limb was generally not used for weight-bearing during the two 1-hour loading periods. However, weight-bearing may sometimes have coincided with the applied loading in some animals, causing a higher peak stress for that particular load cycle. The effect of such high-stress cycles cannot yet be quantified because there are no published data on the normal *in vivo* stresses on the ovine metacarpus, nor has the relationship between the number of load cycles and the subsequent bone response been established.

In one sheep (No. 107) which had been subjected to a high level of stress, very little new bone had been formed and periosteal, endosteal and intracortical activities were still at a low level at the end of the test. It is possible that Sheep No. 107 was an old animal inadvertently included in a batch of 1–2-year-old sheep, as supported by the presence of relatively large areas of lamellar bone on the dorsal aspect of bone cross-sections. However, it is also possible that, where bone activity is initially at a very low level, a period longer than 28 days is required to initiate any substantial remodelling activity. Such factors can only be elucidated by tests using a larger number of animals and different durations.

The apparent difference between the original areas of the experimental and normal bones (Table 2) has arisen because the original cross-sectional areas can not be measured directly, but must be inferred from measurements obtained at the end of the test. Since the cross-sectional areas of left and right metacarpi not subjected to surgery (Table 3) were found equal, the −2.5% difference between the experimental and normal bone areas shown in Table 2 has been interpreted as a measure of the periosteal surface resorption that occurred during the test period. This resorption is presumably the first step in the bone apposition process.[14,15] The microscopically observed loss of some portions of the initial tetracycline marker is consistent with the above area discrepancy.

The mean area difference of −2.5% for the test animals may be compared with the −1.4% mean difference previously reported for the nonloaded surgical control sheep.[4] There is no significant difference between these two means, and hence the resorption must occur predominantly (although perhaps not entirely) as a result of the surgical intervention rather than the applied loading.

Assuming that the observed periosteal resorption in the experimental bones is the first step in the bone apposition process, the overall area change on these bones may be presented in two ways. The corrected area ΔA_C, which presumably includes most (if not all) of the resorption, may be defined as a measure of the total active cross-sectional area resulting from the applied loading. The results are presented in this form in Figure 13, with ΔA_C as ordinate. Alternatively, the 2.5% (or at least a large proportion of it) may be regarded as nothing more than a surface remodelling required to provide a bond for the newly-forming bone. If this is the case, the *true* bone proliferation area could be estimated by subtracting from ΔA_C, a figure which compensates for the resorption on the individual bones. However, because the precise resorption figure can not be determined for individual bones, and the amount of resorption does not depend significantly on loading,[4] 2.5% has been subtracted from all values of ΔA_C to give the *net* area, ΔA_n, shown as the right-sided ordinate in Figure 13.

From Figure 13, based on the values of ΔA_n, the threshold stress for bone proliferation lies below 4.8 N/mm^2, with a probable value of 3.7 N/mm^2. However, in view of the uncertainty in the total loading, which may have occurred because of possible weight-bearing on the experimental limb coinciding with the applied loading, these threshold values must be interpreted with caution.

SUMMARY

To obtain carried load, quantitative data relating functional adaptation of bone to controlled, sinusoidally-varying, compressive loading of constant amplitude was applied to the right metacarpal bones via Steinmann's pins inserted through the metaphyses in 13 sheep. Loading was applied for two 1-hr periods/day at 24 cycle/minute, throughout a test period of 28 days. The amplitude of the applied loading was varied from test to test, giving peak stresses on the mid-diaphyseal cross-section that ranged from 2.2–8.3 N/mm^2. In the mid-diaphyseal region,

the bone responded by periosteal apposition, with maximum proliferation usually occurring on the medio- and laterovolar borders and relatively little new bone on the dorsal and volar aspects. The cross-sectional area of new bone was roughly proportional to the applied stress, with a maximum increase of aproximately 8% in the most highly stressed bones. There was also evidence that periosteal resorption had occurred, presumably as the first step in the apposition process. A significantly increased level of intracortical activity was found in the right metacarpus as compared with the contralateral bone.

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