Calc. Tiss. Res. 2, 20—29 (1968)

Original Papers

Effect of Disuse Osteoporosis on Bone Composition: the Fate of Bone Matrix*

LeRoy Klein, Donald G. Kanefield, and Kingsbury G. Heiple
Division of Orthopaedic Surgery, Department of Surgery, Case Western Reserve University, School of Medicine, Cleveland, Ohio

Received December 29, 1967

Disuse bone atrophy was induced in 10 adult dogs by means of brachial plexus section and/or elbow disarticulation. After 9 to 12 weeks of disuse intact humeri were examined by X-ray, and their physical properties determined. The *whole* humeri were isolated, defatted, dried to constant weight, and their mineral and collagen content determined.

Eight out of 10 experimental (non-used) humeri demonstrated evidence of decreased radiodensity. The non-used limb demonstrated parallel loss in dry, fat-free weight (−23.2%), in collagen (−25.3%), and in mineral (−26.1%), as compared to the normal limb. The data indicated that the major portion of the lost bone tissue in disuse osteoporosis is replaced by water, fat, and other unidentified organic materials rather than fibrous tissue, and that collagen is lost in equal proportion to mineral. The proportionately greater loss of collagen and mineral than of dry, fat-free weight appears to be due to an increase of non-collagenous, non-lipid organic material, presumably protein.

Key words: Bone Atrophy — Bone Resorption — Calcium — Collagen — Osteoporosis.

Atrophie osseuse d'immobilisation était induite par section de la plexus brachial et/ou désarticulation de la coude. Après 9 bis 12 semaines de désuétude huméri intactes furent examinés par rayons X et leur qualités physiques déterminés. Les huméri *entiers* furent isolés, dégraissés, desséchés au poids constant, et leur teneur en cendre d'os et collagène déterminés.

Huit des 10 huméri immobilisés monstrèrent radiodensité diminué. La jambe inusitée monstra perte parallèle en poids sec et poids sans gras (−23.2%), en collagène (−25.3%), et en cendre d'os (−26.1%) en comparaison de la jambe normale. Les données monstrèrent que la portion plus grande du tissu osseux perdu en atrophie osseuse d'immobilisation est remplacée par de l'eau, du gras, et des autres matériels organiques inconnus plutôt que par tissu fibreux, et que collagène est perdu en proportion du minéral. La perte proportionellement plus grande du collagène et de la cendre d'os que du poids sec et poids san gras semble dû à une augmentation du matériel organique, non-collagène, non-lipide, probablement protéine.

Immobilisations-Knochenatrophie wurde in 10 erwachsenen Hunden herbeigeführt durch Brachialplexusschnitt und/oder Ellenbogenexartikulation. Nach 9—12 Wochen der Immobilisation wurden die intakten Humeri unter Röntgenstrahlen untersucht und ihre physischen Eigenschaften bestimmt. Der *intakte* Humerus wurde isoliert, entfettet, zu konstantem Gewicht getrocknet und der Knochenaschengehalt und Kollagengehalt bestimmt.

Acht der 10 experimentellen (immobilisierten) Humeri demonstrierten Beweis von verringerter Röntgendichte. Die immobilisierte Extremität zeigte ähnlichen Verlust in trockenem, fettfreiem Gewicht (−23,2%) in Kollagen (−25,3%) und Knochenasche (−26,1%) im Vergleich zur normalen Extremität. Die Daten deuten an, daß der Hauptanteil des Verlustes

* This investigation was supported in part by Public Health Service Grants Tl-AM-5437 and HD-00669, and in part by the Rainbow Hospital Research Fund and Easter Seal Research Foundation.

For reprints: Dr. LeRoy Klein, University Hospitals of Cleveland, 2065 Adelbert Road, Cleveland, Ohio 44106, USA.

des Knochengewebes (bei Immobilisations-Knochenatrophie) von Wasser, Fett und anderen unidentifizierten organischen Substanzen ersetzt wird, als von Faserngewebe und daß Kollagen und Knochenasche im gleichen Verhältnis verloren gehen. Der größere, proportionale Verlust an Kollagen und Knochenasche, eher als der Verlust an fettfreiem Gewicht, scheint in der Zunahme an nicht kollagener, nicht lipoider, organischer Substanz, vermutlich Protein, zu liegen.

Introduction

The fate of bone matrix in disuse osteoporosis has been defined usually in terms of the matrix being lost in equal proportion to the mineral (Allison and Brooks, 1921; Landoff, 1942). Radiographic studies which were the first used to study disuse osteoporosis, demonstrated a loss of bone mass (Grey and Carr, 1915). These studies give no compositional information in that they are an index only of the mineral mass of cortical and cancellous bone. They are further limited in a quantitative sense by a lack of sensitivity to small changes in bone mass.

Histological study of disuse osteoporotic bone has been interpreted in various ways. Allison and Brooks (1921) produced disuse osteoporosis of humeri by brachial plexus section in immature and adult dogs. They described the marrow as containing increased amounts of fat. Other workers (Landoff, 1942; Burdeaux and Hutchison, 1952) have described granulation and loose vascular connective tissue replacement of resorbed bone in disuse osteoporosis (plaster immobilization) of adult rabbits and dogs. No evidence of fibrous tissue replacement of osteoporotic calcanei was observed in adult rabbits with heel cord section (Geiser and Trueta, 1958). These histological studies varied in their interpretations, the replacement of resorbed bone being attributed to fat (Allison and Brooks, 1921), water (Landoff, 1942) or fibrous tissue (Landoff, 1942; Burdeaux and Hutchison, 1952). It is often difficult to evaluate histological observations due to the various methods used in cleaning and extracting organic components of bone and bone marrow prior to its fixation and staining.

Compositional studies of disuse osteoporotic bone began with the work of Allison and Brooks (1921). They determined the per cent of water, organic matter and mineral in *intact* humeri and *biopsies* of compact bone in young and adult dogs. The dogs were subjected to brachial plexus section and sacrificed from 24 to 256 days postoperatively. The content of water was calculated from the difference between fresh and dry weights. The amount of organic component was determined as the difference between dry and ash weights on dry, but not fat-free bone. In bone samples obtained by *biopsy* there was little difference between the mineral content of the disused and control bone. However, in the *whole* experimental humerus there was a decrease in the mineral content of a young bone from 23.1% to 17.2% and in adult bone from 35.7% to 26.1%. From Allison and Brooks' (1921) data on *whole* humeri we calculated that there was a 29% loss of mineral mass, but only a 2.2% loss of organic matter. Armstrong *et al.* (1945); Armstrong (1946), utilizing young and mature rats, demonstrated a greater net loss of total mineral than of organic material in dry defatted bone of paralyzed whole humeri. These observations have been confirmed (Gedalia *et al.*, 1966) on intact femurs from disuse atrophy induced by sciatic and femoral nerve sections in young adult rats. Thus, there appeared to be a difference in mineral and organic composition between whole bones and biopsy samples.

Physical changes in disuse osteoporotic bone have been obtained via the determination of weight, volume, and specific gravity (Allison and Brooks, 1921). These physical measurements have shown varying losses in weight and density of osteoporotic bone, depending on the species of experimental animal, the age of the animal, and the method of rendering the bone osteoporotic. Since mineral is the largest component of bone by weight, weight and specific gravity measurements largely depend on change in mineral content. Usually the content of organic matter of bone has been determined indirectly by the difference between the dry, fat-free weight of bone and the ash weight.

On the basis of histological studies in disuse osteoporosis, it has been reported that the bone marrow is fibrous (McLean and Urist, 1961) and compositional studies have shown that the intact atrophic bones exhibited greater net losses of total mineral mass than of organic matter (Armstrong *et al.*, 1945; Gedalia *et al.*, 1966). The purpose of this investigation was to determine in adult dogs the relative losses of mineral and collagen in intact (whole) humerus. Chemical analysis for hydroxyproline, an amino acid that is relatively specific for collagen (Neuman and Logan, 1950a), was used to quantitate collagen.

Adult dogs were used to avoid the complicating factors of growth (Pottorf, 1916; Armstrong, 1946). The use of whole intact humeri instead of biopsy material circumvents the heterogeneity of bone in reference to the amount of cancellous and cortical bone found in osteoporotic and control extremities, and the differences in composition of trabecular and cortical bone (Strobino and Farr, 1949; Gong *et al.*, 1964). This approach permits a study of bones as an *organ* rather than a tissue (Weinmann and Sicher, 1955).

Methods

Animals

Ten adult mongrel dogs weighing from 10.0 to 26.6 kgm were used as the experimental group. Seven females and three males were employed. The animals were judged to be in good health prior to selection. All animals were weighed preoperatively and at two-week intervals postoperatively. Three additional non-operated dogs weighing 13 to 28 kgm were used to confirm bilateral equality of humeri.

X-Rays

The right humeri of the ten experimental dogs were X-rayed preoperatively after small doses of intravenous sodium pentothal. X-rays were taken of both proximal and distal epiphyses. Only animals showing bony closure of both proximal and distal humeral epiphyses were selected as adult animals.

Operative Procedure

Six dogs were anesthetized with intravenous sodium pentothal, and a right elbow disarticulation was performed. A right elbow disarticulation as well as a right brachial plexus section were performed on two animals. The brachial plexus was isolated over the first rib by a medial suprascapular incision. Two dogs were subjected to only a right brachial plexus section. The experimental animals were relatively inactive for the first week postoperatively, and then ambulated well on three legs. All wounds healed well and there were no postoperative infections. Table 1 gives the sex, weight changes and operative procedure for the experimental animals.

Table 1. *Animal Protocol*

Animal	Sex	Days from procedure to sacrifice	Body weight change at sacrifice (kg)	Operative procedure
1	Male	88	+2.9	RED[b]
2	Male	82	+0.6	RED
3	Female	64	+2.2	RED
4	Female	88	+0.2	RED
5[a]	Female	82	−2.5	RED
6	Female	78	+2.5	RED
7	Female	70	+5.1	RED+BS
8	Female	74	−0.3	RED+BS
9	Female	67	+0.5	BS[c]
10	Male	63	+1.9	BS

[a] *Distemper post operatively, but recovered.*
[b] RED = Right elbow disarticulation.
[c] BS = Brachial plexus section.

Bone Preparation

The ten experimental animals were sacrificed 9 to 12 weeks postoperatively using lethal doses of sodium pentothal. Whole intact humeri were dissected free from the animals. After careful cleaning (sharp dissection) of articular cartilage, soft tissue and periosteum, both the experimental and opposite control humeri were X-rayed simultaneously. In 5 of the 10 experimental dogs, both right and left femora were similarly handled to confirm physical equality between left and right sides. In three additional non-operated dogs, the humeri were similarly studied to confirm the radiological and physical equality of the intact humeri.

All of the above bones (whole, intact humeri and femora) were weighed wet in air and water to determine volume and specific gravity (Steindler, 1936; Stover and Jee, 1963). The bones were then fragmented in a vise, weighed, and placed in individual beakers. For the removal of lipids, 300 ml of an alcohol-ether mixture (1:3) were added to the fragmented bone and heated to boiling on a steam table. The alcohol-ether was decanted and the entire procedure was repeated three times. The samples were dehydrated twice by the addition of 100 ml of acetone. After decanting the acetone, the samples were allowed to dry at room temperature for one hour. They were then dried at room temperature for four to seven days in a desiccator at a pressure of 1 mm of mercury over sodium hydroxide and the dry weight determined (Robinson and Elliott, 1957).

Analysis

The entire specimen of cortical bone and marrow content was hydrolyzed in 20 volumes of 6 N HCl at 121° for 4 hours in an autoclave. Aliquots of the hydrolyzate were evaporated to dryness in a vacuum desiccator over sodium hydroxide. The dried residue was made up to a known volume with water and analyzed for hydroxyproline (Neuman and Logan, 1950b; Klein, 1958). A second aliquot of hydrolysate was dried before being ashed in a muffle furnace at 800° for 3 hours; the ash was cooled in a desiccator and weighed.

Results

Radiographic Measurements

Eight out of ten experimental humeri showed varying but obvious decreased radiodensity in situ. These changes were more apparent when experimental and control humeri were removed from the dogs and X-rayed simultaneously (Fig. 1). Both cancellous and cortical bone showed evidence of demineralization. There were large areas of rarefaction in the metaphyses and diaphyses, and the endosteal surfaces of the cortical bone showed scalloping and striations. Those dogs showing the greatest loss of dry weight exhibited loss of normal trabecular patterns and thinning of the cortices.

Fig. 1. Radiograph of the whole femur and humerus from the left (*L*) and right (*R*) sides of dog #1. The right humerus was 88 days after disarticulation of the right elbow

Physical measurements (Tables 2 and 3). There was a small decrease in the average volume of the experimental humeri (—2.6%) as compared to the control side. A decrease was observed in eight of the ten experimental dogs. The specific gravity of the disused (right) humeri was decreased in all animals, with an average decrease of 8.9% as compared to the control (left) side. The wet weights of the experimental humeri were decreased in all animals, with an average loss of 11.0%. The dry defatted weights of experimental bones were decreased 13.2 to 39.0%, with an average loss of 23.2% as compared to the control sides. The *absolute* differences between wet and dry defatted weight, which represent the amount of fat and water removed, are greater in the paralyzed humeri (average, 18.53 gm) as compared to the control side (average 16.86 gm). This is reflected in the per cent water plus lipid which is greater in the paralyzed side (45.6%) than in the control bones (36.7%). The per cent of water plus lipid averaged 36.6% in 5 pairs of femurs from experimental dogs and from 3 pairs of humeri from non-operative dogs. There was essentially no difference in the volume,

Table 2. *Physical measurements — Experimental*

Animal	Bone	Volume (cc)	Specific gravity	Wet weight (gm)	Dry, fat-free weight (gm)	Difference in wet and dry weight (gm)	Water plus lipid content (%)
1	Right Humerus	31.11	1.284	39.95	23.60	16.35	41.0
	Left Humerus	32.64	1.445	47.21	32.92	14.29	30.3
2	Right Humerus	36.38	1.302	47.30	25.95	21.35	45.1
	Left Humerus	36.12	1.410	50.90	31.87	19.03	37.4
3	Right Humerus	24.30	1.220	29.48	13.92	15.56	52.8
	Left Humerus	25.57	1.370	35.16	21.20	13.96	39.7
4	Right Humerus	26.79	1.290	34.54	19.75	14.79	42.8
	Left Humerus	28.30	1.448	40.84	27.20	13.64	33.4
5	Right Humerus	26.50	1.310	34.66	19.70	14.96	43.2
	Left Humerus	26.70	1.405	37.57	23.90	13.67	36.4
6	Right Humerus	18.13	1.305	23.65	13.81	9.84	41.6
	Left Humerus	18.85	1.450	27.33	18.10	9.23	33.8
7	Right Humerus	33.45	1.187	39.64	19.37	20.27	51.1
	Left Humerus	33.80	1.356	45.74	27.63	18.11	39.6
8	Right Humerus	45.30	1.188	53.83	23.14	30.69	57.0
	Left Humerus	48.14	1.357	65.32	37.93	27.39	41.9
9	Right Humerus	20.05	1.333	26.78	15.30	11.48	42.9
	Left Humerus	20.47	1.400	28.64	17.65	10.99	38.4
10	Right Humerus	55.82	1.393	77.67	47.64	30.03	38.7
	Left Humerus	55.62	1.420	79.05	50.75	28.30	35.8
Average	Right Humerus	31.78	1.281	40.75	22.22	18.53	45.6
	Left Humerus	32.62	1.406	45.78	28.92	16.86	36.7
Mean difference		−2.6%	−8.9%	−11.0%	−23.2%	+ 9.9%	—

specific gravity, wet weight and dry, fat-free weight of the control femurs and humeri.

Chemical Measurements (Tables 4 and 5). The effect of disuse on whole humeri was evaluated by determining the *composition* of each humerus, and by comparing the *total* amounts of collagen and mineral in the operative limb and the non-operative limb. The content of organic plus inorganic solids after the removal of fat and water was similar in the control bones of both operative and non-operative dogs, 63.3 and 63.4%, respectively, as compared to the experimental humeri 54.4%. The collagen and ash compositions of the non-used humeri were 23.1—24.2% and 57.5—67.5%, respectively. The collagen and ash contents of the control side were 23.2—25.6% and 62.0—67.3%.

A comparison of the total amounts of collagen and ash in the operative humeri to the unoperative side showed that the average loss of collagen was 25.3% and the loss of ash was 26.1%. The loss of dry, fat-free weight was 23.2%. The three control pairs of humeri varied from +4.3% to −3.0% in dry weight, total hydroxyproline and bone ash, and the five pairs of femurs from operative and non-operative dogs varied from +5.0% to −3.0%.

Table 3. *Physical measurements — Control*

Animal	Bone	Volume (cc)	Specific gravity	Wet weight (gm)	Dry fat-free, weight (gm)	Difference in wet and dry weight (gm)	Water plus lipid content (%)
3	Right Femur	27.02	1.340	36.34	21.20	15.14	41.7
	Left Femur	27.31	1.290	35.33	20.21	15.12	42.8
4	Right Femur	30.76	1.424	43.87	28.67	15.20	34.6
	Left Femur	31.23	1.423	44.47	29.28	15.19	34.2
6	Right Femur	17.06	1.460	24.89	16.96	7.93	31.9
	Left Femur	17.18	1.460	25.11	17.18	7.93	31.6
7	Right Femur	33.19	1.330	44.13	26.49	17.64	40.0
	Left Femur	33.19	1.318	43.71	25.78	17.93	41.0
8	Right Femur	47.01	1.330	62.48	35.62	26.86	43.0
	Left Femur	46.98	1.332	62.55	36.17	26.38	42.2
11	Right Humerus	91.10	1.350	123.00	76.55	46.45	37.8
	Left Humerus	93.30	1.340	125.00	74.17	50.83	40.7
12	Right Humerus	30.75	1.440	44.20	30.40	13.80	31.2
	Left Humerus	30.51	1.390	42.56	30.88	11.68	27.4
13	Right Humerus	29.93	1.468	43.97	29.80	14.17	32.2
	Left Humerus	29.47	1.462	43.11	29.06	14.05	32.6
Average	Right Humerus	38.35	1.393	52.86	33.21	19.65	36.6
	Left Humerus	38.46	1.377	52.73	32.84	19.89	36.6
Mean difference		− 0.8%	+1.2%	+ 0.2%	+ 1.1%	− 1.2%	—

Discussion

The data indicate that there was an equal loss of bone mineral and collagen from whole humeri after 2 to 3 months of disuse in adult dogs. The greater loss of mineral (—26.1%) and collagen (—25.3%) in the experimental humeri as compared to the loss of dry, fat-free weight (—23.2%) can be explained by an influx of organic material presumable non-collagenous protein. The data obtained here cannot be compared closely to that of previous workers. Allison and Brooks (1921); Armstrong *et al.* (1945), Armstrong (1946) used the subtraction of ash weight from dry weight as a means of determining *total* organic matter and *per cent* organic matter. The gravimetric method does not permit estimation of changes between collagen and non-collagenous materials. Allison and Brooks' (1921) data were further complicated by the use of dry but not fat-free bone, thus fat replacement was included in the total organic fraction. The replacement of lost bone collagen by fat and other organic compounds would account for Allison and Brooks' (1921) small loss of organic matter found in *intact* osteoporotic humeri. The replacement of lost bone mineral and collagen by other organic materials (carbohydrate or protein) would account for the greater loss of total ash than dry, fat-free weight observed by Armstrong (1946); Gedalia *et al.* (1966). The latter workers observed that the per cent ash on a weight basis was similar in both experimental and control femurs, although the per cent calcium was lower in the paralyzed femur. This would suggest a change in

Table 4. *Percent changes in dry weight, collagen and ash — Experimental*

Animal	Bone	Dry, fat-free weight (gm)	Dry, fat-free weight loss (%)	Hydroxy-proline (mg)	Collagen loss[a] (%)	Ash (gm)	Ash loss (%)
1	Right Humerus	23.60	−28.3	733.0	−29.2	14.33	−30.8
	Left Humerus	32.92		1034.0		20.72	
2	Right Humerus	25.95	−18.6	804.6	−23.0	14.92	−26.5
	Left Humerus	31.87		1047.0		20.31	
3	Right Humerus	13.92	−34.3	453.8	−33.9	8.40	−36.4
	Left Humerus	21.20		687.0		13.22	
4	Right Humerus	19.75	−27.3	644.6	−31.0	11.72	−30.5
	Left Humerus	27.20		934.3		16.86	
5	Right Humerus	19.70	−17.4	627.9	−21.2	13.31	−16.6
	Left Humerus	23.90		797.2		15.95	
6	Right Humerus	13.81	−23.7	435.6	−26.1	8.46	−28.2
	Left Humerus	18.10		589.1		11.79	
7	Right Humerus	19.37	−29.9	606.5	−29.3	11.54	−32.8
	Left Humerus	27.63		858.0		17.18	
8	Right Humerus	23.14	−39.0	759.2	−41.5	14.56	−40.4
	Left Humerus	37.93		1296.0		24.49	
9	Right Humerus	15.30	−13.3	492.9	−15.1	10.08	−15.1
	Left Humerus	17.65		580.6		11.87	
10	Right Humerus	47.64	− 6.1	1481.0	− 2.5	31.63	− 3.4
	Left Humerus	50.75		1519.0		32.75	
Average		—	−23.2	—	−25.3	—	−26.1

[a] mg Collagen = mg hydroxyproline X 7.46.

composition of osteoporotic bone or replacement of lost bone by non-skeletal minerals. In the present study the lower per cent ash and collagen found in the experimental humeri in the presence of a constant ratio of ash to collagen (range was 2.42—2.69) is consistent with the replacement of lost bone by non-collagenous organic material.

Recent studies by one of us (Klein *et al.*, 1966) on acute paraplegia in humans have shown that large losses of peptide hydroxyproline and calcium in the urine occurred early during the first week following injury. The temporal pattern of acute skeletal catabolism demonstrated an *earlier* and greater excretion of urinary hydroxyproline than urinary calcium. However, Mishima (1964) on the basis of a higher percentage of total nitrogen and hydroxyproline in the compact bone of the non-used tibia from adult dogs as compared with the control tibia suggested that the resorption of bone mineral occurred earlier than the destruction of bone collagen during the acute course of bone atrophy.

In summary, disuse atrophy of bone can be characterized by a late reduction in bone mass as seen by X-ray, by a simultaneous loss of mineral and collagen from the skeleton which is manifested early as hypercalcemia and increased excretion of calcium and hydroxyproline in the urine. The simultaneous loss of

Table 5. *Percent changes in dry weight, collagen and ash — Control*

Animal	Bone	Dry, fat-free weight (gm)	Dry, fat-free weight change %	Hydroxy-proline (mg)	Collagen loss[a] (%)	Ash (gm)	Ash loss (%)
3	Right Femur	21.20	+4.8	682.8	+5.0	13.25	+3.5
	Left Femur	20.21		650.4		12.82	
4	Right Femur	28.67	−2.0	941.0	−1.8	18.26	−3.0
	Left Femur	29.28		959.4		18.83	
6	Right Femur	16.96	−1.3	550.0	0.0	11.28	+1.0
	Left Femur	17.18		549.6		11.16	
7	Right Femur	26.49	+2.7	832.8	−1.3	16.11	0.0
	Left Femur	25.78		843.8		16.08	
8	Right Femur	35.62	−1.6	1228.7	+2.9	23.14	−1.7
	Left Femur	36.17		1193.9		23.54	
11	Right Humerus	76.55	+1.8	2300.0	−3.0	41.66	−0.2
	Left Humerus	74.17		2371.0		41.73	
12	Right Humerus	30.40	−1.6	945.0	+1.9	17.58	+1.0
	Left Humerus	30.88		927.0		17.46	
13	Right Humerus	29.80	+2.3	939.2	+4.3	19.06	+2.9
	Left Humerus	29.06		900.5		18.52	
Average		—	+0.6	—	+0.9	—	+0.4

[a] mg Collagen = mg hydroxyproline X 7.46.

mineral and collagen is deduced from *chronic* experiments and cannot be used for interpreting the mechanism of bone resorption in more *acute* experiments.

References

Allison, N., and B. Brooks: Bone atrophy. An experimental and clinical study of the changes in bone which result from non-use. Surg. Gynec. Obstet. **33**, 250—260 (1921).

Armstrong, W. D.: Bone growth in paralyzed limbs. Proc. Soc. exp. Biol. (N.Y.) **61**, 358—362 (1946).

— M. Knowlton, and M. Gouze: Influence of estradiol and testosterone propionates on skeletal atrophy from disuse and on normal bones of mature rats. Endocrinology **36**, 313—322 (1945).

Burdeaux, B. D., and W. J. Hutchinson: Studies in osteoporosis following fractures. Surg. Forum **2**, 434—437 (1952).

Gedalia, I., A. Schwartz, J. Sela, and E. Gazenfield: Effects of fluoride intake on disuse atrophy of bone in rats. Proc. Soc. exp. Biol. (N.Y.) **122**, 657—660 (1966).

Geiser, M., and J. Trueta: Muscle action, bone rarefaction and bone formation. J. Bone Jt Surg. B**40**, 282—311 (1958).

Gong, J. K., J. S. Arnold, and S. H. Cohn: Composition of trabecular and cortical bone. Anat. Rec. **149**, 325—332 (1964).

Grey, E. G., and G. L. Carr: An experimental study of the factors responsible for non-infectious bone atrophy. Bull. Johns Hopk. Hosp. **26**, 381—385 (1915).

Klein, L.: The action of collagenase on native collagen. Ph. D. Thesis, Boston University 1958.

— S. van den Noort, and J. J. de Jak: Sequential changes of urinary hydroxyproline and serum alkaline phosphatase in acute paraplegia. Med. Serv. J. Can. **22**, 524—533 (1966).

Landoff, G. A.: Experimental studies on bone atrophy resulting from immobilization and acute arthritis. Acta chir. scand., Suppl. **71**, 1—198 (1942).
McLean, F. C., and M. R. Urist: Bone. An introduction to the physiology of skeletal tissue, 2nd ed, p. 215. Chicago: Chicago University Press 1961.
Mishima, H.: Biochemical studies on metabolic mechanism in experimental bone atrophy. Sapporo med. J. **25**, 123—140 (1964).
Neuman, R. E., and M. A. Logan: The determination of collagen and elastin in tissues. J. biol. Chem. **182**, 549—556 (1950a).
— — The determination of hydroxyproline. J. biol. Chem. **184**, 299—306 (1950b).
Pottorf, J. L.: An experimental study of bone growth in the dog. Anat. Rec. **10**, 234—235 (1916).
Robinson, R. A., and S. R. Elliott: The water content of bone. J. Bone Jt Surg. A**39**, 167—187 (1957).
Steindler, A.: Physical properties of bone. Arch. phys. Ther. (Omaha) **17**, 336—345 (1936).
Stover, B. J., and W. S. S. Jee: Some effects of long-term alpha irradiation on the composition and structure of bone. Hlth Phys. **9**, 267—275 (1963).
Strobino, L. J., and L. E. Farr: The relation of age and function of regional variations in nitrogen and ash content of bovine bones. J. biol. Chem. **178**, 599—609 (1949).
Weinmann, J. P., and H. Sicher: Bone and bones, fundamentals of bone biology, 2nd ed., p. 15. St. Louis: C. V. Mosby Co. 1955.