Abstract:

Bone remodelling activity in the avian ulna was assessed under conditions of disuse alone, disuse with a superimposed continuous compressive load, and disuse interrupted by a short daily period of intermittent loading. The ulna preparation is made by two submetaphyseal osteotomies, the cut ends of the bone being covered with stainless steel caps which, together with the bone they enclosed, are pierced by pins emerging transcutaneously on the dorsal and ventral surfaces of the wing. The 110 mm long undisturbed section of the bone shaft can be protected from functional loading, loaded continuously in compression by joining the pins with springs, or loaded intermittently in compression by engaging the pins in an Instron machine. Similar loads (525 N) were used in both static and dynamic cases engendering similar peak strains at the bone's midshaft (-2000 x 10^-6). The intermittent load was applied at a frequency of 1 Hz during a single 100 second period per day as a ramped square wave, with a rate of change of strain during the ramp of 0.01 per second. Peak strain in the same region during wing flapping in the intact bone was recorded with strain gauges in vivo as -3300 x 10^-6 with a maximum strain rate of 0.056 per second.

After an eight week period, transverse sections from the midshafts of the experimental and contra lateral intact bones were compared. Both non-loaded and statically loaded bones showed an increase in endosteal diameter and intra cortical porosity

resulting in a similar decrease in cross sectional area (-13 percent). Intermittently loaded bones however showed an increase in new bone formation predominantly, but not exclusively, on the periosteal surface, and a 24 percent increase in cross sectional area. It appears from these data that a static load has no effect on bone remodelling, whereas a short daily period of a strain regime involving physiological strains and strain rates but an unnatural strain distribution can be associated with a substantial increase in bone mass.

Introduction:

The first systematic series of experiments designed to investigate the mechanism of functional adaptation in bone tissue was that conducted by Hert and his coworkers using artificial loads applied to the tibias of rabbits (Hert et al., 1969, 1971; Liskova and Hert, 1971). The earliest parameter they investigated was the effect of static versus dynamic loading. Their conclusion was that, whereas the remodelling process was influenced by dynamic loading, static loads had no such effect. Since this observation, there has been increasing interest in the mechanism of Wolff's Law, and artificial loading experiments using controlled dynamic loads have been employed on sheep (Churches et al, 1979; O'Connor et al, 1982), chickens (Rubin and Lanyon, 1981, 1983a) and turkeys (Rubin and Lanyon, 1983b). In addition to these studies, reports have appeared from static loading studies which, contrary to Hert's findings, suggest an association between static load and remodelling activity (Hassler, 1980; Meade et al, 1981; Hart et al, 1983). In all these static loading studies mathematical models were also developed which, by their reasonableness, appeared to support the existence of a relationship between the remodelling observed and the static stresses produced within the bone tissue.

In any artificial loading experiment in vivo, there are two major pitfalls:

1) bone remodelling is sensitive to many factors other than mechanical ones and so the direct and indirect effects of trauma and vascular disturbance can easily obliterate any remodelling

related to physiological changes in the bone's mechanical situation.

2) when a continuous load is applied to a bone which is also being functionally loaded, it not only induces static strains onto which the functional strains are superimposed, but it also modulates the pattern of dynamic strain produced by functional activity.

The first of these dangers can be avoided, or at least reduced, by developing preparations in which the sites of surgical interference are kept remote from those where the remodelling is assessed. The second can be overcome by the use of models in which artificial loads are applied to a bone which is retained in vivo but which is isolated from alternative (natural) sources of loading.

The functionally isolated externably loadable avian ulna model (Rubin and Lanyon, 1981, 1983) is well suited in both these respects and so we used it to determine the relative effects of functional deprivation alone and functional deprivation modified by a chronic static load applied by springs.

Materials and Methods

The experimental animals used were skeletally mature male turkeys. The preparation consists of the 110mm long diaphysis of the ulna which is deprived of functional loading by subarticular osteotomies at either end, the cut ends of the bone being covered by stainless steel caps. These caps and the bone they enclose are pierced by Steinman pins which emerge through the skin on the dorsal and ventral surfaces of the wing. The ends of these protruding pins are used for the application of external load. The strains at the bone's midshaft resulting from both natural and experimental loading were assessed using rosette strain gauges attached to the bone's surface.

In one group of "calibration" ulnas three rosette strain gauges were attached in vivo around the midshaft of the intact bone under general anesthesia. After 2 days recovery from the surgery, the animal was encouraged to flap its wings vigorously and data recorded from the implanted gauges. These data allowed calculation of the peak physiological strains and strain rates in that region. The animal was then reanesthetized and the bone prepared with caps and pins and the pins clamped between external fixators. The strains recorded during wing flapping were again recorded. Finally, the fixators were removed and the bone loaded between the loading forks of a modified Instron machine. The strains from the implanted gauges were again recorded establishing the relationship between strain at the midshaft and load applied between the pins.

In the groups in which remodelling was to be assessed, no gauges were attached to the bone but all observations were confined to the midshaft region for which strain data had been obtained in the calibration series. This region is 55 mm away from each osteotomy site. In the study reported here, remodelling was assessed in three groups of birds

1) in which the preparation was made and the pins continuously clamped together by external fixators with no load applied;

2) in which the preparation was made and the pins joined by springs, (Fig. 1), producing a combined load of 525 N and a maximum longitudinal strain around the circumference of the bone midshaft of 2000 microstrain;

3) in which the preparation was made and the pins clamped together with external fixators which were removed once daily and the pins engaged in a loading apparatus. One hundred consecutive 1 Hz cycles of a peak force of 528 N were imposed with a constant strain rate on the up and down ramp of 10,000 microstrain per second.

The birds in each group were maintained on their respective protocols for 8 weeks following the initial surgery after which they were killed and transverse sections taken of the ulna midshaft on the prepared and contralateral (intact) sides. In addition to routine histology, microradiographs were taken of undecalcified sections 100 micron thick, and the area of bone digitized for left:right comparisons.

Results:

During both wing flapping and external loading from the Instron machine, the midshaft of the ulna was subjected to both axial loads and bending moments so that the neutral axis passed through the marrow cavity. During wing flapping in the intact bone the peak longitudinal strain was -3300 microstrain and the maximum strain rate 56,000 microstrain per second. In the prepared situation with the external fixators attached, the pattern of strain change was irregular and all the strains were below 100 microstrain. Thus, in the non-loaded group the prepared bones experienced only negligible dynamic strains. In the static loaded group, similarly trivial dynamic strains would have been superimposed upon a constant maximum strain of -2000 microstrain. In the dynamically loaded group, trivial dynamic strains would be interrupted once daily for a brief period, during which significant strains (peak -2,000 microstrain) would be engendered at physiological strain rates (10,000 microstrain per second). Although each of these strain parameters was well within the physiological range, the distribution of strain across the section was different from that naturally engendered during flapping (Fig. 2).

The transverse cross sectional areas of left (prepared) and right (intact) digitized microradiographs for non-loaded, statically loaded, and dynamically loaded bones are shown in Table 1, and the comparisons of these data expressed as percentages in Table 2. Since the numbers in each group were small and there were some discrepancies in area between left and right sides which were not due to remodelling over the experimental period, each histological section was checked for signs of periosteal resorption and the left:right comparisons "normalised" to the same periosteal enclosed area. These data are also presented in Table 2 (in parentheses).

It can be seen that if the ulna preparation is made and the bone maintained in an unloaded situation, then there is practically no difference in the periosteal enclosed area (0.6 + S.E. 1 percent) whereas the endosteal area increases by 11 (+ S.E. 2.4) percent and the absolute area of intracortical porosity from a mean of 0.55 sq mm (0 in 3 animals and 1.1 sq. mm in 1 animal) to a mean of 1.07 (+.09) sq mm, an increase between 0.3 and 2.4 sq. mm. evident in all 4 animals, Fig. 3c. These changes combine to produce a reduction in total bone area of 13 (+ S.E. 4) percent, or if normalised for similar periosteal area, a reduction of 13.5 percent.

In those individuals in which the pins were continuously joined by external fixators, the comparison between left (prepared) and right (intact) bones showed that there was also a widening of the endosteal area and increase in intracortical porosity, Fig. 3d. These bones in spring loaded and non loaded groups were also similar in that there was no evidence of any periosteal or endosteal new bone formation. The degree of bone loss as evidenced by the area change was similar in the two groups, (-13 percent, or if normalised, -13.5 to -8 percent).

In each bird in the dynamically loaded group, instead of a loss of bone, the cross-sectional area actually increased (24 percent) as a result of new bone formation primarily on the periosteal surface, Fig. 3b.

Discussion

The data presented here suggest that a static load sufficient to maintain a peak longitudinal strain of 2000 microstrain at the midshaft does not modulate the amount of bone loss which would occur with functional deprivation alone. These data are in contrast to those in which a short daily period of dynamic loading in a similar bone preparation not only prevented bone loss but was associated with a substantial increase in bone cross-sectional area. Using this same preparation in another study, we have also been able to show (Rubin and Lanyon, 1981, 1983b) that for peak strains between 0 and 4,000 microstrain, there is a fairly linear "dose:response" relationship between the amount of change in area and the peak strain magnitude. Since a peak strain of 3000 microstrain is still within the physiologically attainable strain range, we ascribe this adaptive response to be due to the altered distribution of dynamic strain rather than exceeding the physiological level of any one strain parameter.

The sensitivity of the remodelling process to short periods of dynamic strain change, and the absence of any response to static strain is consistent with Hert's data (Hert et al., 1969; Liskova and Hert, 1971) obtained from loading the rabbit tibia. It is also consistent with Perren's findings (Perren et al., 1969) applying chronic compressive loads to cortical bone from fracture plates, and with O'Connor et al. (1982) who showed a relationship between the amount of new bone formation and the maximum rate of change of strain.

The absence of a sensitivity to static strain is also consistent with the absence of any natural requirement for the skeleton to adapt to a static load. Furthermore, since a cellular mechanism capable of forming and retaining an 'appreciation' of absolute strain would be extremely difficult to achieve, it is unlikely that such a capacity would evolve unless it provided a significant selection advantage. The factors involved in transducing mechanical strains to chemical signals controlling cellular behaviour are as yet unknown. It seems, however, that the remodelling response in bone is sensitive to a number of aspects of the structure's dynamic strain situation. Those identified up to this time include the magnitude, rate of change and distribution of dynamic strain change throughout the structure (Churches and Howlett, 1979; Rubin and Lanyon, 1983b; O'Connor, Lanyon and MacFie, 1982; Lanyon et al., 1982). There does not, however, appear to be any response to chronic static load.

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Table 1. Total bone area, periosteal enclosed area, endosteal enclosed area and intracortical porosity (sq. mm.) measured from microradiographs of midshaft sections of left (prepared) and right (control) ulnas.

Identity		Total Bone Area		Periosteal		Endosteal		Porosity	
NO LOAD:									
		Left	Right	Left	Right	Left	Right	Left	Right
9	1	49.7	55.3	122.3	120.5	70.2	65.2	2.4	0
18	2	41.1	53.1	117.5	117.8	74.6	63.6	1.8	1.1
20	3	49.4	53.0	109.5	106.3	59.6	53.4	0.3	0
32	4	47.4	53.7	116.9	119.1	69.2	65.4	0.3	0
CONSTANT SPRING LOAD:									
67	5	43.3	54.2	102.8	107.9	57.8	53.6	1.7	0.1
62	6	46	53.9	122.3	131.2	74.2	76.8	2.1	0.5
75	7	53.6	54.0	118.2	115.9	64.2	61.9	0.4	0.0
84	8	51.8	59.9	110.7	120.8	58.4	60.9	0.7	0.1
DAILY INTERMITTENT LOAD:									
5	9	75.1	57.1	144.1	119.8	65.8	62.7	3.2	0
11	10	80.6	55.4	138.1	119.9	57.4	64.5	2.1	0.2
33	11	51.7	47.2	111.2	104.9	59.5	57.7	0	0
36	12	61.7	56.1	132.3	128.1	70.6	72.1	0	0

Table 2. Left:right comparisons of the data presented in Table 1 showing the percentage change in total periosteally enclosed and endosteally enclosed area. The figures in parentheses represent those values adjusted to the same original periosteal enclosed area. The figures for porosity* are expressed as absolute values in sq mm.

LEFT:RIGHT COMPARISONS - percent

	Identity	Total Bone Area	Periosteal	Endosteal	Porosity
	NO LOAD:				
[illegible]	1	-10 (-11)	+1.4 (0)	+8 (+6)	*+2.4
18	2	-23 (-23)	0 (0)	+17 (+17)	*+0.7
20	3	-7 (-10)	+3.0 (0)	+13 (+8)	*+0.9
32	4	-12 (-10)	-2.0 (0)	+6 (+8)	*+0.3
		------	------	------	------
		-13 (-13.5)	+0.6 (0)	+11 (10)	*1.07
		S.D.+6.9 (6.3)	+2.1	+4.9 (4.9)	+0.9
	SPRING LOAD:				
57	5	-21 (-16)	-5.0 (0)	+8 (+13)	*+1.6
62	6	-15 (-9)	-7.0 (0)	-9 (+4)	*+1.6
75	7	-1 (-3)	+2.0 (0)	+4 (+2)	*+0.7
84	8	-14 (-5)	-9.0 (0)	-4 (+5)	*+0.1
		______	______	______	______
		-13 (-8)	-5 (0)	-1 (+6)	*+1.0
		S.D.+8 (8)	+4.7	+7.6 (5)	0.7

INTERMITTENT LOAD:

	9	31 (28)	+20 (17)	+5 (+2)	*+3.2
1	10	45 (51)	+16 (19)	-11 (-8)	*+1.9
3	11	10 (10)	+6 (6)	+3 (+3)	0
6	12	10 (10)	+3 (3)	-2 (-2)	0
		------	------	------	------
		+24 (25)	11.5 (11)	-1 (-1)	*1
		S.D.+17 (19)	+8 (8)	+7 (5)	+1.5

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. 1. A radiograph of the ulna preparation taken post mortem ving the 110 mm portion of the bone's diaphysis, with caps and nsfixing pins in place. The percutaneous pins are shown joined the loading springs which are situated outside the wing.

. 2. Outlines of transverse sections of the ulna midshaft ving the longitudinal strains and the position of the neutral s a) at the time of peak strain during flapping in the intact g and b) during external loading in the bone preparation. nough the external applied load is compressive, the bone's ıral curvature engenders bending.

. 3. Transverse sections taken from the ulna midshaft at the end the experimental period; a) intact right ulna from bird 10; prepared left ulna from bird 10, subjected to 100 cycles per day of ermittent loading; c) prepared ulna from bird 2, protected from nanical loading; d) prepared ulna from bird 5, subjected to tinuous loading from springs.

Table 1. Total bone area, periosteal enclosed area, endosteal enclosed area and intracortical porosity (sq. mm.) measured from microradiographs of midshaft sections of left (prepared) and right (control) ulnas.

Identity	Total Bone Area		Periosteal		Endosteal		Porosity	
NO LOAD:								
	Left	Right	Left	Right	Left	Right	Left	Right
1	49.7	55.3	122.3	120.5	70.2	65.2	2.4	0
2	41.1	53.1	117.5	117.8	74.6	63.6	1.8	1.1
3	49.4	53.0	109.5	106.3	59.6	53.4	0.3	0
4	47.4	53.7	116.9	119.1	69.2	65.4	0.3	0
CONSTANT SPRING LOAD:								
5	43.3	54.2	102.8	107.9	57.8	53.6	1.7	0.1
6	46	53.9	122.3	131.2	74.2	76.8	2.1	0.5
7	53.6	54.0	118.2	115.9	64.2	61.9	0.4	0.0
8	51.8	59.9	110.7	120.8	58.4	60.9	0.7	0.1
DAILY INTERMITTENT LOAD:								
9	75.1	57.1	144.1	119.8	65.8	62.7	3.2	0
10	80.6	55.4	138.1	119.9	57.4	64.5	2.1	0.2
11	51.7	47.2	111.2	104.9	59.5	57.7	0	0
12	61.7	56.1	132.3	128.1	70.6	72.1	0	0

Table 2. Left:right comparisons of the data presented in Table 1 showing the percentage change in total periosteally enclosed and endosteally enclosed area. The figures in parentheses represent those values adjusted to the same original periosteal enclosed area. The figures for porosity* are expressed as absolute values in sq mm.

LEFT:RIGHT COMPARISONS - percent

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3	-7 (-10)	+3.0 (0)	+13 (+8)	*+0.9
4	-12 (-10)	-2.0 (0)	+6 (+8)	*+0.3
	-13 (-13.5)	+0.6 (0)	+11 (10)	*1.07
	S.D.+6.9 (6.3)	+2.1	+4.9 (4.9)	+0.9
SPRING LOAD:				
5	-21 (-16)	-5.0 (0)	+8 (+13)	*+1.6
6	-15 (-9)	-7.0 (0)	-9 (+4)	*+1.6
7	-1 (-3)	+2.0 (0)	+4 (+2)	*+0.7
8	-14 (-5)	-9.0 (0)	-4 (+5)	*+0.1
	-13 (-8)	-5 (0)	-1 (+6)	*+1.0
	S.D.+8 (8)	+4.7	+7.6 (5)	0.7
INTERMITTENT LOAD:				
9	31 (28)	+20 (17)	+5 (+2)	*+3.2
10	45 (51)	+16 (19)	-11 (-8)	*+1.9
11	10 (10)	+6 (6)	+3 (+3)	0
12	10 (10)	+3 (3)	-2 (-2)	0
	+24 (25)	11.5 (11)	-1 (-1)	*1
	S.D.+17 (19)	+8 (8)	+7 (5)	+1.5

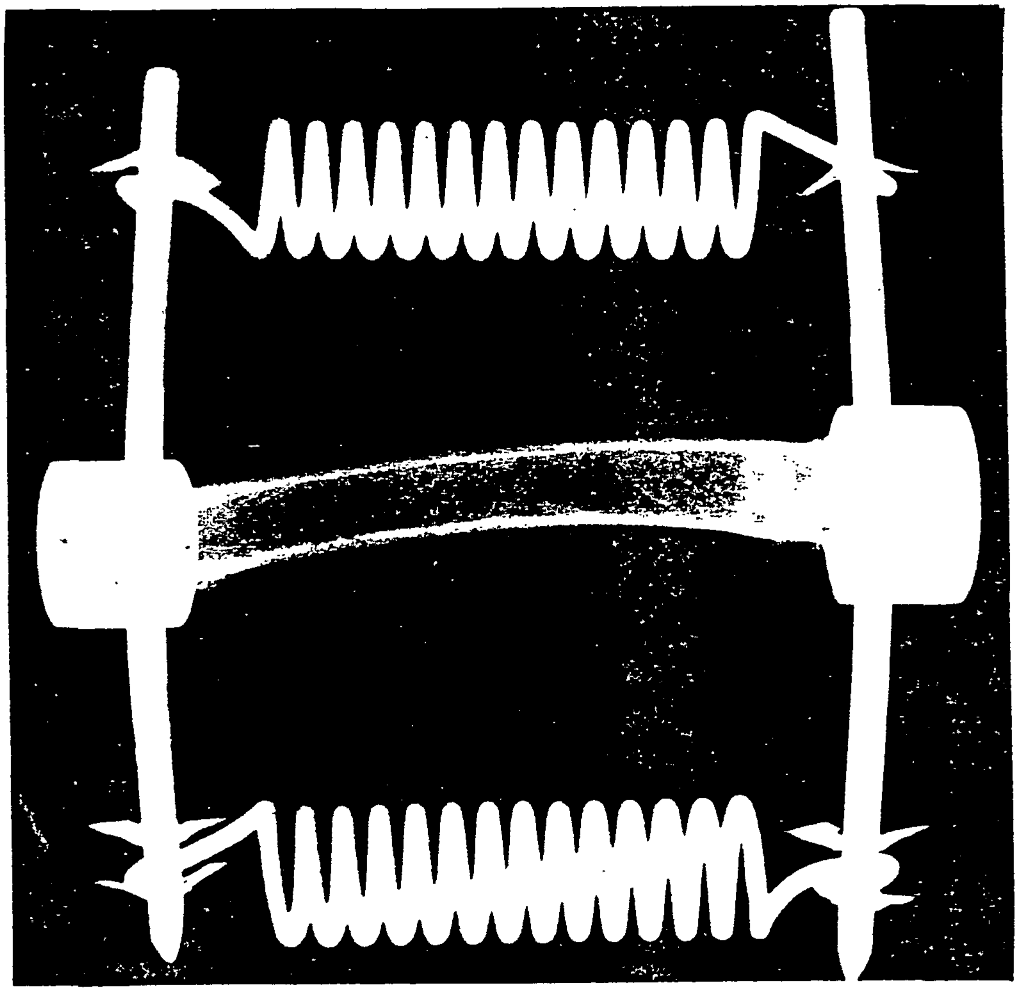
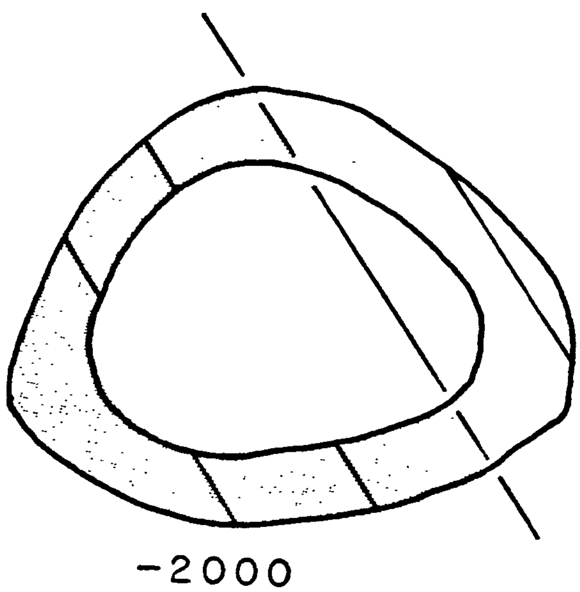


FIG 1



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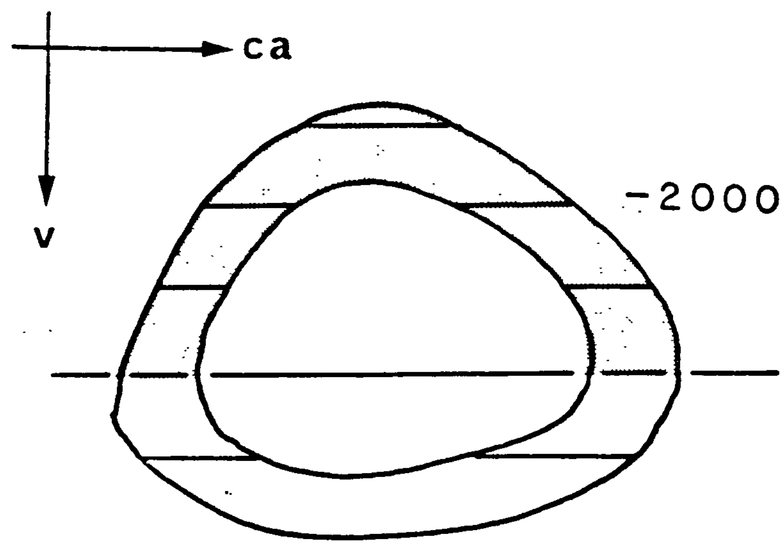

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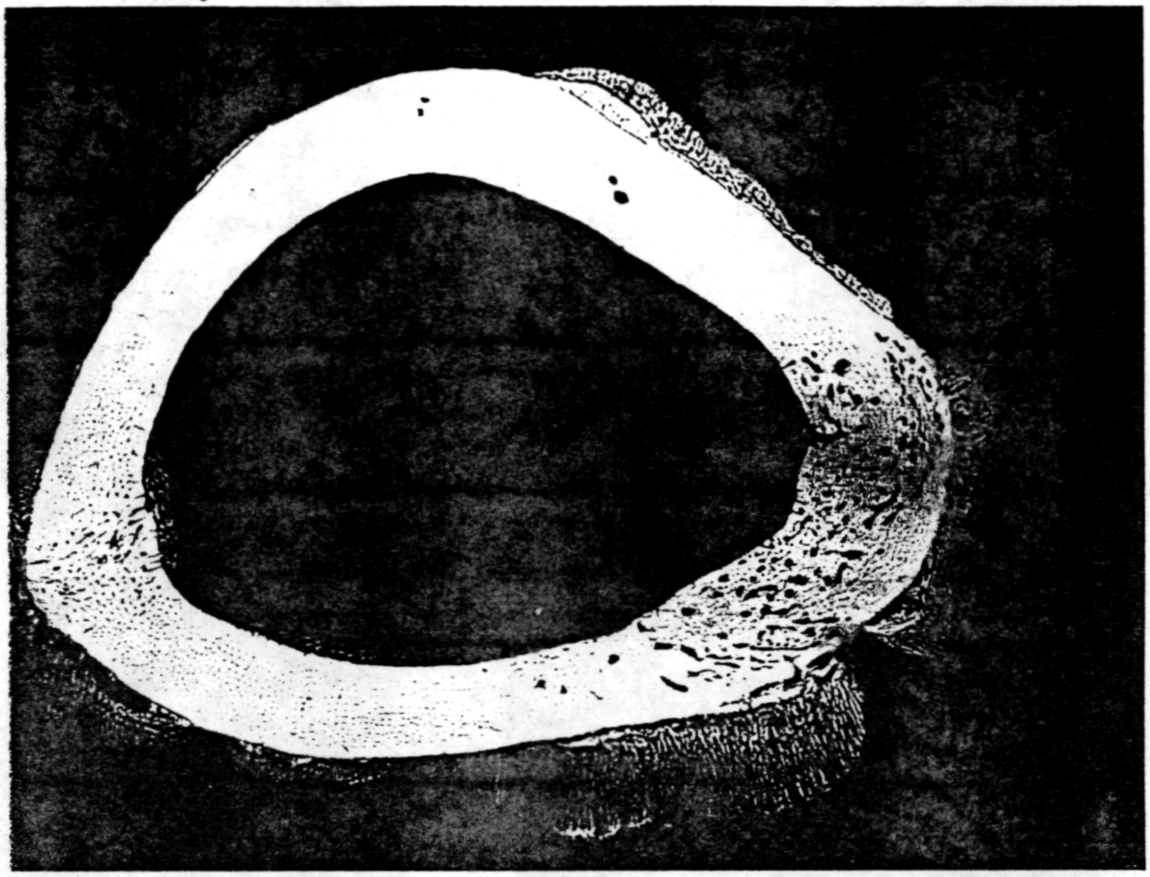
FIG 3



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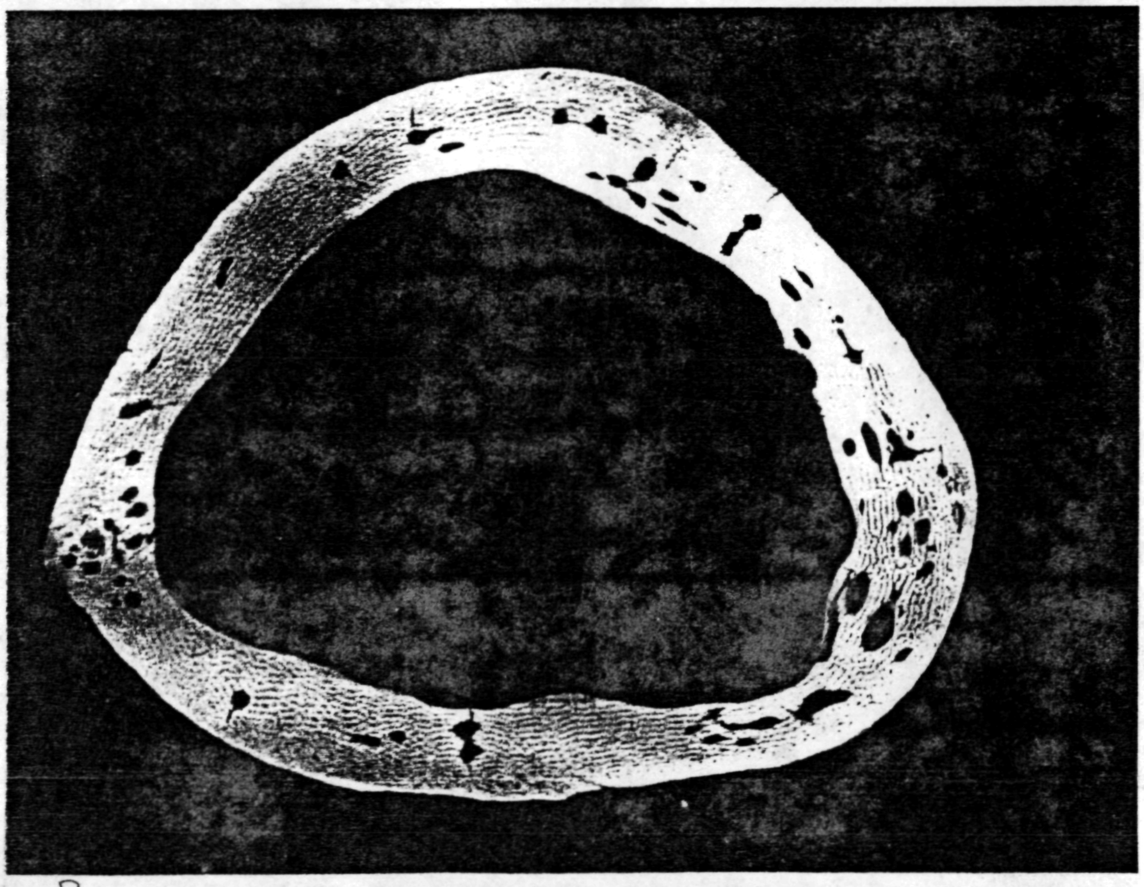

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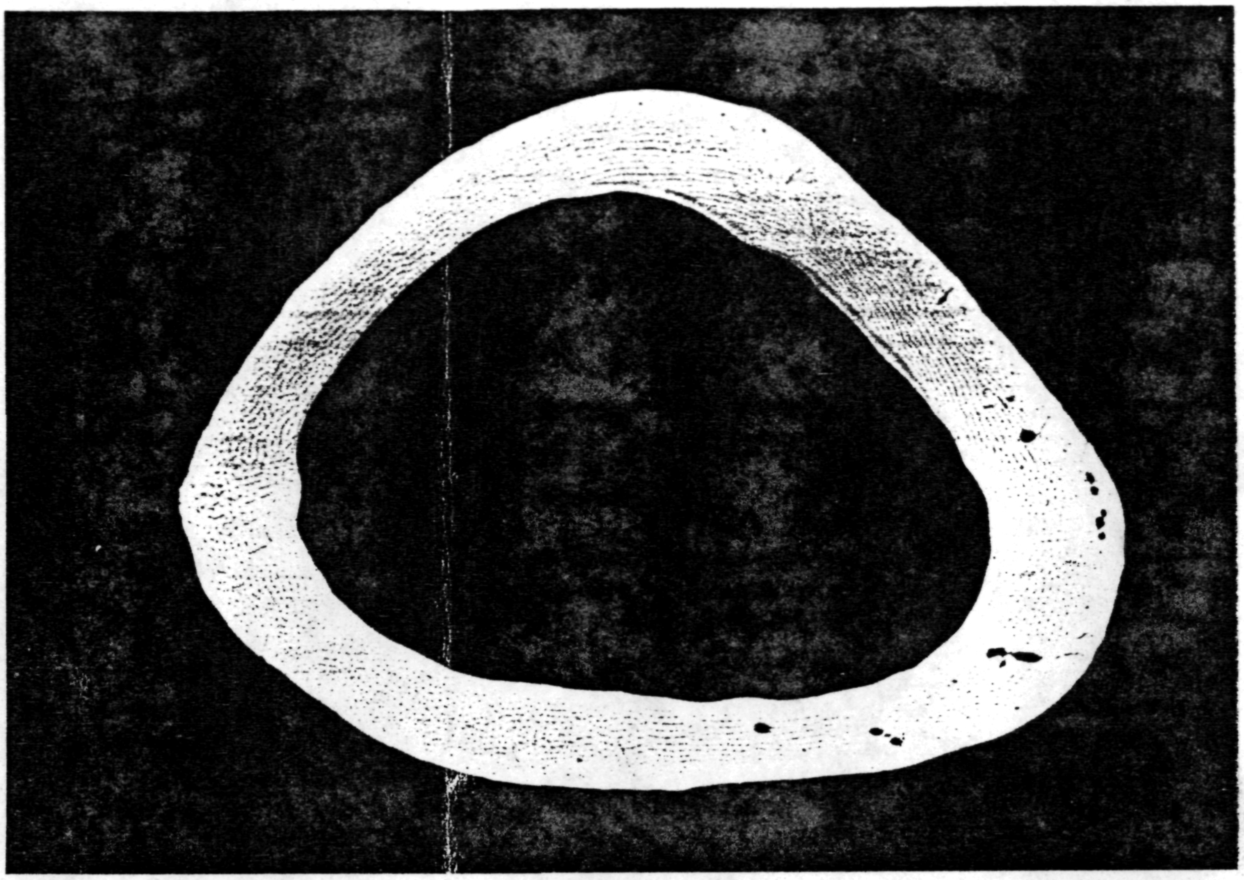
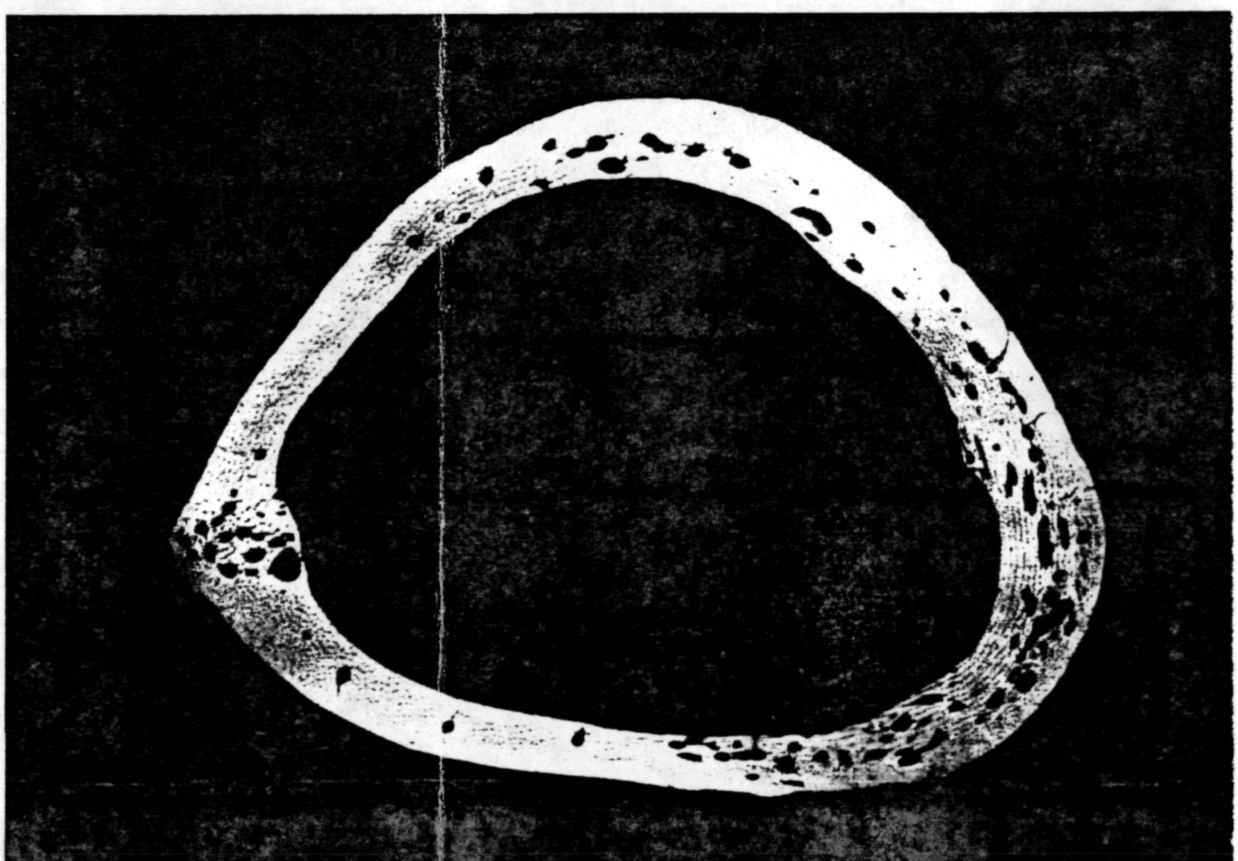


A.



C