

## QUANTITATIVE HISTOLOGIC ANALYSES OF ARTICULAR CARTILAGE AND SUBCHONDRAL BONE FROM OSTEOARTHRITIC AND NORMAL HUMAN HIPS

INGE REIMANN\*, HENRY J. MANKIN & CAROL TRAHAN

Orthopedic Research Laboratories, Massachusetts General Hospital, and  
the Harvard Medical School, Boston, USA.

Twelve femoral heads (two normal, four after fracture and six osteoarthritic) were obtained at surgery or autopsy. Circumferential slices were obtained and five separate areas were analyzed in each for ash content; histological-histochemical grading of the severity of the cartilage changes; and quantitative morphometric analyses to establish the percentage of trabecular area, osteoblastic area and osteoclastic area. Analyses were performed to compare weight-bearing and non-weight-bearing areas of the femoral heads and to determine correlations between the bony and cartilaginous alterations. The data obtained showed wide variations in all parameters in the osteoarthritic specimens but consistently more marked cartilage and bony changes in the weight-bearing areas. Bone structures and metabolic parameters were distinctly increased for the osteoarthritics, differing significantly from both normal and fracture control groups. The bony change correlated directly with the severity of the cartilage lesions, as determined by the histological-histochemical grade.

*Key words:* bone mineral; cartilage degeneration; osteoarthritis; osteoblastic area; osteoclastic area; subchondral bone; trabecular bone area

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Over the years, numerous reports have described the structural abnormalities in cartilage and subchondral bone from osteoarthritic human joints (Collins 1949, Harrison et al. 1953, Sokoloff 1969, Meachim & Fergie 1975). Alterations in these tissues have been shown to be progressive with advancing disease and highly focal, particularly for the femoral

head (Mankin et al. 1971), the site most extensively studied in recent years. Cartilage alterations consist of erosion, ulceration, fibrillation and progressive destruction leading to complete loss of tissue (Collins 1949, Sokoloff 1969, Meachim & Fergie 1975) while the bony changes are those of increased bone formation, thickening trabeculae, sclerosis and cyst formation (Sokoloff 1969, Radin et al. 1970, Foss & Byers 1972, Jeffrey 1973). Although the concept of a relationship between the changes in the cartilaginous and bony tissues exists, it ap-

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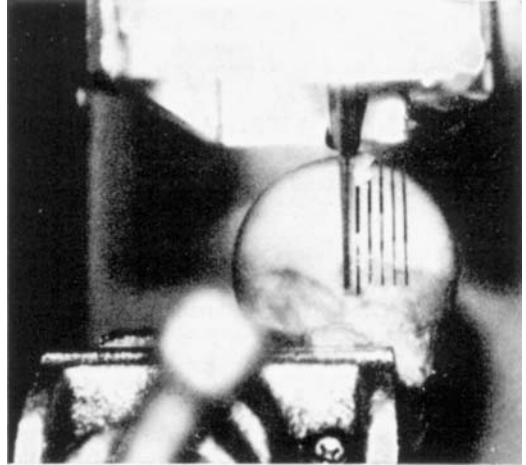
pears to be based principally on biomechanical considerations (Radin et al. 1973, Pugh et al. 1974). To date, no attempt has been made to assess the two processes simultaneously by quantitative histological techniques.

In 1971 a report from this laboratory described a histological-histochemical grading system which provided numerical values for defining the severity of the changes in articular cartilage from osteoarthritic femoral heads (Mankin et al. 1971). Progressive deterioration provided increasingly higher scores on the scale, and these data correlated well with biochemical (Mankin et al. 1971) and enzyme (Ehrlich et al. 1973) abnormalities in the cartilage. The system does not measure changes in the subchondral bone, and no such grading system for the bone is available.

This report describes an experiment which sought to develop morphometric techniques to assess the alterations in subchondral bone and then apply both these and the previously described cartilage grading system to a series of control and osteoarthritic femoral heads. Furthermore, it also assesses the variation in the morphometric parameters from site to site and also compares these for osteoarthritic tissue as well as for the normal groups.

## MATERIAL AND METHODS

Twelve femoral heads were used for this study and divided into three groups. A normal group, consisting of two femoral heads obtained at autopsy from two male patients, aged 60 and 61; a fracture group consisting of four femoral heads obtained at the time of replacement surgery, which took place immediately after the fracture, from female patients with an average age of 79.5; and an osteoarthritic group consisting of six femoral heads obtained at the time of replacement surgery from four female and two male patients with an average age of 57.8. Specimens were fixed for 24 hours in 10 per cent buffered formalin phosphate and then two circumferential slices were cut at



*Figure 1. Demonstration of the use of the band saw to cut parallel circumferential sections of the femoral heads.*

exactly 1.5 mm in thickness, using a Bronwill TSM 77 band saw (Figure 1). The sections were taken in such a way as to include both weight-bearing and non-weight-bearing areas. At the time they were obtained they were photographed and microradiographs were made using a Machlett apparatus (exposure time one hour at 20 KV and 20 MA). Five equally sized rectangular slices were obtained from each section in a manner so as to include both weight-bearing and non-weight-bearing areas (Figure 2).

The five sections from one of the two circumferential slices were used to determine mineral content. The samples were fixed in 80 per cent ethanol with three changes over a period of 48 hours. After freeze drying at  $-50^{\circ}\text{C}$  for three hours, the bone pieces were weighed and then ashed at  $600^{\circ}\text{C}$  for 18 hours and reweighed using a microbalance.

The second group of five sections was fixed in 10 per cent buffered formalin phosphate, then decalcified, dehydrated and embedded in paraffin. Samples were cut at six micra and stained with hematoxylin and eosin and Safranin-O-iron hematoxylin-fast green for histologic study (Mankin et al. 1971). The degree of cartilage degeneration and destruction was evaluated for each of the five segments from the Safranin-O-stained preparations according to the histological-histochemical grading system of Mankin et al. (1971). Four microscopic areas of the subchondral bone from each of the five samples were randomly selected for study. Two of these were considered to be at the "surface" and the other two lay one lattice depth subjacent to that region ("deep"). In all, a total of 240 areas were

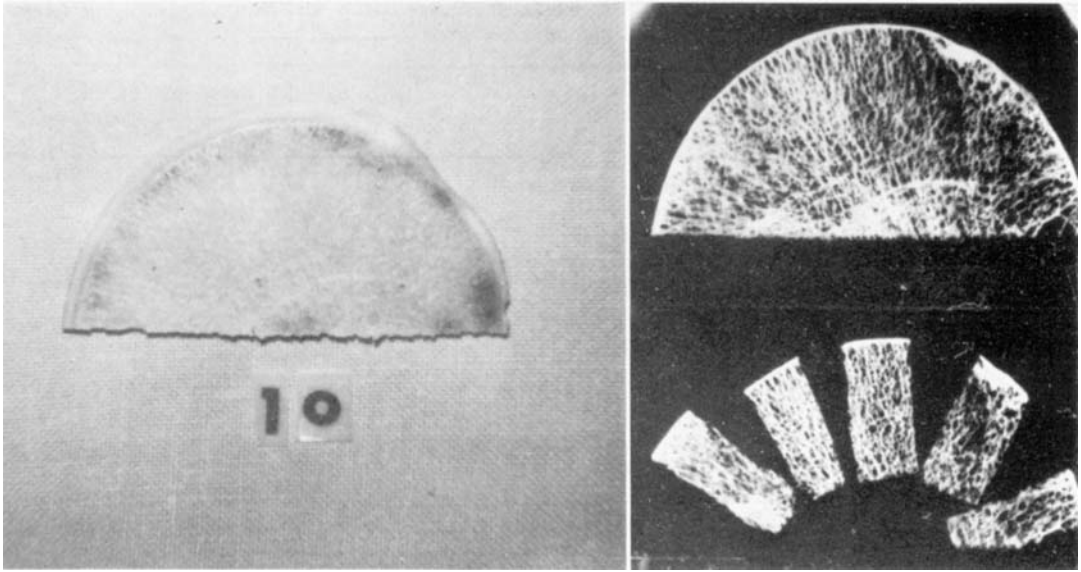


Figure 2. Slice from the normal femoral head of a male aged 61 (left). The x-ray shown in the right side of the figure shows the distribution of the bone segments.

evaluated by specialized morphometric techniques.

The morphometric techniques utilized for this study were designed to provide a measure of total bone present (trabecular bone area: percentage of trabecular bone per total area); anabolic activity (osteoblastic area: per cent of total bone surface occupied by osteoblasts); and catabolic activity (osteoclastic area: per cent of total bone surface occupied by either osteoclasts or resorption lacunae). The trabecular bone area measurement utilized a point-counting method with an integrating eyepiece of 22 points in an hexagonal lattice according to the method of Harris & Weinberg (1972). For the osteoblastic and osteoclastic area counts an integrating eyepiece with a square lattice was used according to the technique of Melsen et al. (1975). Values were expressed as percentage of total area for trabecular bone and percentage of bone present for osteoblastic and osteoclastic areas.

## RESULTS

### 1. Alterations in the cartilage

As can be seen from examination of Table 1, the histological-histochemical grades varied considerably from site to site in the five segments studied from each femoral head, particularly in the osteoarthritic group. A total of seven of

the segments from this group had osteoarthrosis of such severity that no cartilage was present on the specimen. All of these were in the weight-bearing areas.

Table 2 shows the mean values for the histological-histochemical grading of cartilage from the three groups and a comparison of the values for the weight-bearing and non-weight-bearing areas. It should be noted that the bare areas were excluded from the calculations, since the grading system provides no score for total loss of cartilage. Despite this, the differences between the osteoarthritic and either normal or fracture group are significant at the  $P < 0.001$  level. The fracture group also differed significantly from the normal, but with considerably less deviation. Only the osteoarthritic group showed a difference between weight-bearing and non-weight-bearing areas ( $P < 0.01$ ).

### 2. Mineral content

The distribution of the per cent of mineral content in relation to disease and site is shown in Table 3.

Table 1. Distribution of the histological-histochemical grades in the cartilage with relation to age, sex, diagnosis and area.

Specimen No.	Age	Sex	Diagnosis	Histological-histochemical grade	
				WB	Non-WB
2	66	F	Osteoarthritis	-, -, -	3, 8
4	65	F	Osteoarthritis	13, 8, 8,	5, 4
5	46	M	Osteoarthritis	5, 5, 7	4, 2
7	59	F	Osteoarthritis	-, -, 12	1, 2
9	59	F	Osteoarthritis	12, 12, -, -	13
11	52	M	Osteoarthritis	6, 13, 13	2, 13
3	73	F	Fracture	0, 2	3, 2, 7
6	78	F	Fracture	1, 4, 1	1, 2
8	82	F	Fracture	2, 3, 2, 4	5
12	85	F	Fracture	2, 2, 2	2, 2
10	61	M	Normal	0, 2, 2	0, 1
13	60	M	Normal	0, 1, 2	0, 1

—: No cartilage remaining.

Table 2. Variation of histological-histochemical grade with diagnosis and site.

	All sites	Weight-bearing	Non-weight-bearing
Normal (10)	0.90 ± 0.88 <sup>+</sup>	1.17 ± 0.98 (6)	0.50 ± 0.58 (4)
		n.s.	
Fracture (20)	2.45 ± 1.57	2.08 ± 1.16 (12)	3.00 ± 2.0 (8)
	$P < 0.001^{\circ}$	n.s.	
OA (23)	7.30 ± 4.51 <sup>*</sup>	9.50 ± 3.29 <sup>*</sup> (12)	5.18 ± 4.31 (11)
	$P < 0.001$ $P < 0.001$	$P < 0.01$	

\* Excludes 7 of 16 sites on the weight-bearing areas of the osteoarthritic femoral heads which were bare of cartilage.

<sup>+</sup> Standard deviation.

<sup>°</sup> As determined by Student's t-test.

As can be noted, the osteoarthritic samples showed a significant ( $P < 0.001$ ) increase in ash content compared with either normal or fracture groups. Differences between weight-bearing and non-weight-bearing areas for the normal and fracture groups were just barely significant ( $P < 0.02$  and  $P < 0.10$ , respectively) but for the osteoarthritic sub-

chondral bone it was highly significant ( $P < 0.005$ ). The difference between the ash content of the non-weight-bearing areas was moderate (a 17 per cent increase in the osteoarthritic group over the other two at the 10 per cent confidence level) but marked in the weight-bearing areas (~ 27 per cent increase for the osteoarthritic group with  $P < 0.005$ ).

Table 3. Variation of percentage mineral content with disease and site studied.

	All sites	Weight-bearing area	Non-weight-bearing area
Normal (10)	30.42 ± 4.03*	32.72 ± 2.64 (6)	26.99 ± 3.27 (4)
		P < 0.02	
Fracture (20)	29.39 ± 4.64	31.16 ± 4.15 (12)	26.99 ± 4.71 (8)
	n.s.°	n.s.	n.s.
		P < 0.10	
OA (30)	38.78 ± 7.54	40.69 ± 10.01 (19)	31.82 ± 5.71 (11)
	P < 0.001°	P < 0.005	
	P < 0.001†	P < 0.005	P < 0.10
		P < 0.001	P < 0.10

\* Standard deviation.

° Significance of variation from the control group as determined by Student's t-test.

† Significance of variation from the fracture group.

Table 4. Comparison of morphometric parameters for surface and deep zones.

	Per cent trabecular bone area	Per cent osteoblastic area	Per cent osteoclastic area
Normal (10)			
Surface	20.45 ± 2.22†	24.32 ± 2.53	1.45 ± 0.82
Deep	19.68 ± 3.60	20.28 ± 4.80	1.59 ± 0.63
Fracture (20)			
Surface	16.14 ± 4.61+	22.53 ± 12.10	3.34 ± 1.21+
Deep	16.02 ± 3.51+	20.29 ± 12.52	3.23 ± 1.39+
OA (30)			
Surface	32.58 ± 10.97+°	44.20 ± 13.84+°	4.21 ± 2.06+
Deep	26.29 ± 8.20+°	* 31.60 ± 8.98+°	3.64 ± 1.73+

† Standard deviation.

\* Indicates significant difference between deep and superficial zones (as determined by a Student's t-test value of  $P < 0.01$ ).

+ Indicates significant difference from normal group.

° Indicates significant difference from fracture group.

### 3. Bone morphometric analyses

In Table 4 all three morphometric parameters for both superficial and deep zones are compared. Table 5 shows a comparison of the weight-bearing and non-weight-bearing areas for the same measurements.

(a) *Per cent of trabecular bone area.* A significant decrease was noted in the

per cent of trabecular bone area in the fracture population compared with the controls, presumably reflecting the fairly marked difference in average age of the two groups and sex variation (see below). This is perhaps an indication of the sensitivity of the method since it is possible to detect the small difference in bone density associated with aging.

Table 5. Variation in morphometric parameters for surface and deep zones in weight-bearing and non-weight-bearing areas.

	Per cent trabecular bone area		Per cent osteoblastic area		Per cent osteoclastic area	
	WB	NWB	WB	NWB	WB	NWB
Normal						
Surface	20.83 ± 2.24†	19.88 ± 2.18	25.81 ± 1.80	22.09 ± 3.62	1.57 ± 0.78	1.28 ± 0.90
Deep	21.59 ± 1.91	16.82 ± 6.14	22.22 ± 3.16	17.37 ± 7.25	1.51 ± 0.43	1.27 ± 0.88
Fracture						
Surface	18.75 ± 5.41	12.22 ± 3.42*	20.85 ± 11.62	25.04 ± 12.84	3.02 ± 1.11	3.81 ± 1.37
Deep	18.18 ± 3.88	12.78 ± 2.96*	19.04 ± 11.75	22.16 ± 13.67	3.17 ± 1.39	3.34 ± 1.39
OA						
Surface	36.97 ± 11.68	25.00 ± 9.75†	48.44 ± 15.05	36.87 ± 11.72*	5.03 ± 2.32	2.80 ± 1.61*
Deep	29.55 ± 8.78	20.66 ± 7.22*	31.85 ± 8.72	31.16 ± 9.43	3.84 ± 2.13	3.30 ± 1.03

† Standard deviation.

\* Significant difference between weight-bearing and non-weight-bearing zones as determined by Student's t-test with  $P < 0.01$ .

Of greater significance to this study, however, was the rather marked increase ( $\sim 60$  per cent) in the percentage of trabecular bone in the osteoarthritic surface zone and the slightly less marked increment for the deeper zone (Table 4). Compared with the fracture group, the osteoarthritics were markedly hyperostotic with an increase of over 100 per cent for the trabecular bone area in the surface zone.

Analyses of the differences between trabecular bone areas for the weight-bearing and non-weight-bearing areas showed no difference for the normal group, but there was a significant increase in the weight-bearing area for both the fracture group and the osteoarthritics ( $\sim 50$  per cent) (Table 5). In both cases the variation was slightly less marked in the deeper zones.

(b) *Per cent of osteoblastic area.* The percentage of bony surface involved in osteoblastic activity ranged between 18 per cent and 25 per cent for both normal and fracture groups and appeared to vary little with either the surface or deep

zones or the weight-bearing and non-weight-bearing areas (Tables 4 and 5). In contrast, the osteoarthritic subchondral bone showed a marked increase in osteoblastic activity with over 40 per cent of the bony surface engaged in new bone formation. This represents a significant variation in relation to both control groups with an almost 100 per cent increase for the surface zone and over 55 per cent for the deep zone (Table 4). The surface zone showed approximately 40 per cent greater activity than the deep zone (Table 4) and the value for the weight-bearing area exceeded that for the non-weight-bearing by over 30 per cent (Table 5).

(c) *Per cent of osteoclastic area.* The percentage of bony surface involved in osteoclastic activity was very low in all groups and at all sites, ranging from 1.27 per cent to 5.03 per cent. The values were least in the normal, greater in the fracture and highest in the osteoarthritic group (Table 4). No significant difference was noted between the surface and deep zones for any of the groups and

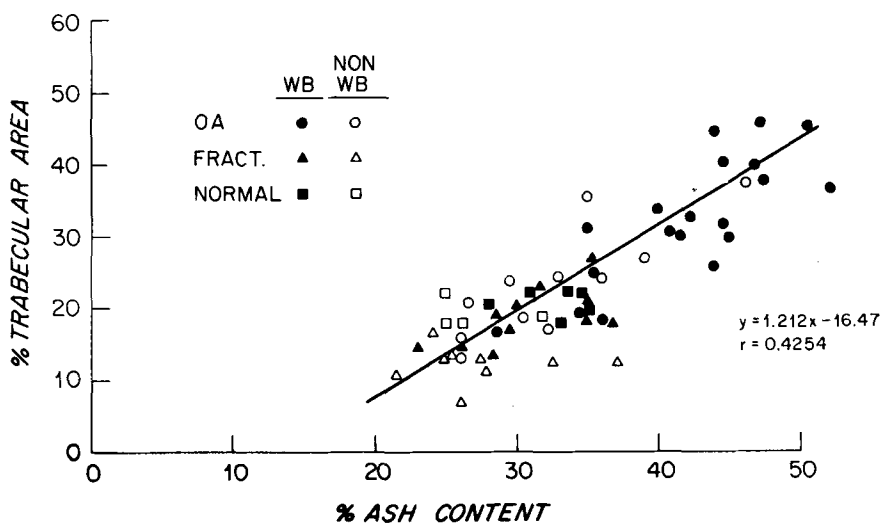


Figure 3. Graphic representation of the relationship between the per cent of trabecular bone area and the per cent of mineral content in the osteoarthritic, fracture and normal femoral head specimens. (Regression analysis was performed by the method of least squares.)

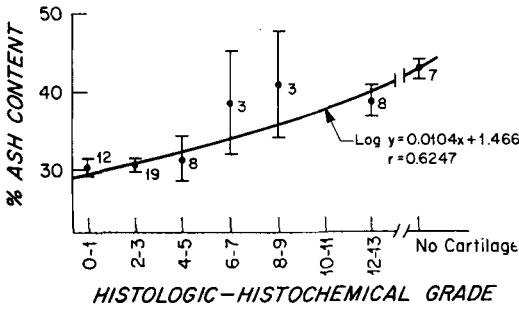


Figure 4. Graphic representation illustrating the relationship between the histological-histochemical grade of the articular cartilage and the per cent of mineral content for the osteoarthritic, fracture and normal femoral heads.

only as a crude corroboration of the methods.

Of greater importance and central to the theme of the study were the correlative data regarding the histological-histochemical grading and the per cent of ash and bone morphometric analyses. Figures 4, 5, 6 and 7 show the correlative data in two forms: groupings with deviations for each data point and correlations as determined by regression analysis. Only the surface morphometric studies were included. As can be seen, the correlations for all but the per cent of trabecular area were of moderate significance. Most

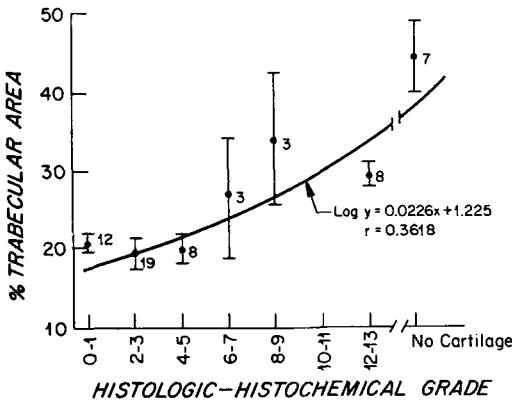


Figure 5. Diagram illustrating the relationship between the histological-histochemical grade and the per cent of trabecular area.

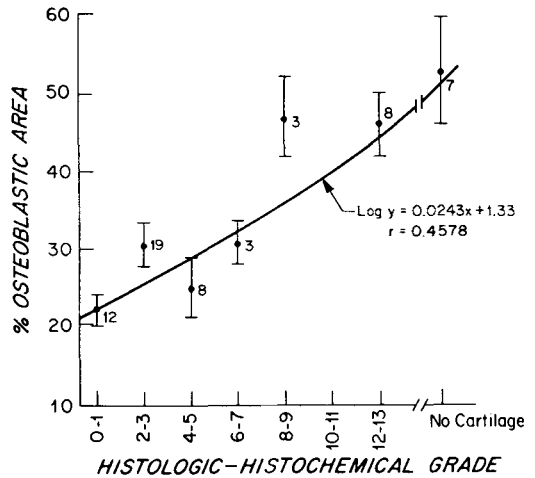


Figure 6. Diagram showing the relationship between the histological-histochemical grade and the per cent of osteoblastic area for all specimens.

only the osteoarthritics showed a difference between weight-bearing and non-weight-bearing areas (Table 5).

#### 4. Correlations

As an "internal control" for the methods, the percentage mineral content was compared with the trabecular bone area. As can be noted in Figure 3, the relationship is linear and has a coefficient of correlation of 0.4254. Since only the per cent of trabecular area for the surface zone was included in the calculations (while the per cent of mineral content determination included both the surface and deep zones), this observation serves

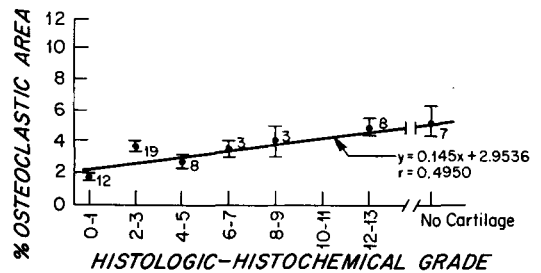


Figure 7. Graphic representation illustrating the relationship between the histological-histochemical grade and the per cent of osteoclastic area for all specimens.

closely correlated was the percentage ash content ( $r = 0.6247$ ) while per cent of osteoblastic and osteoclastic areas were of slightly lesser significance ( $r = 0.4950$  and  $0.4578$ , respectively). The correlations were all non-linear except for the per cent of osteoclastic activity.

## DISCUSSION

Before discussing the significance of the data obtained in this study, it is important to review the methods and some possible sources of error. First, in regard to methods, the histological-histochemical grading system has been shown to correlate well with biochemical abnormalities in osteoarthritic articular cartilage (Mankin et al. 1971) and with increase in enzyme levels in the tissue (Ehrlich et al. 1973). Its principal value is in providing a technique to evaluate a small locus and hence avoid the problems of the highly focal nature of the osteoarthritic process (Sokoloff 1969). Other methods of classification, especially that of Collins (1949), utilize a more macroscopic approach and are of little value in a study such as this.

One problem of the grading system is that no score is possible when the cartilage is totally absent. For purposes of determination of the correlation curves in Figures 4, 5, 6 and 7, an arbitrary value of 16 was awarded to those segments in which no cartilage was found. The shape of the curves (and indeed the coefficient of correlation) might have been different if another value (such as 20 or 25) had been used.

The bone morphometric techniques used in this study are fairly standard and, providing they are randomized, are considered to be reasonably valid (Harris & Weinberg 1972, Melsen et al. 1975). The problem of application to osteoarthritic subchondral bone lies principally in relation to cysts and areas of

microfracture. Since random-site selection was strictly adhered to and thus no attempt made to preselect non-cystic or non-callus areas, this could in theory have introduced a source of error. It should be noted, however, that only the per cent of trabecular area:

$$\frac{\text{trabecular bone}}{\text{total area}} \times 100,$$

could be affected by the presence of cysts or fracture callus since the other determinations are based on total bone present. This may explain the slightly poorer correlation of the per cent of trabecular bone area observed with the histological-histochemical grade (Figure 5).

As previously noted by Harrison et al. (1953), variable amounts of osteonecrosis (as determined by empty lacunae) were noted in the subchondral bone of the osteoarthritic specimens, particularly in the weight-bearing area. Although a presumably important factor in the pathogenesis of the bony changes, the finding did not alter the morphometric observations and was not quantified in this study.

Two "internal controls" were utilized. The first was the inverse correlation of the per cent of trabecular bone area with patient age and sex in the two control groups. The two normal femoral heads were from males aged 60 and 61 years; those in the fracture group were all from females with an average age of 79.5. On the basis of both sex and age, the fracture group should have been considerably more osteopenic than the controls, and this is clearly evident in the data shown in Table 4. As a second internal control, the per cent of ash weight was correlated with the per cent of trabecular bone and showed a linear correlation of moderate significance (Figure 4).

One additional problem in the study is related to selection of specimens. Although the diagnosis was controlled in the sense that the two normal femoral

heads were indeed normal and the osteoarthritics showed extensive changes of disease, the fracture group showed several sites on the femoral heads which displayed mild to moderately severe osteoarthritis. Of perhaps greater importance was the fact that sex and age, both of which have a significant effect on bone mass, were not controlled. The ages for the three groups differed somewhat (especially the fracture group) and the normal specimens were both from males, in the fracture group all from females, and in the osteoarthritic group from four males and two females. For these reasons, the authors utilized the younger, male control specimens upon which to base variations observed in the osteoarthritic group, particularly where a difference was noted between the normal and fracture groups.

In consideration of the experimental results, several points are clearly evident. First, the histological-histochemical grading of the osteoarthritic cartilage showed wide variation from site to site and more severe changes in the weight-bearing areas. These data confirm prior observations from this laboratory (Mankin et al. 1971).

Second, the per cent of mineral content and all bone morphometric parameters showed highly significant increments in the osteoarthritic subchondral bone compared with either the control or fracture group. Thus, not only is there evidence for a hyperostosis in the subchondral area, but also a fairly profound increase in metabolism reflected by increased rates of both osteoblastic and osteoclastic activity.

Of perhaps greater interest was the relationship of these changes to site on the femoral head. In all morphometric measurements, the osteoarthritic weight-bearing area showed greater increases than the non-weight-bearing area with increments above the control values of 27 per cent for ash control, 50 per cent

for the per cent of trabecular area, 30 per cent for the per cent of osteoblastic area and even greater increments for the per cent of osteoclastic area. Furthermore, when looking at the zone extending no more than 5 mm deep to the subchondral surface ("deep" zone), these variations were for the most part significantly reduced. These variations with site are consistent with those reported by Foss & Byers (1972), Radin et al. (1970, 1973), and Pugh et al. (1974), but differ from the observations of Lereim et al. (1975) who found no significant difference in the density of subchondral bone from the weight-bearing area of osteoarthritic joints and that from the non-weight-bearing area or from normal controls.

The principal purpose of this study was to assess if the cartilage changes (which are believed to be an indicator of the severity of the osteoarthritic process) could be correlated with the bony abnormalities as determined by the per cent of mineral content and the morphometric analyses. The correlation curves shown in Figures 4, 5, 6 and 7 indicate a direct relationship between the two groups of parameters of moderately high significance ( $r = 0.500$ ) with the exception of the bone trabecular area which correlated less well ( $r = 0.4254$ ). These data define an association between the two biologic processes: degeneration of the cartilage and sclerosis and hypermetabolism of the subchondral bone.

There are insufficient data from this experiment to indicate whether the two processes, one bony and the other cartilaginous, are related to one another in an etiologic way, as has been suggested by Pugh et al. (1974) and Radin et al. (1970) or whether the two are responding separately (and oppositely) to some other mechanical or chemical stimulus. Although it is possible that the findings are the result of a multiplicity of independent factors, the close inverse

concordance allows speculation that the two processes are interdependent and possibly represent two divergent responses to a single biologic alteration.

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Correspondence to: Inge Reimann, M.D., Department of Orthopaedic Surgery, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark.