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A BIOCHEMICAL ANALYSIS OF SUBCHONDRAL BONE OF THE MEDIAL TIBIAL CONDYLE IN THE NORMAL STATE AND IN OSTEOARTHRITIS AND RHEUMATOID ARTHRITIS

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It has been shown in a previous study (Lereim et al. 1974) that the hardness of subchondral trabecular bone of the medial tibial condyles increases with increasing age in normal individuals irrespective of sex. In patients with osteoarthritis and rheumatoid arthritis the hardness, however, was significantly lower than in the normal group. There was no significant difference between osteoarthritis and rheumatoid arthritis.

The present study concerns a biochemical analysis of subchondral trabecular bone in the medial tibial condyle with special reference to the density of the bone samples and their content of collagen, calcium, phosphorus and magnesium.

MATERIAL

The specimens for this biochemical analysis were identical to those (Figure 1) used in the mechanical study previously reported (Lereim et al. 1974). All specimens were taken from the medial condyle of the tibia and were obtained at autopsy, after amputations or during reconstructive surgery.

Normal

Specimens were obtained from 22 deceased individuals with an age range of 20–90 years (Figure 2). Radiology and naked eye observations verified the normal structure of the bone. The causes of death are seen in Table 1. All individuals had been fully active until 0–2 days before death.

Osteoarthritis

Specimens were obtained from 14 individuals with an age range of 62–91 years (Figure 2). The diagnosis was verified by radiology and naked eye observation,

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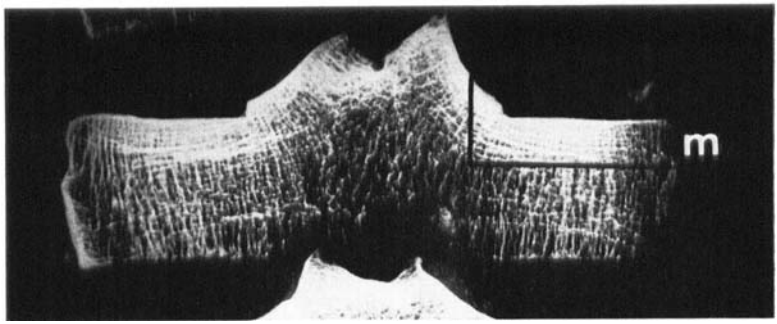

Figure 1. Radiograph of a 5 mm thick slice from normal tibial condyles (m = medial). The demarcated zone was used for biochemical assay.

based on cartilage destruction, osteophytes, subchondral cysts and sclerosis. The cases could be classified as grades II and III according to the classification suggested by Collins (1949). The ailment which prevailed for individuals from which specimens were taken is presented in Table 1. All patients had been fully active until 1–3 days before death.

Rheumatoid arthritis

Specimens were obtained from 12 individuals with an age range of 53–86 years (Figure 2). All were classified as classical rheumatoid arthritis according to the criteria of the American Rheumatism Association (1958). Macroscopically as well as microscopically the changes matched those seen in the osteoarthritis cases very well. Four of the twelve subjects had received general steroid treatment for various periods prior to the time at which the specimen was taken. (In the previous study

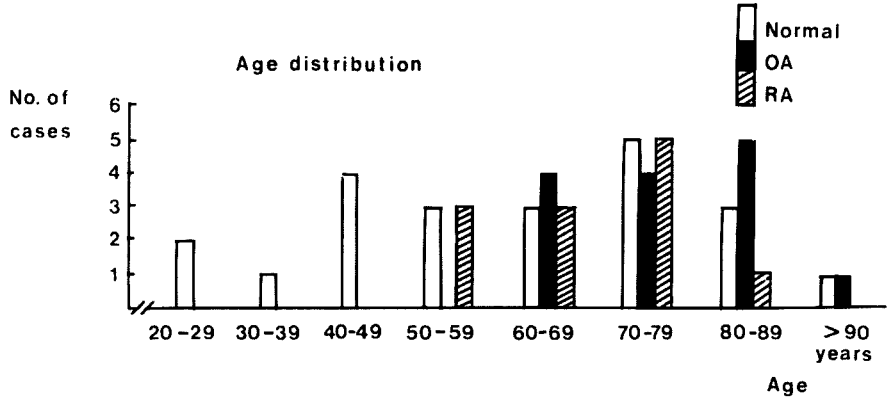

Figure 2. Age distribution of material used for biochemical analysis of subchondral tibial bone in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA).

Table 1. Medical procedure carried out on individuals from whom bone samples from the medial tibial condyle have been obtained for biochemical analysis.

Autopsy		Normal	OA	RA
	1. Cardiovascular disease	8	6	6
	2. Cerebral haemorrage	3	1	
	3. Cancer not affecting the skeletal system	8		
	4. Various diseases	3	1	
Femoral amputation			6	1
Reconstructive surgery				5
Total		22	14	12

on testing of hardness (Lereim et al. 1974) it was shown that steroids had no influence on the hardness. The chemical composition of the subchondral bone of steroid and non-steroid-treated patients is commented upon on page 683. The source of the specimens is seen in Table 1. All individuals had been active until 3–4 days before death.

METHODS

Immediately after removal the specimens were frozen and kept at $-20°$ C. After the Brinell hardness testing at room temperature the specimens were once more frozen to $-20°$ C (Lereim et al. 1974). The frozen specimens were then cut into 5 mm thick slices (Figure 3) which were totally freed from soft tissues and cartilage. For biochemical analysis, slices (a or b in Figure 3) from the weight-bearing area were used.

Volume and weight

The specimens were weighed by attaching them to a surgical needle on a fine wire hanging on a balance. Thereafter they were weighed submerged in distilled water while still suspended from the balance as described. After the specimens had been weighed in water they were weighed in air again. These procedures were undertaken at room temperature (about 22° C). The weight of the bone piece in air returned to the original wet weight after submerging in water. The weight of the water displaced by the bone sample gave the volume of the actual sample at room temperature and the density could be calculated as weight per volume.

The specimens were defatted in two changes of acetone over a period of 48 hours and in two changes of ether for another 48 hours. After this the bone pieces were dried in air at 50° C to constant weight. Then the weight of the dry fat free samples was recorded.

Each sample was placed in hydrolysis vials and 10 ml 6 N HCL was added. After

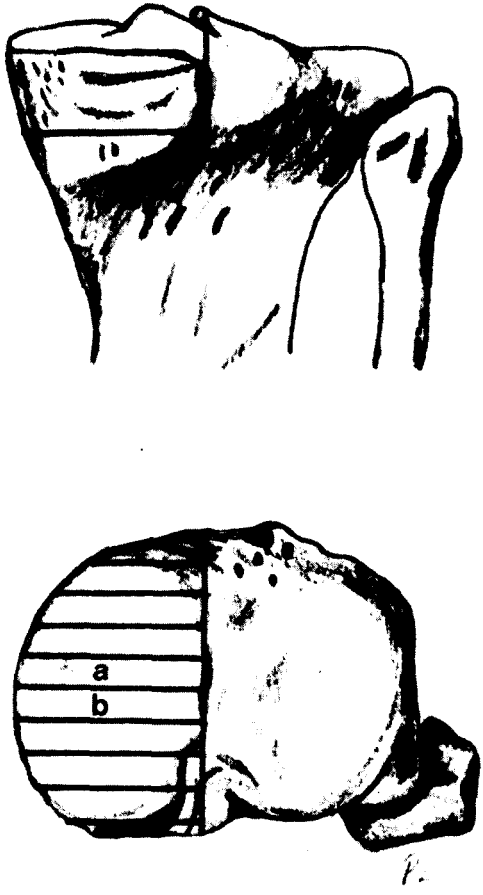

Figure 3. Schematic representation of the tibial condyles to demonstrate the sections used from the medial condyle for various analyses. The biochemical analysis was carried out on bone from the weightbearing area here marked a and b.

sealing the vials, the samples were hydrolysed at 125° C for 18 hours. The hydrolysates were divided into appropriate samples for the different analyses.

Collagen

Collagen was determined according to the method of Firschein & Shill (1966). The part of the hydrolysate used for the estimation of collagen was filtered through a fine porosity fitted glass disc (Pyrex G 3) and the content of hydroxyproline was

measured. The content of collagen was calculated assuming that 14.5 per cent of the collagen was hydroxyproline (McLean & Urist 1968).

Calcium – Phosphorus – Magnesium

Part of the hydrolysate was diluted 1:100 with distilled water. Calcium was determined by the SMA 12/60 System Calcium method, which is a modification (Gitelman 1967) of the method of Kessler & Wolfman (1964). Phosphorus was determined by the SMA 12/60 System Inorganic Phosphate method, which is based on the formation of phosphomolybdic acid (Kraml 1966).

For the magnesium determination part of the hydrolysate was diluted 1:10 with distilled water. This mixture was diluted 1:26 with 2 per cent lanthanum chloride and analysed in a Unicam SP 90 atomic absorptions spectrophotometer. Day to day variation coefficients were: Ca. 1.9 per cent, P 3.7 per cent, and Mg 5.1 per cent.

Statistical analysis

For statistical analysis Student's *t*-test was used as well as chi square analysis (Ulf Runze, B. A.).

RESULTS

Density

The density (w/v) of the individual specimens is shown in Figure 4. A presentation of mean values is made in Table 2. In the normal group there was a decrease in density with advancing age ($P < 0.05$). Over the age of 54 there was no significant difference between the three groups. In the entire material there was a tendency for a decrease in density with advancing age ($0.05 < P < 0.10$). The age group 70–79 years had the lowest density

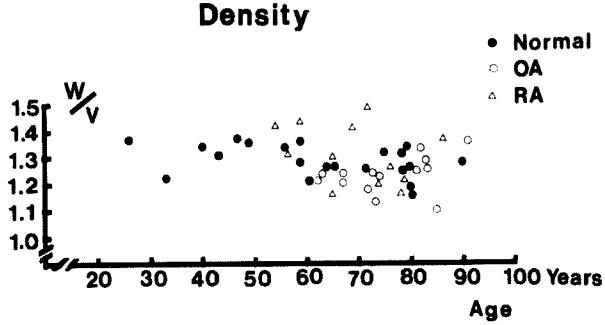

Figure 4. Density in weight per volume of subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and rheumatoid arthritis, related to age.

Table 2. Density of subchondral tibial bone. Mean values in different age groups of normal individuals and those with osteoarthritis (OA) or rheumatoid arthritis (RA).

Age	Normal	n	O.A.	n	R.A.	n
< 50	1.33	(7)				
50—59	1.30	(3)	–		1.39	(3)
60—69	1.25	(3)	1.22	(4)	1.29	(3)
70—79	1.29	(5)	1.18	(4)	1.27	(5)
80—89	1.20	(3)	1.24	(5)	1.37	(1)
> 90	1.29	(1)	1.36	(1)	–	
Mean > 56	1.27 ± 0.05		1.23 ± 0.07		1.31 ± 0.11	
Total normal	1.29 ± 0.06					

Chemical determination

The results of the chemical determinations are presented as percentage of dry fat free bone and mg/ml wet bone. Differences between the groups (normal, osteoarthritis and rheumatoid arthritis) have been calculated with the above-mentioned units. Specimens in normal individuals below the age of 56 were excluded as corresponding values for the pathological groups were not obtained. The change with age has been calculated for the normal state in mg/ml. Calculations for possible changes with increasing ages in the pathological groups have not been done.

Table 3. Collagen in subchondral tibial bone. Mean values in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA) expressed in percentage of dry fat free bone weight (%) and mg/ml wet bone.

Age	Normal			O.A.			R.A.		
	%	n	mg/ml	%	n	mg/ml	%	n	mg/ml
< 50	23.28	(7)	135.60						
50—59	23.91	(3)	117.83	–		–	26.37	(3)	180.63
60—69	23.74	(3)	112.23	23.66	(4)	94.80	23.40	(3)	119.03
70—79	23.14	(5)	119.20	23.75	(4)	84.97	23.07	(5)	115.38
80—89	25.33	(3)	98.20	23.53	(5)	108.24	20.91	(1)	148.8
> 90	24.89	(1)	149.20	24.58	(1)	141.2	–		–
Mean > 56	24 ± 1.3		115 ± 17	24 ± 1.4		100 ± 31	24 ± 2.2		135 ± 53
Total normal	24 ± 1.1		121 ± 19						

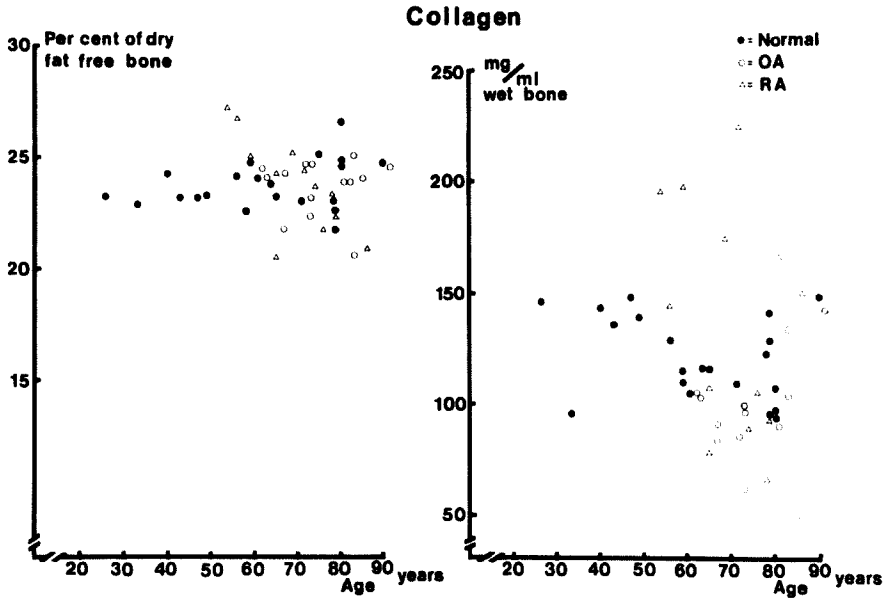

Figure 5. Individual collagen determinations in subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and in rheumatoid arthritis, related to age.

Collagen

There was no significant difference between the three groups (Figure 5, Table 3). However, there was a tendency for a difference between osteoarthritis and rheumatoid arthritis ($0.05 < P < 0.10$), the highest mean value being found in rheumatoid arthritis.

The collagen content remained unchanged with increasing age.

Calcium

The calcium content per volume of tissue was significantly higher in rheumatoid arthritis than in osteoarthritis ($P < 0.05$) while the mean value of the normal bone was found to be between those of the pathological groups (Figure 6, Table 4).

The calcium content in the normal state was constant with increasing age.

Phosphorus

The content of phosphorus per volume of tissue was significantly higher in rheumatoid arthritis than in osteoarthritis ($P < 0.05$) while

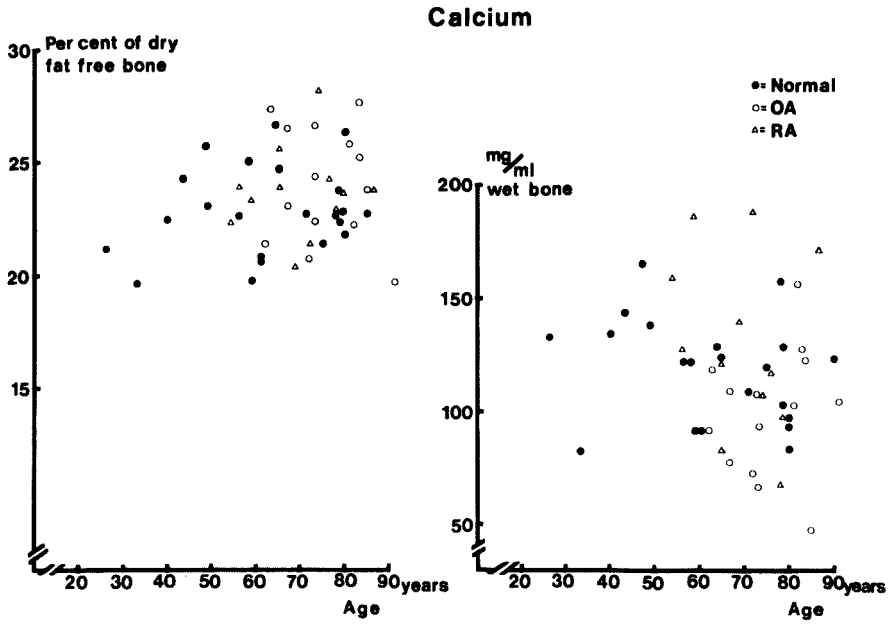

Figure 6. Individual calcium determinations in subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and in rheumatoid arthritis, related to age.

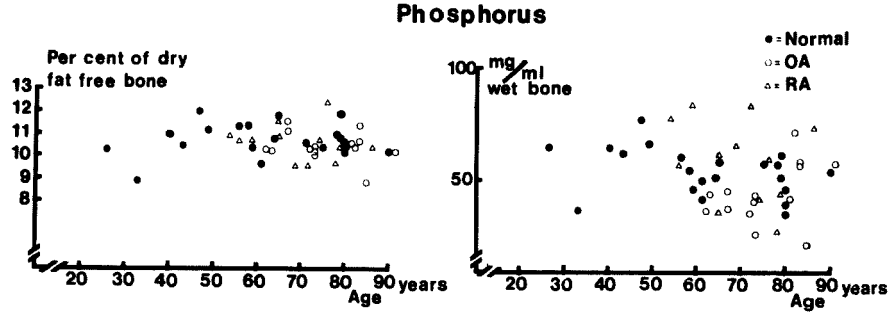

Figure 7. Individual phosphorus determinations in subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and in rheumatoid arthritis, related to age.

the mean value of the normal bone was found to be between those of the other two groups (Figure 7, Table 5).

The content per volume of tissue decreased significantly with age in the normal group ($P < 0.05$).

Table 4. Calcium content of subchondral tibial bone. Mean values in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA) expressed in percentage of dry fat free bone weight (%) and mg/ml wet bone.

Age	Normal			O.A.			R.A.		
	%	n	mg/ml	%	n	mg/ml	%	n	mg/ml
< 50	23.02	(7)	135.3						
50—59	22.48	(3)	111.10	–		–	23.23	(3)	157.46
60—69	24.10	(3)	114.63	24.57	(4)	98.82	23.29	(3)	114.76
70—79	22.66	(5)	123.34	23.55	(4)	84.40	24.06	(5)	114.76
80—89	23.73	(3)	90.96	24.95	(5)	110.27	23.88	(1)	169.90
> 90	22.78	(1)	122.5	19.68	(1)	113.00	–		–
Mean > 56	23 ± 1.9		112 ± 20	24 ± 2.6		100 ± 28	24 ± 2.0		130 ± 40
Total normal	23 ± 2.0		118 ± 23						

Table 5. Phosphorus content of subchondral tibial bone. Mean values in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA) expressed in percentage of dry fat free bone weight (%) and mg/ml wet bone.

Age	Normal			O.A.			R.A.		
	%	n	mg/ml	%	n	mg/ml	%	n	mg/ml
< 50	10.62	(7)	62.33						
50—59	11.01	(3)	54.24	–		–	10.73	(3)	73.08
60—69	10.79	(3)	51.28	10.78	(4)	41.19	10.70	(3)	53.42
70—79	10.94	(5)	56.06	10.30	(4)	37.09	10.51	(5)	50.81
80—89	10.41	(3)	40.40	10.36	(5)	49.75	10.36	(1)	73.72
> 90	10.19	(1)	54.80	10.19	(1)	58.56	–		–
Mean > 56	10.8 ± 0.6		52 ± 4	10.5 ± 0.7		44 ± 14	10.6 ± 0.9		59 ± 19
Total normal	10.7 ± 0.7		55 ± 9						

Ca/P–ratio

There was no difference between rheumatoid arthritis and the normal state whereas there was a tendency for higher values in osteoarthritis ($0.05 < P < 0.10$) (Figure 8, Table 6). The mean value for the normal group was 2.14 and did not change with advancing age.

Magnesium

There was no difference between the three groups (Figure 9, Table 7). In the normal group there was a decrease in magnesium per volume of tissue with advancing age ($P < 0.05$).

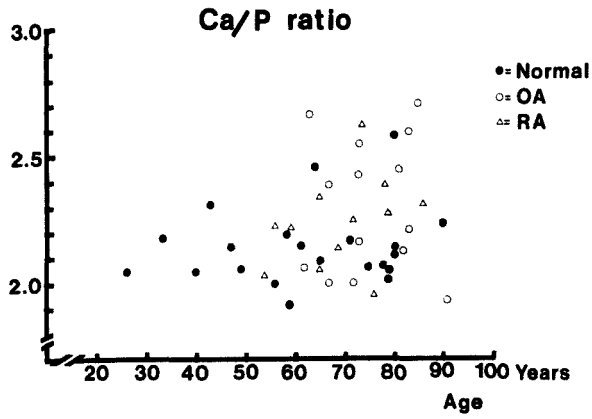

Figure 8. Individual Ca/P ratio in subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and in rheumatoid arthritis, related to age.

Table 6. Ca/P ratio in subchondral tibial bone. Mean values in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA) expressed in percentage of dry fat free bone weight (%) and mg/ml wet bone.

Age	Normal		O.A.		R.A.	
	mg/ml	n	mg/ml	n	mg/ml	n
< 50	2.17	(7)				
50—59	2.04	(3)	–		2.16	(3)
60—69	2.23	(3)	2.27	(4)	2.17	(3)
70—79	2.07	(5)	2.28	(4)	2.28	(5)
80—89	2.27	(3)	2.40	(5)	2.01	(1)
> 90	2.23	(1)	1.93	(1)	–	
Mean > 56	2.15 ± 0.17		2.31 ± 0.19		2.24 ± 0.18	
Total normal	2.14 ± 0.16					

COMMENTS

The reasons for expressing the chemical results both as percentage of dry fat free bone weight and as mg/ml wet bone volume was that this allowed a comparison of the results with those of earlier reports. In these, the percentage of dry fat free bone weight as the basic unit is often used. However, Robinson & Elliot (1957) and especially Strandh & Norlén (1965) showed that in studies of bone atrophy the results would not disclose any major differences when calculations were made on a weight basis. Greater confidence in results for com-

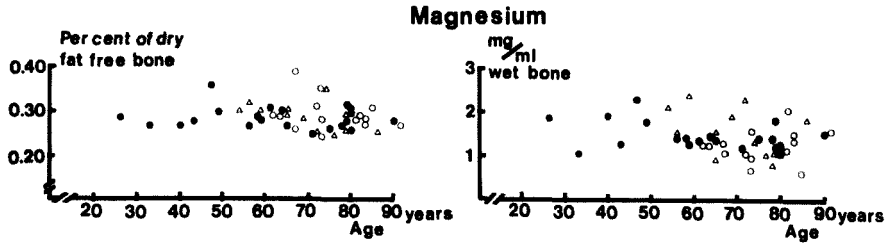

Figure 9. Individual magnesium determinations in subchondral bone from the medial tibial condyle in the normal state, in osteoarthritis and in rheumatoid arthritis, related to age.

Table 7. Magnesium content of subchondral tibial bone. Mean values in the normal state, in osteoarthritis (OA) and in rheumatoid arthritis (RA) expressed in percentage of dry fat free bone weight (%) and mg/ml wet bone.

Age	Normal			O.A.			R.A.		
	%	n	mg/ml	%	n	mg/ml	%	n	mg/ml
< 50	0.30	(7)	1.75						
50—59	0.28	(3)	1.39	–		–	0.31	(3)	2.00
60—69	0.29	(3)	1.39	0.30	(4)	1.22	0.29	(3)	1.49
70—79	0.27	(5)	1.42	0.29	(4)	1.07	0.27	(5)	1.30
80—89	0.29	(3)	1.12	0.28	(5)	1.32	0.25	(1)	1.78
> 90	0.28	(1)	1.51	0.27	(1)	1.56	–		–
Mean > 56	0.28 ± 0.02		1.36 ± 0.19	0.29 ± 0.03		1.24 ± 0.37	0.29 ± 0.03		1.57 ± 0.55
Total normal	0.29 ± 0.02		1.46 ± 0.32						

parison could be obtained in recording mg/ml wet bone volume. In this study the opinion of Strandh & Norlén (1965) was confirmed as the variations in the results were very small when calculated on the weight basis, whereas the calculations on the volume basis revealed greater variation. We found this more suitable as a means of illustrating the age influence and the differences between subchondral bone from individuals with normal and diseased knee joints. It became evident that this procedure was more appropriate when comparison was made with the results obtained at a determination of the physical properties and the chemical composition of bone as presented by Romanus in 1974. The background for our study was to correlate, if possible, the results of a hardness testing of subchondral bone with the chemical analysis. For this reason it was found more acceptable to calculate the

influence of age on the different chemical components, as well as the difference between the various groups, on the basis of volume rather than on percentage of dry fat free bone weight.

In this investigation it was shown that the difference in the chemical content of bone between the three different groups (normal, osteoarthritis and rheumatoid arthritis) and the influence of age were rather small. (In our small material we found no difference in chemical composition of bone from steroid and non-steroid-treated patients with rheumatoid arthritis.) This can only in part suggest an explanation for the possibility of the chemical composition serving as a background for the great differences in hardness in the same subchondral bone material as reported by Lereim et al. (1974). Therefore, material identical to that used for this investigation will be further analysed by histologic and radiologic means in order to throw more light on the factors behind the difference in hardness of subchondral bone in the different groups.

SUMMARY

A chemical analysis has been carried out on specimens from the subchondral weightbearing area of the medial tibial condyle from 22 normal individuals, 14 individuals with osteoarthritis and 12 individuals with rheumatoid arthritis. In the normal group there was a decrease in density with advancing age. Over the age of 50 there was no significant difference between the groups.

The content of collagen, calcium, phosphorus and magnesium in each bone specimen was calculated. When expressed in per cent of dry fat free bone there was no significant difference between the three groups. When calculations were made on the basis of content per volume tissue wet bone some differences were found. There was a tendency for a higher content of collagen in rheumatoid bone than in normal and osteoarthritic bone. The content of calcium was significantly higher in rheumatoid arthritis than in osteoarthritis; the same result was found in the analysis of phosphorus. In the normal group there was a decrease in phosphorus content with advancing age, this was also seen in the magnesium analysis.

REFERENCES

American Rheumatism Association (1958) Diagnostic criteria for rheumatoid arthritis 1958 revision. *Ann. rheum. Dis.* **18**, 49.

Collins, D. H. (1949) *The pathology of articular and spinal diseases.* Williams & Wilkins Co., Baltimore.

Firschein, H. E. & Shill, J. P. (1966) The determination of total hydroxyproline in urine and bone extracts. *Analyt. Biochem.* **14**, 296.

Gitelman, H. J. (1967) An improved procedure for the determination of calcium in biological specimens. *Analyt. Biochem.* **18**, 521.

Kessler, G. & Wolfman, M. (1964) An automated procedure for the simultaneous determination of calcium and phosphorus. *Clin. Chem.* **10**, 686.

Kraml, M. (1966) A semiautomated determination of phospholipids. *Clin. Chem.* **13**, 442.

Lereim, P., Goldie, I. & Dahlberg, E. (1974) Hardness of the subchondral bone of the tibial condyles in the normal state and in osteoarthritis and rheumatoid arthritis. *Acta orthop. scand.* **45**, 614.

McLean, F. C. & Urist, M. R. (1968) *Bone.* The University of Chicago Press, Chicago & London.

Robinson, R. & Elliott, S. (1957) The water content of bone. *J. Bone Jt Surg.* **39**-A, 167.

Romanus, B. (1974) Physical and chemical content of canine femoral cortical bone in nutritional osteopenia. Its reversibility and the effect of fluoride. *Acta orthop. scand.*, Suppl. 155.

Strandh, I. & Norlén, H. (1965) Distribution per volume bone tissue of calcium, distribution per unit weight. *Acta orthop. scand.* **35**, 257.

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