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BONE THICKENING IN OSTEOARTHROSIS

Observations of an Osteoarthrosis-Prone Strain of Mouse

MARK WALTON* & MICHAEL W. ELVES†

Institute of Orthopaedics, Stanmore, England

Estimations were made of the amount of bone (histologically) and the rate of bone formation (^{85}Sr incorporation) in the epiphyses of the knees of osteoarthrosis-prone (STR/ORT) and normal (CBA/ORT) mice. Though the bone was significantly thicker in the STR/ORT mice, this was not the cause of the articular degeneration. Bone sclerosis and cartilage breakdown were chronologically very closely related with perhaps the cartilage changes occurring initially. In male STR/ORT mice bone formation was depressed in the cancellous bone of the epiphyses as, unlike the normal mice, it was at the same level as the compact bone of the femoral shaft. As there was no elevation of the osteoblastic activity in knee joints with developing osteoarthrosis, it would appear that bone sclerosis associated with the disease was due to decreased osteoclasis.

Key words: bone formation; bone thickening; mouse; osteoarthrosis

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The thickening of subchondral bone under degenerating articular cartilage is an integral part of the disease process in osteoarthrosis (degenerative joint disease) (Collins 1949, Sokoloff 1969). It has been generally accepted that the bone thickening is a secondary reaction of the tissue to the disintegration of the overlying cartilage. Contrary to this belief, Radin et al. (1972) have postulated that initially the bone becomes thickened as a result of the repair of microfractures caused by excessive impulse loading of the joint. Consequently, they claimed, the weight-bearing tissues become less pliable so that there is greater shock absorption by the cartilage itself, which, as a result, degenerates. However the exact sequential appearance of subchondral bone sclerosis relative to cartilage breakdown cannot be investigated in the human and has not been studied in a naturally occurring form of osteoarthrosis in animals.

A murine form of osteoarthrosis in the knee joint of the STR/ORT strain, which has been described in some detail (Walton 1977a, b and c, 1978, 1979), appeared to be a suitable model for examining the sequential appearance of subchondral bone sclerosis and cartilage degeneration and to study the rate of bone turnover in the development of the disease.

Present addresses:
* Dept. of Orthopaedic Surgery, Univ. of Otago Medical School, P.O. Box 913, Dunedin, New Zealand.

† Microbiology Division, Glaxo-Allenburys Research, Greenford, England.

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MATERIALS AND METHODS

Mice from the osteoarthrosis-prone strain STR/ORT were taken at 2 (mostly pre-arthrotic), 7 (mostly early arthrotic) and 10 (mostly advanced arthrotic) months of age. As controls, mice of the same ages were used from the non-osteoarthrosis-prone strain CBA/ORT (Walton 1977a).

Epiphyseal bone content. Histological preparations of coronal sections of the knee joints from male mice were used to assess the proportion of the epiphyses that consisted of lamellar bone. Under a magnification of ×160 and with a Chalkley random point graticule inserted in one eyepiece, the medial and lateral, tibial and femoral epiphyses were positioned in turn such that only cancellous bone filled the field covered by the graticule. Of the 25 randomly scattered dots on the graticule, only those that were on or partly on lamellar bone were counted. Serial sections from a minimum of eight knee joints from four STR/ORT mice and ten from six CBA/ORT mice were scored in each age group. The results were calculated as a percentage of the epiphysis which was occupied by lamellar bone. This was repeated for the medial and lateral sides of the tibial and femoral epiphyses for each strain in the three age groups. In addition each joint was scored for the severity of osteoarthrosis as previously described (Walton 1977b). Student's t test was applied to assess the level of significance of the bone content (a) between the various ages and (b) between the various grades of osteoarthrosis.

Rates of bone accretion. The method used was modified from that of Elves (1974). Portions of bone from a total of 82 male and female, CBA/ORT and STR/ORT mice were used. Each animal was given 0.08 μC ^{85}Sr/10g body weight by the intraperitoneal route, and was killed $3\frac{1}{2}$ days later. Lateral and antero-posterior radiographic views of the knee joints were taken. The femur and proximal end of the tibia were removed from each side of the animal and scraped clean. Two portions of the femur were excised, (a) the distal epiphysis (including the growth plate) which was then divided, through the patella groove and intercondylar notch, into medial and lateral halves, and (b) the shaft. The proximal epiphysis of the tibia was also taken and divided between the two condyles into medial and lateral portions. After immediate weighing, the radioactivity of each piece of bone was measured using a Nuclear Enterprise 8312 β/γ counter. The results were calculated as specific activity of ^{85}Sr/mg of bone. In order to ascertain the type of bone that constituted the femoral shaft, histological examinations were made of samples taken from each age group of both strains and sexes.

RESULTS

Epiphyseal bone content

The results were very consistent within each group as shown by the low values for the standard errors (Figure 1.). Generally in individual non-arthrotic joints the values of bone content were reasonably consistent throughout all the serial sections. However in joints with early osteoarthrosis the bone was thicker in sections showing degenerative changes in the articular cartilage.

In the normal young mice of the CBA/ORT strain the bone was significantly thicker in their medial compared with each of their lateral tibial ($P = 0.05$–0.025) and femoral ($P = 0.05$) epiphyses. However only in the medial tibial epiphysis, where there was a rise of 7.7 per cent between 2 and 7 months of age, did the density of bone increase significantly with age ($P = 0.025$).

The tibial epiphyses of male STR/ORT mice had significantly more bone on the

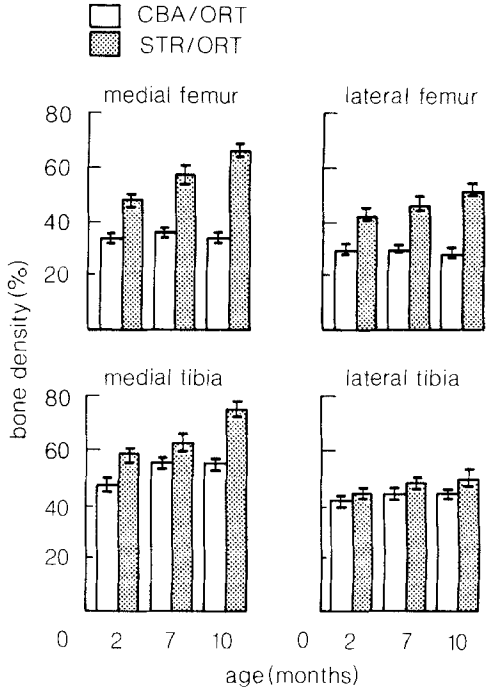


Figure 1. Density of bone in the epiphyses of male STR/ORT and CBA/ORT mice. The standard error is indicated at the top of each column.

medial side at 2 months of age ($P < 0.005$) but no such asymmetry occurred in the femoral epiphyses. With age in all the bone portions the content of bone increased significantly. Between 2 and 10 months the amount of bone in the tibial epiphyses increased twice as much on the medial side (17.4 per cent) compared with the lateral side (8.7 per cent). Over the same period the medial femoral epiphyses consisted of 19.1 per cent more lamellar bone compared with an increase of only 9.0 per cent laterally.

Comparing the results from the two strains, the medial tibial epiphyses of the STR/ORT mice contained 10.7 per cent more bone than the comparable region of the CBA/ORT mice at 2 months of age ($P < 0.005$). At all ages, three regions were significantly more dense ($P < 0.005$) than the corresponding CBA/ORT epiphyses. The exception was the lateral tibial epiphysis which had a greater density of bone than the equivalent CBA/ORT, but the difference between the two was not significant.

Table 1 shows the mean content of bone in the medial tibial epiphyses of those joints with each grade of severity of osteoarthrosis. From this data it will be seen that the greater the severity of the lesion the greater was the content of bone in the epiphysis. There were statistically significant differences between grades 0 and 1 and between grades 2 and 4 ($P = 0.005$ and <0.005, respectively). There was also a significant difference between grade 0 STR/ORT knee joints and those of 2-month-old CBA/ORT mice ($P = 0.025$).

Table 1. Correlation between the grade of severity of osteoarthrosis and the degree of bone sclerosis in the medial tibial epiphyses

Grade of osteoarthrosis	No. of joints	Content of bone (% volume of epiphysis ± s.e.)
STR/ORT		
0	7	55.1 ± 2.1
1	7	65.8 ± 2.7
2	5	68.1 ± 3.2
3	1	75.3
4	5	90.6 ± 2.1
CBA/ORT		
0	30	53.0 ± 3.2

Rates of bone accretion

A minimum of six results were obtained from each sex and strain for every age and each of the bone portions. A mean of the six results was calculated. In some instances a high value of the standard error reflected considerable deviation of individual readings from the mean value. The medial epiphyses of male STR/ORT tibiae incorporated more radiostrontium than the lateral side at 2 months of age ($P = 0.05$). However, as the differences in uptake between lateral and medial epiphyses in all the other groups were not statistically significant, the data from both sides were combined to give the final values. The results from the left and right femoral shafts were also combined. The results are presented in Figures 2, 3 and 4.

Overall there was a general decline in the uptake of the radionuclide with age. The male CBA/ORT and the females of both strains incorporated more ^{85}Sr in the cancellous bone of their epiphyses than in the compact bone of their femoral shafts. In contrast, the levels of radioactivity in both the shafts and epiphyses of the male STR/ORT were similar. Thus though the levels in males of both strains were very similar in the compact bone, the levels were higher in the epiphyses of the CBA/ORT strain than the STR/ORT. Female CBA/ORT mice showed lower incorporation of the radionuclide than their male counterparts, whereas the female STR/ORT mice always had much higher levels than the male of that strain.

Severe degenerative lesions in the form of deep erosions could be seen by the naked eye on the medial condyles of some of the male STR/ORT specimens. In some of these joints both the medial and lateral epiphyses had incorporated approximately half the ^{85}Sr than the mean for their age group. However, the other osteoarthrotic joints deviated little from the age group mean.

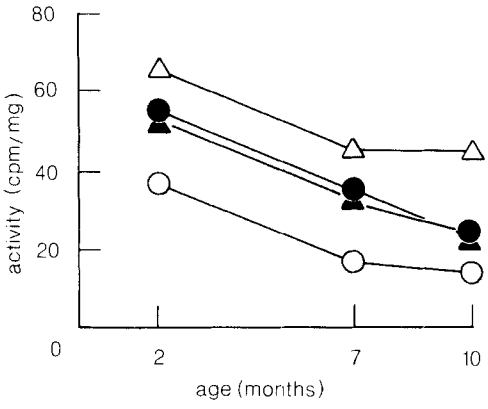


Figure 2. Femoral shafts

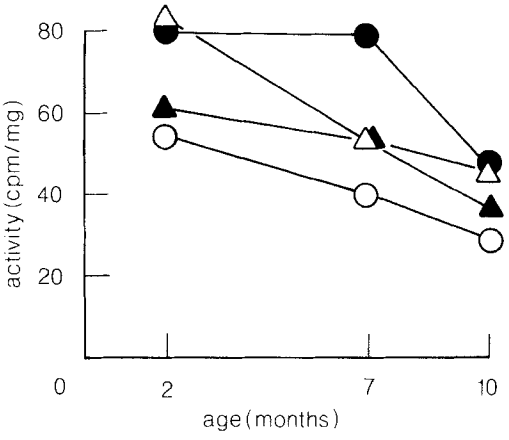


Figure 4. Proximal tibial epiphyses

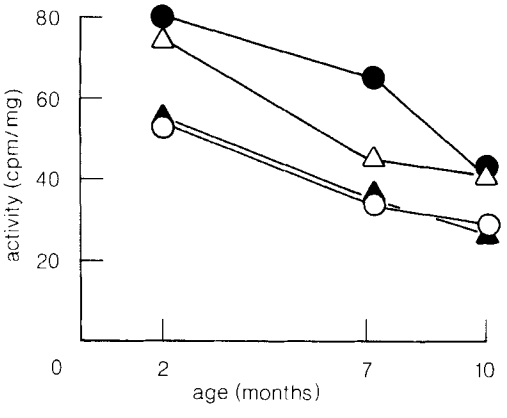
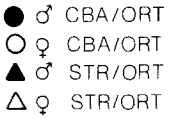


Figure 3. Distal femoral epiphyses



Figures 2, 3 and 4.
Radioactivity of portions of bone 3½ days after intraperitoneal administration of 0.08 μC ^{85}Sr/10g body weight.

DISCUSSION

Some degree of flexibility will enable a structure to widely distribute through its mass mechanical forces applied to it. On the other hand if the structure is rigid, then there is little dissipation of the applied forces, which are therefore borne by a smaller mass of the structure. Thus weight-bearing by an articulating surface of a joint is not likely to damage the tissues if the force-attenuating properties of the cartilage and underlying bone are such that they can absorb and spread the load. However, Radin & Paul (1970) have pointed out that cartilage has visco-elastic properties which means that there is a time delay in its response to load-bearing; consequently the subchondral bone probably takes the brunt of the impulsive loads applied to the joint. The amount, and thus the stiffness of this bone is therefore a major factor in the nature of the mechanical response of the joint and consequently the health of the tissues. If the volume of subchondral bone relative to marrow space increases, then mechanically it will be stronger but less flexible (Behrens et al. 1974).

The present study has shown that the

amount of bone in the medial tibial epiphyses of CBA/ORT and non-arthrotic STR/ORT mice is greater than that of the lateral epiphyses. A similar situation is present in man (Behrens et al. 1974, Lereim et al. 1974). Between strains at 2 months of age there was 10.7 per cent more bone in the medial tibial epiphyses of the STR/ORT strain than in the corresponding region in the CBA/ORT strain. However this difference is not an explanation for the differential occurrence of osteoarthrosis between the strains. The onset of the disease in individual STR/ORT mice is spread over a wide span of ages (2–11 months, Walton 1977b). As some 10-month-old mice had no histological signs of osteoarthrosis (grade 0) yet had a higher epiphyseal bone content than the CBA/ORT mice, it would appear that the abnormal amounts of bone do not as such give rise to the articular degeneration.

According to Radin et al. (1973) the initial process of bone thickening in osteoarthrosis is due to callus formation at the sites of multiple fatigue microfractures. However, in an identical experiment, Serink et al. (1977) found that microfractures were rare and they considered them to be artefactual. Bone sclerosis in STR/ORT mice appears only under degenerating articular cartilage. It forms first in the small inner portion of the medial tibial epiphysis and then slowly extends outwards as the cartilage lesion spreads across the condyle. At all stages of development the bone thickens by the laying down of normal lamellar bone on existing trabeculae. Callus in the form of woven bone was never seen. When the disease in STR/ORT mouse knee joints was prevented by surgical means the subchondral bone sclerosis did not occur (Walton 1979).

If active thickening of the bone was a prerequisite for the disease then a wide variability in the content of bone in grade 0 joints would be expected; some individual joints would have been abnormally thickened prior to an early onset and there would have been others with normal amounts of bone which would develop the disease later in life. However, this was not the case, as the grade 0 joints included some from 2, 7 and 10 months of age, and yet the variation between the individual joints was small as demonstrated by a low standard error. It would seem more likely that if one of the pathological features had preceded the other, then the cartilage degeneration had occurred before bone thickening.

The actual amount of bone present in any skeletal system is determined by a dynamic equilibrium between formation and resorption. The incorporation of radio-strontium into the mouse bones indicates the level of bone accretion over a $3\frac{1}{2}$ day period, after which non-incorporated radionuclide has disappeared from the tissues (Elves 1974). With the larger osteogenic surfaces of cancellous bone as opposed to compact cortical bone, greater formation would be expected, and was indeed found in CBA/ORT mice, in the bone of the epiphyses compared with that in the bone of the shafts. However, incorporation of the radionuclide in the bones of the male STR/ORT strain was similar in the epiphyses and shafts. This could be explained by either the thicker bone of the STR/ORT epiphyses, resulting in reduced surface area for appositional bone formation, or it may be due to an abnormal depression of the osteoblastic activity in the epiphyses. Certainly in both femoral and tibial epiphyses of the male STR/ORT mice the levels of radionuclide were very much lower than in the CBA/ORT mice, even at 2 months of age before any significant amount of sclerosis was evident. This would indicate that there is a reduced rate of bone formation in these animals.

The epiphyses of the femur and tibia were divided in order to detect any differences in bone formation rates between the medial side, where osteoarthrosis always occurs in the male STR/ORT mice, and the lateral side which invariably remains normal. Only in 2-month-old male STR/ORT mice was there any significant difference between medial and lateral sides. This is unlikely to be due to early sclerosis as the incidence of osteo-

arthrosis is low in this age group. At subsequent ages in male STR/ORT mice, medial and lateral sides were not statistically different in their ^{85}Sr uptake, which was unexpected in view of the fact that sclerosis was actively developing medially in a high proportion of the 7-month-old mice. The explanation for this must be that bone sclerosis in degenerative joint disease of male STR/ORT mice is due to reduced osteoclasis rather than enhanced osteoblastic activity.

Thus the epiphyses of male STR/ORT mice have an abnormally low bone turnover with reduced bone formation and resorption. In addition it appears from the estimations of epiphyseal bone content that the articular cartilage in the knee joints of male STR/ORT mice may be degenerating before the underlying bone thickens, though both processes are closely related pathologically and chronologically. Certainly in animal models of osteoarthrosis in which cartilage is enzymatically denatured, bone sclerosis is a secondary event (Bentley 1971). However Radin et al. (1973) found that increased stiffness of subchondral bone preceded cartilage degeneration in mechanically induced osteoarthrosis in rabbits. Contrary to this finding, Serink et al. (1977) in an identical experiment found that Young's modulus of the subchondral bone decreased (i.e. the tissues softened) after the same amount of stress that caused cartilage degeneration.

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Correspondence to: Dr. Mark Walton, Dept. of Orthopaedic Surgery, University of Otago Medical School, P.O. Box 913, Dunedin, New Zealand.