Age-Related Changes in Bone Mass in the Senescence-Accelerated Mouse (SAM)

SAM-R/3 and SAM-P/6 as New Murine Models for Senile Osteoporosis

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Age-related changes of the femoral bone mass in several strains of the senescence-accelerated mouse (SAM) were investigated. Microdensitometrically, all strains exhibited essentially the same patterns of age changes, that is, bone mass corrected by the diameter of the shaft reached the peak value when the mice were 4 or 5 months of age and then fell linearly with age up to over 20 months of age. Two strains, SAM-R/3 and SAM-P/6, which originated from the same ancestry on pedigree, had a significantly lower peak bone mass than other strains (SAM-R/1, SAM-R/2, SAM-P/1, and SAM-P/2). On the other hand, the strains with a low peak bone mass had the same rate of decrease as other strains. Mineral and collagen contents per dry weight of bone showed little difference among the strains. Histologic studies of tibia, femur, and lumbar spine revealed that the osteopenia was not due to osteomalacia but, rather, to osteoporosis. The elderly mice in these two strains were prone to fracture, thus should be important models for study of senile osteoporosis seen clinically. (Am J Pathol 1986, 125:276–283)

LOSS OF BONE MASS is one of the physiologic changes associated with aging.[1] Epidemiologic studies on bone mass revealed the normal pattern of age changes and a close negative correlation with the incidence of fracture.[2,3] Some racial difference in bone mass or the incidence of fracture[4–6] and studies on the bone mass of hetero- and homozygotic twins[7] suggested that both genetic and environmental factors are linked to bone mass and to the susceptibility to osteoporosis.

There are few data on age-related changes of bone mass in experimental animals.[8–14] In many studies of osteoporosis, the osteopenic condition has been induced in relatively young animals by ovariectomy,[15,16] nutritional treatments,[17,18] and so forth. Such experimental models are thought to be inappropriate for the study of senile osteoporosis because the induced decrease in bone mass is rapid, compared with the natural age-associated loss.

Beginning in the 1970s, we developed a murine model of accelerated senescence (senescence accelerated mouse, SAM), which consists of two series.[19,20] In general, aging is accelerated in the SAM-P series, determined by the shortened life span and early manifestation of various signs of senescence, including changes in physical activity, skin, hair, eyes, and spinal curvature, as compared with the SAM-R series used as the control. Diseases closely associated with senescence observed in SAM are senile amyloidosis[21–29] and cataract.[28]

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In the course of these studies, we became aware of the spontaneous leg fracture in a few aged SAM-R/3 mice, which motivated us to examine the age-related changes of bone systematically using several strains of SAM (SAM-R/1, R/2, R/3, SAM-P/1, and P/2). We detected osteoporosis in SAM-R/3, and there was one group with more severe osteoporosis on the pedigree of SAM-R/3, which was newly named SAM-P/6. This is the first report of the development of a murine model of senile osteoporosis.

Materials and Methods

Animals

All mice were maintained under conventional conditions and fed a commercial diet (CE-2, Nihon CLEA, containing calcium, 1.01%, and phosphorus, 0.85%) *ad libitum*. Both killed mice, and those found dead were studied. All mice were checked twice daily, and only those mice without severe autolytic changes were studied. The bilateral femurs were excised and stored in 70% ethanol. SAM-R/1, R/2, R/3, SAM-P/1, P/2, and P/6 were examined in this study.

Microdensitometry

Bone mass was assayed roentgenologically.[29,30] Soft X-ray of the exact lateral view of the femur was taken under the following condition, focus-film distance, 50 cm, 5 mA, 40 kVp, and 1 minute of exposure. Beneath the bone we placed an aluminum plate 2 mm thick in order to shift the density of bone and base to the most sensitive range of the film (Fuji Softex Film FG, Fuji Photo Film, Japan). A standard aluminum wedge was also placed on the border of the film. The film was developed and fixed in the usual manner. The film was then scanned at the midpoint of the femoral shaft at right angles to the shaft by microphotodensitometer (Sakura Densitometer PDS15 Sakura Kohki, Japan). The width and height of the slit were 10 μ and 1 mm. Scanning speed was 0.04 mm/min. The optical density of the bone was converted to the thickness of aluminum by the pattern of the density of the standard aluminum wedge, and an M-shaped bone pattern was obtained (Figure 1). The integration of the pattern (=sigmaGS) corresponds to the absolute mineral mass on the scanned plane. The absolute matrix mass (area of the cortex on the scanned plane) can be given as follows:

$$\text{Cortical area} = (D^2 - d^2) \times \pi / 4$$

where D and d indicate the diameters of the shaft and medullary canal, respectively. Because the size of the bone changes considerably with age, bone mass must be corrected by the size (relative bone mass). As indexes of the relative bone mass we used sigmaGS/D^2 and the cortical thickness index (CTI = (D − d) / D), which indicate mineral mass and matrix mass, respectively. The number of animals used was 194, 166, 195, 197, 156, and 155 in SAM-R/1, R/2, R/3, SAM-P/1, P/2, and P/6, respectively.

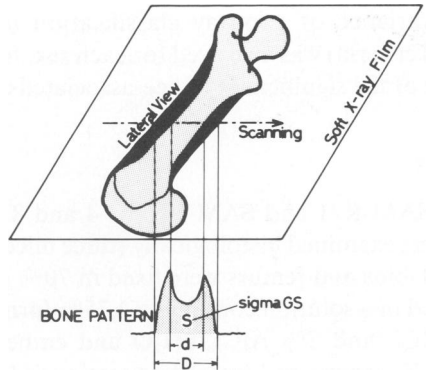

Figure 1—Roentgenologic assay of the bone mass of the shaft of long bone (microdensitometry). The density of the X-ray film is scanned at right angles and is converted to the thickness of aluminum by the standard aluminum wedge, and the M-shaped bone pattern is gained. *D*, diameter of the shaft; *d*, diameter of the medullary canal; *sigmaGS*, integration of the bone pattern and represents mineral mass on the scanned plane. Following indices were calculated: sigmaGS/D^2; cortical thickness index (CTI) = (D − d) / D; cortical area = $(D^2 - d^2) \times \pi / 4$.

Specific Gravity

Specific gravity of the whole dry femur was measured as a more direct index of the relative bone mass corrected size (volume). The dry weight was divided by the external volume of the wet femur determined by Alchimedes' principle. Two hundred twenty-nine mice of various strains and ages were examined.

Chemical Analysis

The femur was dried in a vacuum oven at 100 C overnight and pulverized in liquid nitrogen. About 10 mg of the bone powder was made into ash in an electric furnace at 600 C for 24 hours and was then dissolved in small amount of 1 N HCl. After dilution, calcium and phosphorus were determined by atomnic absorption and Fiske–Subbarow's method,[32] respectively. Collagen content was determined as hydroxyproline, using the remaining bone powder, about 10 or 15 mg, by Woessner's method.[33] Five mice of each age group (4–5, 6–10, 11–15, 16–20 months) of both sexes and all strains were used. The significance of the interstrain difference in each age group was tested by analysis of variance of one-way classification (strains as the criteria). Anal-

ysis of variance of two-way classification (age and strains as criteria) was also used for each sex, for determination of the significance of age-associated changes.

Histology

Male SAM-R/1 and SAM-P/6 of 4 and 8 months of age were examined histologically (three mice of each group). Tibias and femurs were fixed in 70% ethanol, decalcified in a solution containing 4.75% formic acid, 3.15% HCl, and 7% $AlCl_3 \cdot 6H_2O$ and embedded in paraffin. Coronary and transverse sections (4 μ) were made for the epiphyseal parts and the shaft, respectively. Hematoxylin and eosin and elastic van Gieson stains were used for decalcified sections. The lumbar spine was fixed in 70% ethanol for 3 days, followed by block staining in Villanueva's bone stain solution[34] for 3 days. The tissue was then dehydrated and embedded in methylmetacrylate. The block was sliced into a thickness of approximately 100 μ, ground to 20 μ, and examined under a photomicroscope.

Results

Microdensitometric Study

Both of the microdensitometry indexes, sigmaGS/D² and CTI, showed a good correlation to the specific gravity of the whole dry femur (correlation coefficients were 0.771 and 0.840 to sigmaGS/D² and CTI, respectively, in 229 mice of various strains and ages). Thus, microdensitometry was thought to be a convenient and accurate method for the assay of the bone mass of these small rodents.

Figure 2 indicates the age-related changes of sigmaGS/D², which is an index of relative mineral mass. There was a common pattern of age changes. The peak value was seen in SAM at 4 or 5 months of age and fell linearly just after the peak. The approximate rate of decrease was 15% of peak value per 1 year. There are two distinctive groups: one includes SAM-R/1, SAM-R/2, SAM-P/1, and SAM-P/2 and is called the normal bone group; the other includes SAM-R/3 and SAM-P/6 and is called the low bone group. The latter had a significantly lower peak bone mass, but the rate of decrease was the same as the former. The only exceptions were the females of SAM-R/3, which had a normal bone mass at a younger age, then rapidly lost bone. Another index of the bone mass, CTI, an index of relative matrix mass, correlated closely to sigmaGS/D² (correlation coefficient, 0.810) and showed the same pattern of age changes as sigmaGS/D² (Figure 3). The normal and low bone groups were also distinctive with this index, but within the normal bone group, the SAM-P series tended to have a larger bone mass than did the SAM-R series. Comparing the groups which were 4 or 5 months of age (peak period), the difference was about 24% of the normal bone group. The rate of decrease was 14% of the peak value per year in both groups.

Figure 4 indicates the diameters of the shaft (D) and medullary canal (d). D increased rapidly up to 10 months of age and then continued to expand at a decelerated rate until the mice were over 20 months of age. There were no significant differences among the strains. On the other hand, d increased constantly throughout the life span and exceeded the rate of D. Thus, the thickness of the cortex (D − d) decreased after 6 months

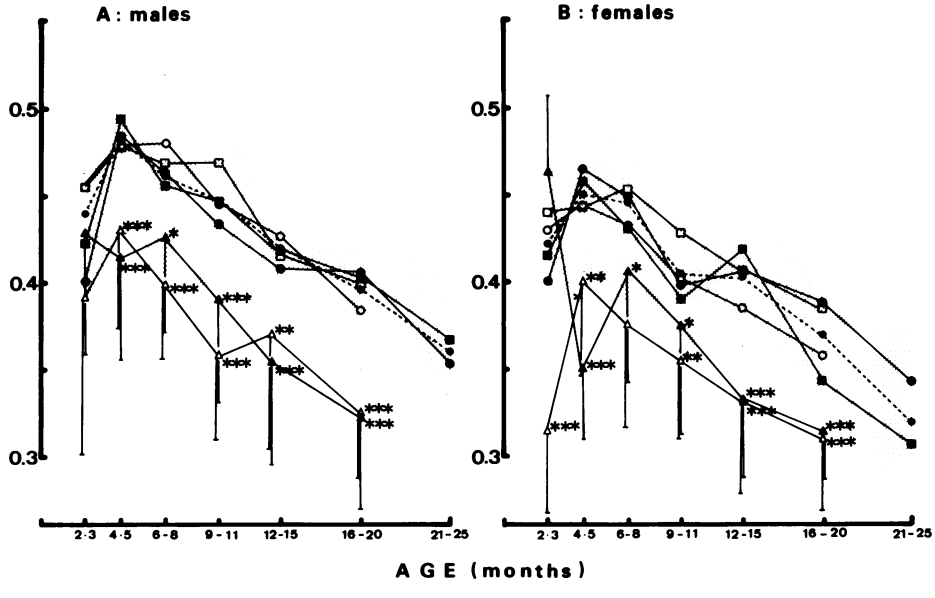

Figure 2—Age-related changes of sigmaGS/D². ●——●, ■——■, ▲——▲, ○——○, □——□, and △——△ indicate SAM-R/1, R/2, R/3, SAM-P/1, P/2, and P/6, respectively. ● – – – ● indicates the summation of the normal bone group (SAM-R/1, R/2, SAM-P/1, and P/2) and the mesh area represents the mean ± 1 SD of the normal bone group. Vertical bars indicate 1 SD of SAM-R/3 and SAM-P/6 (low bone group). Note the parallel downward shift of the normal bone group. Statistical significance was tested against the normal bone group by the Student *t* test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

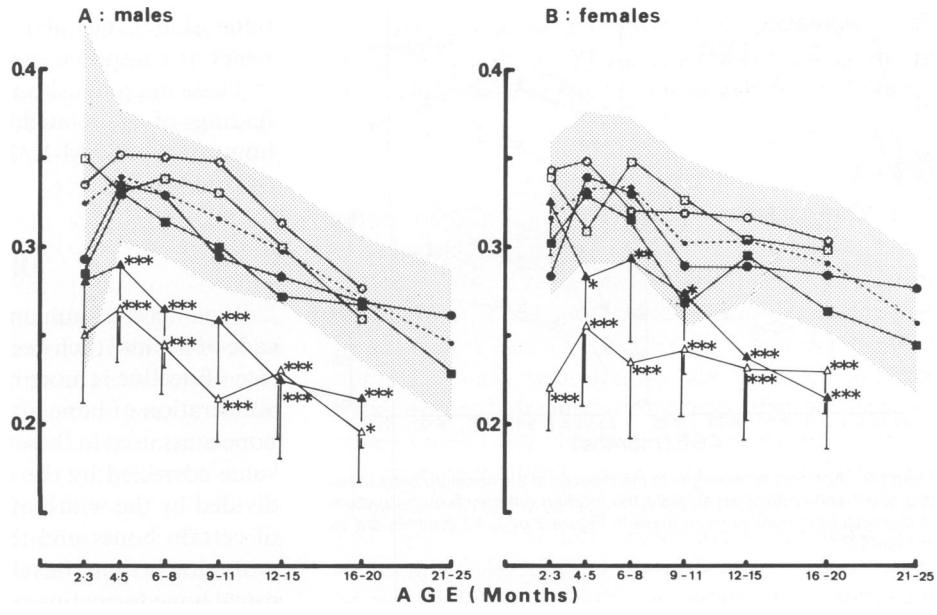

Figure 3—Age-related changes of CTI. The pattern of age change is much the same as sigmaGS/D². All symbols are as in Figure 2.

of age. The changes of d of the low bone group appear as a parallel upward shift of those of the normal bone group.

Longitudinal growth in the epiphyseal plate ceased at about 10 months of age, as shown in Figure 5. Thus, after 10 months of age, the femur had a stumpy proportion as the result of a continuous expansion of D and the ratio L/D decreased, where L represents the length of the femur.

The absolute bone mass (sigmaGS as mineral mass index and cortical area as matrix mass index) did not decrease linearly (Figure 6), unlike the diameter-corrected relative bone mass (Figures 2 and 3). In some strains, the increase continued up to 20 months of ·e.

Chemical Study

Table 1 shows the calcium contents per dry weight of bone. Although a one-way analysis of variance revealed interstrain differences in the old-age groups in both sexes, the absolute differences were small. A two-way analysis of variance revealed no significant difference with regard to age.

Tables 2 and 3 show the phosphorus and hydroxyproline contents per dry weight of bone, respectively. A one-way analysis of variance also revealed significant interstrain differences in some age groups, but the absolute difference was small. A two-way analysis of variance revealed significant differences with regard to age in both

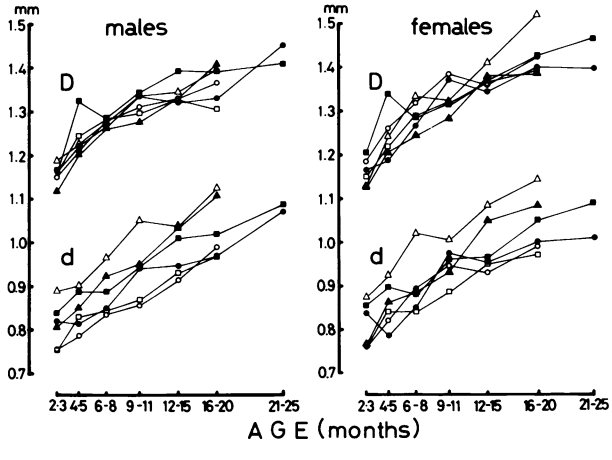

Figure 4—Age-related changes of the diameter of the shaft (*D*) and the medullary canal (*d*). All symbols are as in Figure 2.

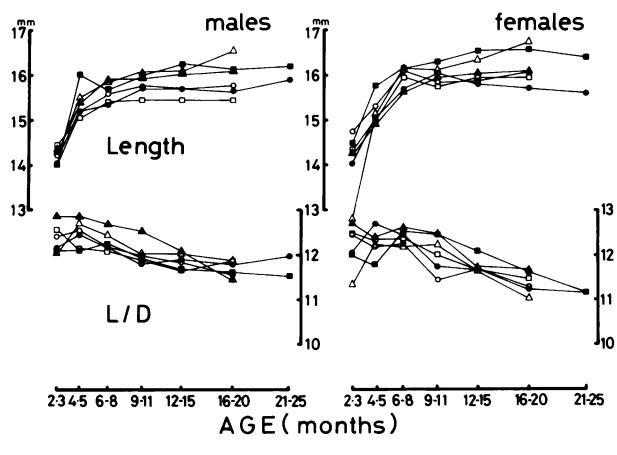

Figure 5—Age-related changes of the length (*L*) and the proportion (*L/D*) of the femur. All symbols are as in Figure 2.

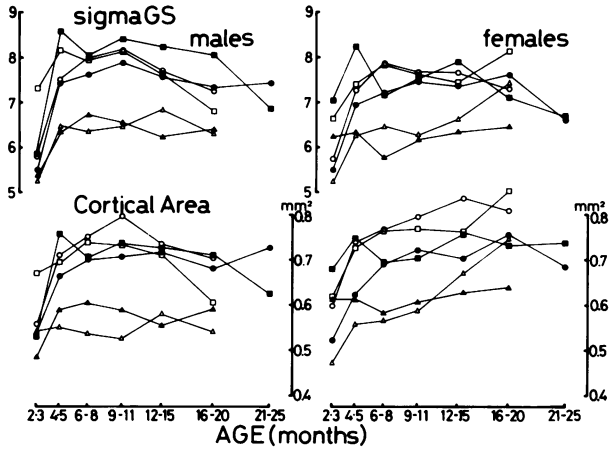

Figure 6—Age-related changes of the indexes of the absolute bone mass (sigmaGS and cortical area). Note the marked difference of the pattern of changes from relative bone mass in Figure 2 or 3. All symbols are as in Figure 2.

phosphorus and hydroxyproline, that is, a decrease with advancing age.

Histologic Study

Decalcified histologically prepared specimens revealed that in SAM-R/1, thinning of the cortex and trabeculas of tibias and femurs occurred in 8-month-old mice, compared with 4-month-old mice, and 4-month-old male SAM-P/6 already showed severe depletion of trabeculas and thinning of the cortex (not shown). Undecalcified specimens (Figure 7) revealed that the lumbar vertebral body of SAM-P/6 had also thin and sparse trabeculas and cortex, compared with SAM-R/1 of the same age and sex. An increase of osteoid tissue did not accompany the low bone mass in SAM-P/6. Thus, osteomalacia could be ruled out. It was also suggested that the low bone mass was not restricted to the femoral shaft (composed of compact bone alone), but also existed in the vertebral body, which is composed mainly of spongiosa.

These microdensitometric, chemical, and histologic findings of age-related bone loss of all strains and low bone mass of SAM-R/3 and SAM-P/6 were indicative of osteoporosis.

Discussion

Bone mass of humans increases up to the third decade of life and then declines gradually.[3] In women, the rate of decline is more rapid, and there is a temporary acceleration of bone loss at menopause. All indexes of bone mass used in these human studies are of a relative value corrected by the size of the bone, for example, divided by the width of the bone. The absolute mass of certain bones and their strength may well decline more slowly than the relative bone mass, because periosteal bone formation occurs throughout life and bone width continues to expand.[35] Periosteal bone formation after the termination of the longitudinal growth is more prominent in small animals.[12] In our data on the femurs of SAM, the longitudinal growth decelerated at about 5 months of age and ceased up to 10 months of age, while the diameter continued to expand. The initiation of bone loss varies considerably, depending on which index is used for the bone mass. In studies on the effect of spleen cell transplantation on bone loss, Tylan reported age-related changes of femoral bone mass in long-lived F1 mice, as a control.[36] He used the total ash weight as the index of bone mass, which represents absolute bone mass. There was no significant decrease before 20 months of age, then a slow decrease up to over 40 months of age. In the case of SAM, few mice can survive until they begin to lose absolute bone mass (after 20 months of age), because the mean life span is 12.4, 16.1, 12.8, 10.3, 8.8, and 7.9 months in SAM-R/1, R/2, R/3, SAM-P/1, P/2, and P/6, respec-

Table 1—Calcium Content of the Femur*

Age (months)	SAM-R/1	SAM-R/2	SAM-R/3	SAM-P/1	SAM-P/2	SAM-P/6
Male						
4–5	234.2 ± 7.2	224.3 ± 5.5	232.5 ± 4.0	228.8 ± 2.6	229.5 ± 4.7	225.3 ± 4.1
6–10	238.5 ± 4.9	229.8 ± 1.7	227.9 ± 6.5	233.7 ± 4.4	230.5 ± 10.5	225.3 ± 7.1
11–15	227.8 ± 5.2	226.6 ± 5.0	229.9 ± 8.5	234.9 ± 5.0	233.4 ± 5.3	233.8 ± 4.6
16–20†	225.9 ± 5.6	222.8 ± 4.8	225.1 ± 7.3	234.4 ± 5.2	241.0 ± 2.4	223.5 ± 7.7
Female						
4–5	226.8 ± 9.9	225.1 ± 5.0	231.0 ± 9.8	235.7 ± 3.1	231.3 ± 8.2	225.6 ± 5.5
6–10	233.3 ± 7.1	229.6 ± 2.5	233.9 ± 3.3	234.9 ± 6.1	230.7 ± 4.2	225.6 ± 3.9
11–15‡	225.4 ± 5.3	235.5 ± 9.7	233.3 ± 3.1	239.4 ± 4.4	232.8 ± 0.9	225.7 ± 3.3
16–20‡	226.2 ± 4.6	229.1 ± 6.1	223.9 ± 5.8	233.7 ± 5.4	237.8 ± 3.7	228.1 ± 3.8

* In milligrams per gram dry weight. Calcium was determined by atomic absorption. Each value is the mean ± standard deviation (SD) of 5 animals. Statistical significance was tested by analysis of variance of one-way classification (strains as the criteria).

† $P < 0.001$.

‡ $P < 0.01$.

Table 2—Phosphorus Content of the Femur*

Age (months)	SAM-R/1	SAM-R/2	SAM-R/3	SAM-P/1	SAM-P/2	SAM-P/6
Male						
4–5*	123.5 ± 4.3	120.2 ± 2.3	120.2 ± 2.6	125.7 ± 2.7	125.2 ± 3.1	121.6 ± 2.6
6–10	124.3 ± 1.0	119.5 ± 3.1	120.2 ± 5.1	123.4 ± 5.1	124.0 ± 5.1	118.3 ± 2.9
11–15	116.7 ± 0.9	120.2 ± 3.1	116.8 ± 4.2	121.4 ± 2.6	122.1 ± 1.7	121.5 ± 2.7
16–20	117.1 ± 1.2	115.5 ± 3.7	116.0 ± 1.7	117.2 ± 1.8	121.3 ± 2.3	118.3 ± 5.0
Female						
4–5	121.2 ± 6.1	126.0 ± 2.1	123.7 ± 7.2	124.5 ± 1.2	122.2 ± 3.9	123.4 ± 2.3
6–10	123.4 ± 2.2	123.4 ± 2.5	123.7 ± 3.8	124.6 ± 2.9	121.1 ± 3.3	120.2 ± 4.1
11–15	121.5 ± 3.1	124.4 ± 3.9	121.9 ± 4.1	121.4 ± 2.0	120.4 ± 2.9	119.3 ± 3.3
16–20†	121.9 ± 1.7	116.9 ± 1.4	117.4 ± 2.0	122.8 ± 3.0	117.3 ± 4.0	119.4 ± 4.2

* In milligrams per gram dry weight. Phosphorus was determined by the Fiske–Subbarow method.[32] Each value is the mean ± SD of 5 animals. By one-way analysis of variance (strains as the criteria) weak significance was noted in a few age groups. Strong significance ($P < 0.0001$) of age, was noted, by the two-way analysis of variance, in both sexes.

† $P < 0.05$.

tively. During the range of age under 20 months, the microdensitometry index of absolute bone mass (Figure 6) remained unchanged. Judged from the basic definition of osteoporosis, that is, the depletion of bone in unit volume of bone tissue, it is reasonable to choose the relative bone mass as the index of osteoporosis, though it cannot be directly related to the mechanical strength of bone.[37] In SAM, the relative bone mass (Figures 2 and 3) showed a clear peak at age 4–5 months and then decreased linearly. The tendency toward a smaller bone mass or a more rapid bone loss in females was not demonstrated in SAM, unlike events seen in humans.

Although signs of senility, such as loss of activity, changes of skin, hair, and eyes, and rigidity of spinal curvature, are more rapid in the SAM-P series than in the SAM-R series, the rate of bone loss does not differ significantly. Various local or systemic conditions may influence bone mass, but femoral bone mass in the wasted SAM-P mice bearing skin ulcers, contracted kidneys, or other disease was never less than that of healthy age-matched SAM-R mice. Rate of bone loss of the long bone is determined by the difference between the rates of periosteal bone formation and endosteal bone resorption, and the uniformity of these rates in various strains of SAM suggests that they might be genetically controlled.

Newton-John and Morgan proposed a simple hypothesis based upon massive data on human age-related changes of bone mass.[2] They assumed that the rate of bone loss is common and constant in any individual in a population. One who has low bone mass at peak age is expected to fall below the fracture threshold and to have symptoms of osteoporosis earlier. Nordin,[38] Riggs,[39] and Parfitt[40] classified bone loss into two types with different hormonal and cellular mechanisms and clinical features. One is a slow and constant loss with age (simple or senile osteoporosis, type B fracture, by Riggs) and is thought to be equivalent to Newton-John's hypothesis. The other is a rapid and temporary loss by various causes, for example, estrogen depletion (postmenopausal osteoporosis, type A fracture, by Riggs). Thus, the early onset of osteoporosis might be due to a low peak bone mass and/or a history of rapid bone

Table 3—Hydroxyproline Content of the Femur*

Age (months)	SAM-R/1	SAM-R/2	SAM-R/3	SAM-P/1	SAM-P/2	SAM-P/6
Male						
4–5	15.91 ± 0.58	15.37 ± 0.96	15.66 ± 0.81	15.33 ± 0.87	16.50 ± 0.71	16.56 ± 0.48
6–10	16.70 ± 0.86	16.77 ± 1.14	15.26 ± 1.04	15.58 ± 0.84	15.95 ± 0.36	16.18 ± 0.41
11–15	16.03 ± 0.75	15.53 ± 0.55	14.78 ± 1.67	15.22 ± 0.46	15.38 ± 0.40	15.28 ± 1.17
16–20	14.72 ± 0.56	14.18 ± 0.51	14.59 ± 0.73	14.04 ± 0.97	14.88 ± 0.50	15.28 ± 1.17
Female						
4–5†	16.37 ± 0.56	16.92 ± 0.22	16.22 ± 0.44	17.55 ± 0.95	16.54 ± 0.34	16.15 ± 0.62
6–10	16.05 ± 0.90	15.95 ± 0.50	15.65 ± 0.49	16.25 ± 0.77	16.42 ± 0.35	15.62 ± 0.32
11–15	15.51 ± 0.44	15.31 ± 0.65	14.79 ± 0.49	16.04 ± 0.84	15.51 ± 0.20	15.21 ± 0.73
16–20	15.17 ± 0.93	16.07 ± 0.55	14.36 ± 0.98	14.78 ± 0.71	15.29 ± 0.75	15.00 ± 0.56

* In milligrams per gram dry weight. Hydroxyproline was determined by Woessner's method.[33] Each value is the mean ± SD deviation of 5 animals. By one-way analysis of variance (strains as the criteria) weak significance was noted in one age-group. Strong significance ($P < 0.0001$) of age, by the two-way analysis of variance, was noted in both sexes.

† $P < 0.05$.

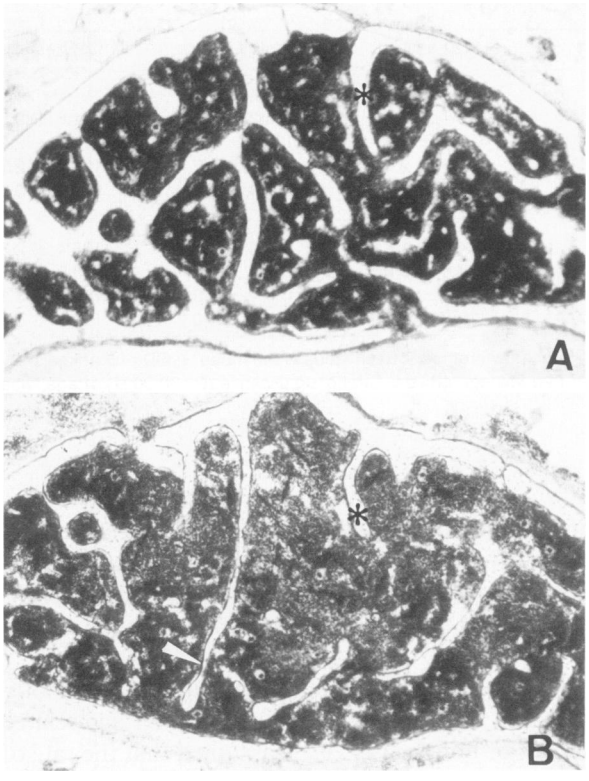

Figure 7—Undecalcified transverse section of the lumbar vertebral body. **A**—SAM-R/1 of 4 months, male. **B**—SAM-P/6 of 4 months, male. Note the thin cortex and trabeculas (*) of SAM-P/6. This did not accompany the increase of osteoid tissue (slightly dark lining on trabeculas, *white arrows*). (Villanueva's bone stain,[34] × 40)

loss. Although the exact pattern of age changes of bone mass cannot be determined in a cross-sectional study, it appears that a significant rapid bone loss does not occur in SAM because the variance of bone mass is constant in most age groups, of any strain studied. On the other hand, considerable interstrain differences were noted in case of peak bone mass; that is, SAM-R/3 and SAM-P/6 had a lower peak mass than the others, without an acceleration of bone loss. These low bone strains are expected to be susceptible to fracture earlier; indeed, 3 mice in these strains and over 20 months of age had spontaneous tibial fracture (Figure 8). The low incidence of fracture was probably because SAM-R/3 and SAM-P/6 have such a short life span that few can survive until fracture occurs. Spontaneous fracture in only the low bone group might be supporting evidence for Newton-John's hypothesis and type B fracture of Riggs' concept.

There are reports of spontaneous fracture in animals but such occurs in utero or early in life and is thought to be a model for osteogenesis imperfecta (OI).[41,42] Shapiro and Rowe emphasized the importance of growth period in the pathogenesis of osteoporosis in the elderly.[43] They used the term "mild OI" and stated that race, sex, hormonal factors, or other factors such as collagen abnormality may have an influence. In the case of SAM-R/3 and SAM-P/6, in which temporary acceleration of the expansion of the medullary canal resulted in a low peak bone mass, systemic factor(s) may cause this phenomenon.

SAM-R/3 and SAM-P/6 have the same origin on pedigree and 18 generations of the latter have been produced since the separtion. The low bone mass determined by microdensitometry of femurs of these strains was confirmed to be osteoporosis, both chemically and histologically. The observation that only the mice of this origin specifically showed a low bone mass strongly suggests the involvement of genetic factors. This low bone group (SAM-R/3 and SAM-P/6) is a unique strain which will serve for research on the pathogenesis, genetic background, and possibly prevention of senile osteoporosis.

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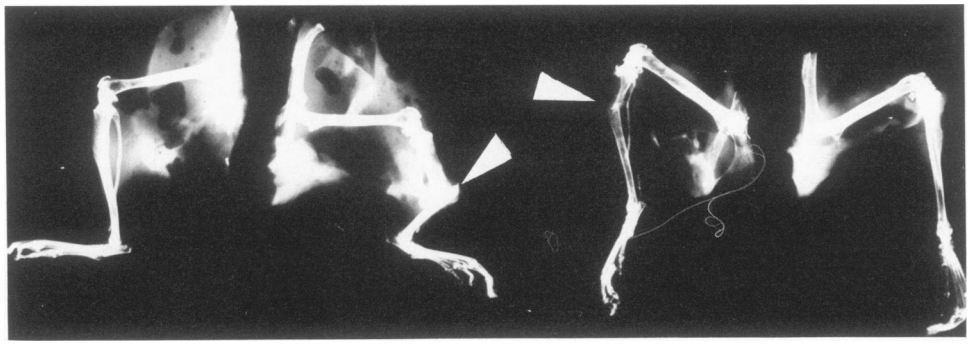

Figure 8—Soft X-ray of spontaneous fractures of tibia in aged mice of the low bone group. **Left**—Twenty-three-month-old male SAM-P/6 mouse. **Right**—Twenty-seven month old female SAM-P/6 mouse. In both cases, fractures occurred in the upper third of the leg (*arrows*). Spontaneous fracture occurred exclusively in old SAM-R/3 and SAM-P/6 mice.

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