DEFECTS IN THE CARTILAGINOUS GROWTH PLATES OF BRACHYMORPHIC MICE

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ABSTRACT

Homozygous brachymorphic (*bm/bm*) mice are characterized by disproportionately short stature. Newborn *bm/bm* epiphyseal cartilages are shorter than normal although the cells in the different zones of growth are relatively well organized. The extracellular matrix reacts poorly with stains specific for sulfated glycosaminoglycans. The ultrastructural appearance of the cartilage matrix indicates normal collagen fibrils; however, proteoglycan aggregate granules are smaller than normal and are present in reduced numbers, particularly in the columnar and hypertrophic zones of the growth plate. In addition, a prominent network of fine filaments, which are extractable in 4 M guanidine hydrochloride, are present in the *bm/bm* cartilage matrix. These findings suggest that a defect affecting the proteoglycan component of cartilage occurs in *bm/bm* mice.

Numerous inherited disorders affect cartilage structure and function in man and other animals. Recently, we have studied cartilage from brachymorphic (*bm/bm*) mice. This mutant was first described by Lane and Dickie (12), and established by genetic studies as an autosomal recessive condition. Homozygous *bm/bm* mice are vigorous but have shortened long bones, shortened tails, and dome-shaped skulls. Such findings are consistent with a defect in cartilage development, and Lane and Dickie found the knee joints of 17–24 day old *bm/bm* mice to be shorter and thicker than normal, although chondrocytes in these epiphyseal growth plates retained good alignment (12).

Cartilage is composed predominantly of one cell type, chondrocytes, surrounded by large quantities of extracellular matrix. Of the constituents of the extracellular matrix, two macromolecules, cartilage-type collagen (19, 36) and cartilage-specific proteoglycans (13, 22), are synthesized by the differentiated chondrocytes. Ultrastructurally, this extracellular matrix consists of thin, 200–300 Å wide cartilage-type collagen fibrils and polygonal matrix granules of precipitated proteoglycan aggregates (1, 6, 18, 25, 33–35).

In examining the cartilaginous epiphyses from *bm/bm* mice, we have observed alterations in the morphological appearance of the epiphyseal growth plates at different stages of development, as well as differences in the extracellular matrix material which may be related to the abnormalities in tissue development found in this mutant.

MATERIALS AND METHODS

Isolation of Tissue

Homozygous brachymorphic (*bm/bm*) mice were identified by inspection and genetic analysis and bred. Litters of *bm/bm* mice as well as age-matched *C57Bl/6J* (control) mice were sacrificed on days 5, 9, and 16 after birth. Hind limbs were excised and the cartilaginous knee joint region was dissected free of adjacent tissue. The epiphyseal cartilages of the knee joint, the distal femoral head, and the proximal tibial head were examined. These cartilages were chosen because the femur and the tibia show the greatest effect from the *bm* gene (12).

Light Microscopy

For light microscopy, whole knee joints from the hind limbs of *bm/bm* and normal animals including attached femoral and tibial segments were cleaned of superficial skin and soft tissue, fixed by exposure for several days in 10% neutral phosphate-buffered formalin at 4°C, then decalcified in 5% HCl-50% ethanol for several hours. Samples were washed, dehydrated, and infiltrated with paraffin in a Technicon automatic tissue processing machine (Technicon Corporation, Ardsley, N. Y.). 6-μm paraffin tissue sections were cut and stained with a variety of stains including hematoxylin and eosin (4), safranin-0 (27), Alcian blue at pH 2.5, and pH 1 (17), and periodic acid-Schiff (PAS) reagent with and without prior diastase digestion (17).

Measurements of the growth plates of proximal tibial epiphyseal cartilages were made on hematoxylin- and eosin-stained sections with a Leitz Ortholux microscope, using a calibrated eyepiece micrometer. The statistical significance of these measurements was determined by the Cochran approximation of the Behrens-Fisher solution (31). This approximation of the Student *t*-test was used because in most cases the observed statistical variances between mutant and normal samples differed and the *t*-test assumes equality of variances. The *P* values for the growth plate measurements are indicated in the text. The sizes of individual lacunae were defined by measuring the largest dimensions of each lacunar space. Because chondrocytes and their lacunae in the proliferative-columnar zone are angular or wedge shaped (24), some are sectioned through the center and therefore appear larger than those sectioned on end. The latter appear only as small fragments. The dimensions of such fragments were not measured; however, since lacunae are presumed to be cell sites, these fragments were noted for cell number determinations.

Electron Microscopy

Distal femoral and proximal tibial cartilages from *bm/bm* and normal *C57Bl/6J* mice, 5 days old, were studied by electron microscopy. Cartilage plus adjacent segments of bone were dissected from the joint, divided along a sagittal plane, and then sliced into 0.5 to 1-mm thick longitudinal pieces. Tissues were routinely fixed in a solution of 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2–7.4, at 4°C for periods of 3 h to 3 days; postfixed in 2% OsO_4 in 0.1 M cacodylate buffer, pH 7.2–7.4, for 2 h; dehydrated through graded ethanols; and flat-embedded in low viscosity plastic resin (32). Some specimens were postfixed in OsO_4 containing 1% ruthenium red (see reference 15).

Thick sections through the whole growth plate, stained with toluidine blue 0, were examined before retrimming blocks of embedded tissue to include only specific regions of the epiphyseal growth plates, e.g., reserve, proliferative-columnar, or hypertrophic zones. Adjacent thin (500–600 Å) sections were stained for 15 min in saturated, filtered, aqueous uranyl acetate followed by staining for 2 min in 0.2% lead citrate (37) before examination with a JEOL JEM 100B electron microscope.

For quantitation of matrix granules, pictures of extracellular matrix of the reserve, proliferative-columnar, and hypertrophic zones of four animals of each group were taken at magnifications of 30,000 and 72,000. The pictures were photographically enlarged three times. Routinely, measurements were made on 2-inch square areas of the enlarged micrographs. Estimates of the number and size of matrix proteoglycan granules were made by counting and measuring granules in micrographs of random regions of known and constant size (34). Only polygonal leaflets were scored since lightly stained, round, or oval figures may be cross or tangential sections through collagen fibrils or proteoglycan precipitated in another configuration.

For ultrastructural studies of cartilage from which proteoglycans had been extracted, 0.5-mm slices of tissue were treated before fixation with 4 M guanidine hydrochloride containing 0.1 M 6-aminohexanoic acid, 0.005 M benzamidine, and 0.05 M Tris, pH 7.3, for 3 days at 4°C in order to extract the proteoglycans (1, 35). Since collagen is somewhat denatured by this procedure, samples were then put through a series of decreasing concentrations of the guanidine hydrochloride-salt solution (4 M, 2.5 M, 1.5 M of guanidine hydrochloride) over an 8-h period in order to permit renaturation of the collagen fibrils (1, 8). The tissue was then embedded, sectioned, stained, and examined as described previously.

RESULTS

Appearance of the Epiphyseal Cartilages of Brachymorphic Mice

Epiphyseal cartilage consists of three readily distinguishable zones of growth (3). The reserve zone in 5-day postnatal mice is adjacent to the

synovial space, and consists of cells residing in regular lacunar spaces surrounded by large quantities of extracellular matrix. The intermediate region, designated here as the proliferative-columnar zone, contains chondrocytes first aggregated and then regularly arranged in distinct columns. The third region, or hypertrophic zone, is adjacent to the bone shaft (metaphysis) and consists of cells that are markedly larger than those in the other zones. Provisional calcification of the cartilage and endochondral ossification begin in the hypertrophic zone.

In the 5-day normal, *C57Bl/6J* mouse, the growth plate is well developed with three zones of growth well demarcated from one another (Fig. 1 *a*). At this time, the epiphyseal plate is still completely cartilaginous and stains homogeneously with hematoxylin and eosin. The hypertrophic zone is 10–12 chondrocytes thick and is invaded on its metaphyseal side by vascular buds. Primary trabeculae of the metaphysis are regularly shaped, and a thin layer of periosteal bone (not shown) surrounds the growth plate, starting at the mid-portion of the columnar zone and extending over the hypertrophic zone towards the metaphysis.

By 9 days, the height of the columnar zone has diminished somewhat in the normal growth plate (Table I) and the zones of growth have become less well defined. Large numbers of hypertrophic cells are present in the developing secondary ossification center. By day 16, a bony secondary ossification center has formed in the epiphysis and a clear resting zone of chondrocytes is difficult to discern (Fig. 2*a*). In normal control animals on day 16, the columnar zone continues to diminish in height and the chondrocytes in this region have become somewhat rounder before

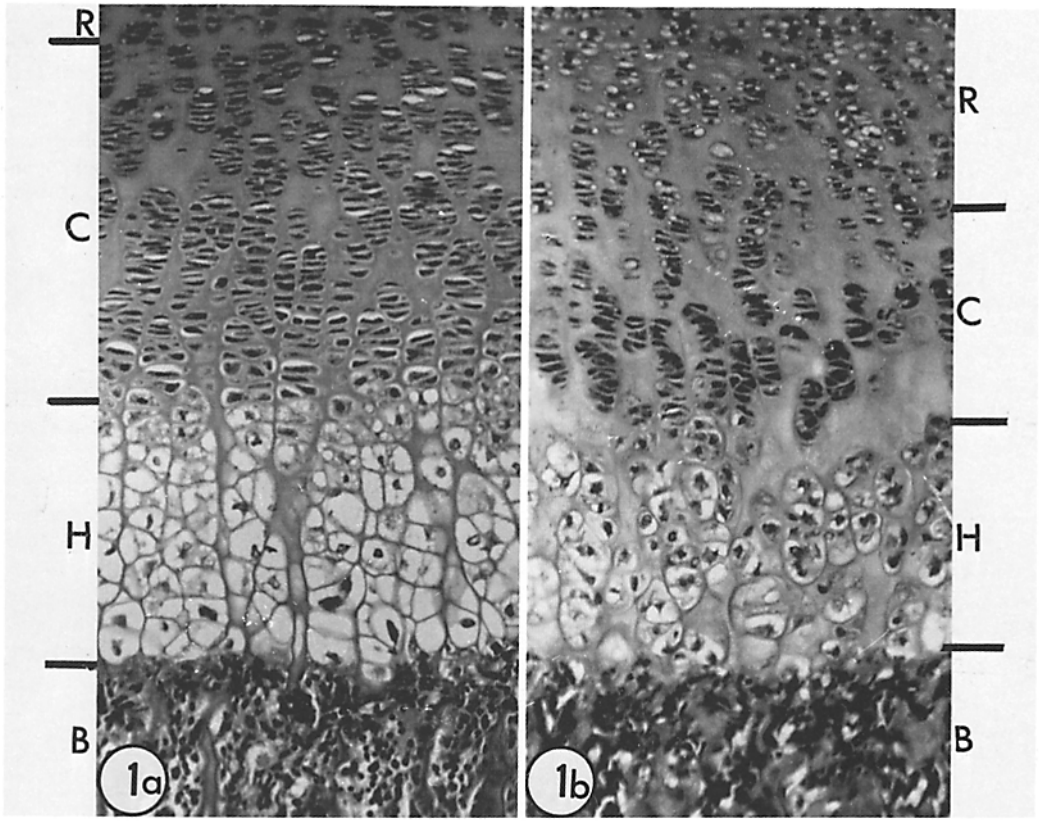

FIGURE 1 Epiphyseal growth plates from 5-day-old normal *C57Bl/6J* (*a*) and mutant *bm/bm* (*b*) tibias. Each zone of growth is clearly demarcated. Cells in the *bm/bm* epiphyses are relatively well aligned, as in the normal growth plate. However, each mutant growth zone is reduced in size. *R*: reserve zone; *C*: proliferative-columnar zone; *H*: hypertrophic zone; and *B*: bone (tibial metaphysis). × 125.

TABLE I

Dimensions of Brachymorphic (bm/bm) and Normal (C57Bl/6J) Epiphyseal Growth Plates

	Day 5		Day 9		Day 16	
	C57Bl/6J	*bm/bm*	*C57Bl/6J*	*bm/bm*	*C57Bl/6J*	*bm/bm*
Total growth plate	960 ± 15.8	778 ± 6.9	893 ± 15.4	736 ± 8.3	318* – 486‡	214 ± 4.0
Columnar zone (ht)	349 ± 5.6	237 ± 5.1	245 ± 3.6	220 ± 12.7	176 ± 4.6	100 ± 2.3
Hypertrophic zone (ht)	201 ± 8.3	143 ± 4.2	180 ± 0.0	147 ± 3.0	189 ± 12.9	57 ± 2.0
Columnar lacuna (ht)	4.8 ± 0.0	4.8 ± 0.0	4.2 ± 0.0	3.8 ± 0.0	8.6 ± 0.1	4.8 ± 0.0
Columnar lacuna (width)	20.8 ± 0.8	15.2 ± 0.3	24.5 ± 0.9	18.9 ± 0.5	21.8 ± 0.8	17.7 ± 0.7
Hypertrophic lacuna (ht)	26.1 ± 0.8	22.6 ± 1.4	22.1 ± 0.8	19.3 ± 0.6	18.3 ± 0.9	20.7 ± 0.7
Hypertrophic lacuna (width)	25.7 ± 0.7	28.5 ± 1.7	29.4 ± 0.9	22.0 ± 0.8	28.3 ± 1.8	15.5 ± 0.7
Average no. columnar cells/column	32 ± 2.1	21 ± 0.6	21 ± 1.3	15 ± 0.9	12 ± 0.5	9 ± 0.2
Average no. hypertrophic cells/column	9 ± 0.5	7 ± 0.3	14 ± 1.1	8 ± 0.4	10 ± 0.6	4 ± 0.6

All measurements are expressed as micrometers from proximal tibial regions and represent the average of 10–20 measurements, ±SEM.

* Height through center of growth plate.

‡ Height through sides of growth plate.

entering the hypertrophic zone proper. At this stage, the hypertrophic zone remains approximately the same size as on days 5 and 9 (Table I).

In comparison to normal age-matched controls, epiphyseal cartilages from *bm/bm* mice display a reduction in size in each zone of growth (Table I). In 5-day-old *bm/bm* mice (Fig. 1*b*), the cartilaginous epiphyseal plate, from synovial space to metaphysis, is 81% ($P < 0.001$) the size of that found in normal animals, and remains at approximately this level through day 9. By day 16, however, the epiphyseal plate from mutant animals is only 44–67% that of normal (Table I), depending on whether measurements through the center or sides of the growth plates are compared. Whereas the normal growth plate is curved, that of the mutant is abnormally straight. This difference in architecture accounts for the range in measured reduction of the mutant growth plate on day 16. Both the columnar and hypertrophic zones are markedly reduced in size by day 16 (columnar zone: 68% of normal on day 5, $P < 0.001$; 57% of normal on day 16, $P < 0.001$; hypertrophic zone: 71% of normal on day 5, $P < 0.001$; 30% of normal on day 16, $P < 0.001$). In particular, the hypertrophic region is often only three to four cells in height in 16-day-old mutant animals (Fig. 2*b*).

Like that in normal growth plates, each zone of growth in the epiphyses from 5-day-old mutant animals is well demarcated, but with time the zones become less distinct. Cells in both the columnar and hypertrophic regions of the mutant cartilages are relatively well aligned, though not so rigidly ordered as in the normals (Figs. 1 and 2). In general, the extracellular matrix in *bm/bm* cartilages stains less intensely than normal with hematoxylin and eosin and, unlike that in normal cartilage, exhibits a somewhat nonhomogenous appearance suggesting localized variations in the distribution of the matrix components of *bm/bm* cartilage. Capillary ingrowth from the metaphysis appears normal throughout the developmental period studied; however, the cartilage-bone junction is straighter than normal. The collar of periosteal derived bone, a noncartilaginous bone which surrounds the growth plate (3), appears normal in brachymorphic animals (not shown). Femoral epiphyses from *bm/bm* mice show reductions in size, similar to those of the mutant tibial epiphyses.

Epiphyseal cartilages from 5-, 9-, and 16-day *bm/bm* and normal *C57Bl/6J* mice were also treated with a number of histochemical stains specific for sulfated proteoglycans including safranin-O (27) and Alcian blue (17). Extracellular matrix material in the mutant cartilages consistently stains less intensely than does tissue from normal animals. Moreover, unlike normal matrix material which stains uniformly throughout the growth plate, mutant cartilages exhibit, in addition to the nonhomogeneity between cells, a gra-

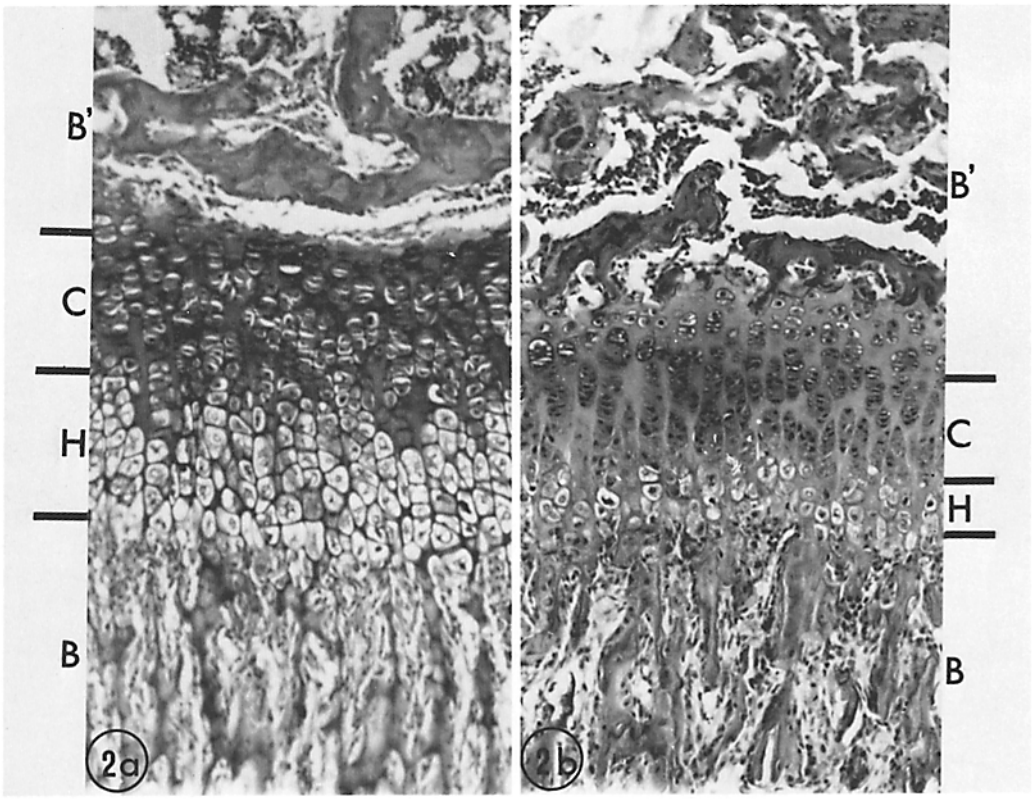

FIGURE 2 Day-16 epiphyseal growth plates from normal *C57Bl/6J* (*a*) and mutant *bm/bm* (*b*) tibias. The mutant growth plate is markedly shorter than normal. By this stage, the reserve zone can no longer be delineated, but the proliferative-columnar (*C*) and hypertrophic (*H*) zones are easily distinguished. Although each zone of growth is reduced in the mutant, the hypertrophic zone is most affected, being only 30% of normal. The bony tibial shaft (*B*) appears normal in the mutant as does the well developed secondary ossification site (*B'*). × 63.

dation of staining intensity, being most intensely stained in the reserve zone and least intensely stained in the hypertrophic region. These histochemical findings suggest that the proteoglycans present in the brachymorphic cartilage contain reduced levels of carboxyl and/or sulfate groups. The PAS stain which reacts with the viscinal hydroxyl groups (14) present primarily in the carbohydrate moieties linked to collagen and in glycoprotein components of the matrix, rather than with the carboxyl and sulfate groups present in proteoglycans, showed no differences in matrix staining between normal and mutant cartilage.

Ultrastructure of the Cartilage Matrix of Brachymorphic Mice

No striking differences in the appearance of chondrocytes were noted between 5-day-old normal and mutant animals; however, the mutant exhibits alterations in the morphology of the extracellular matrix. In normal cartilage, polygonal matrix granules of proteoglycan aggregates and thin (200 Å), indistinctly banded collagen fibrils characteristic of type II collagen are distributed throughout the extracellular matrix of the epiphyseal growth plates (Figs. 3 and 4). In addition, a fine filamentous material is also present, generally associated with collagen fibrils (Fig. 4). Ruthenium red-OsO_4 postfixation of normal cartilage matrix results in an enhancement of the thin filamentous components, in all zones of the normal growth plate (Fig. 5*a*). On the basis of this and other histochemical data from a number of laboratories (16, 20), this material has been designated as proteoglycan.

In the brachymorphic epiphyseal growth plates, the reserve zone (Fig. 3) extracellular matrix contains equal numbers of polygonal pro-

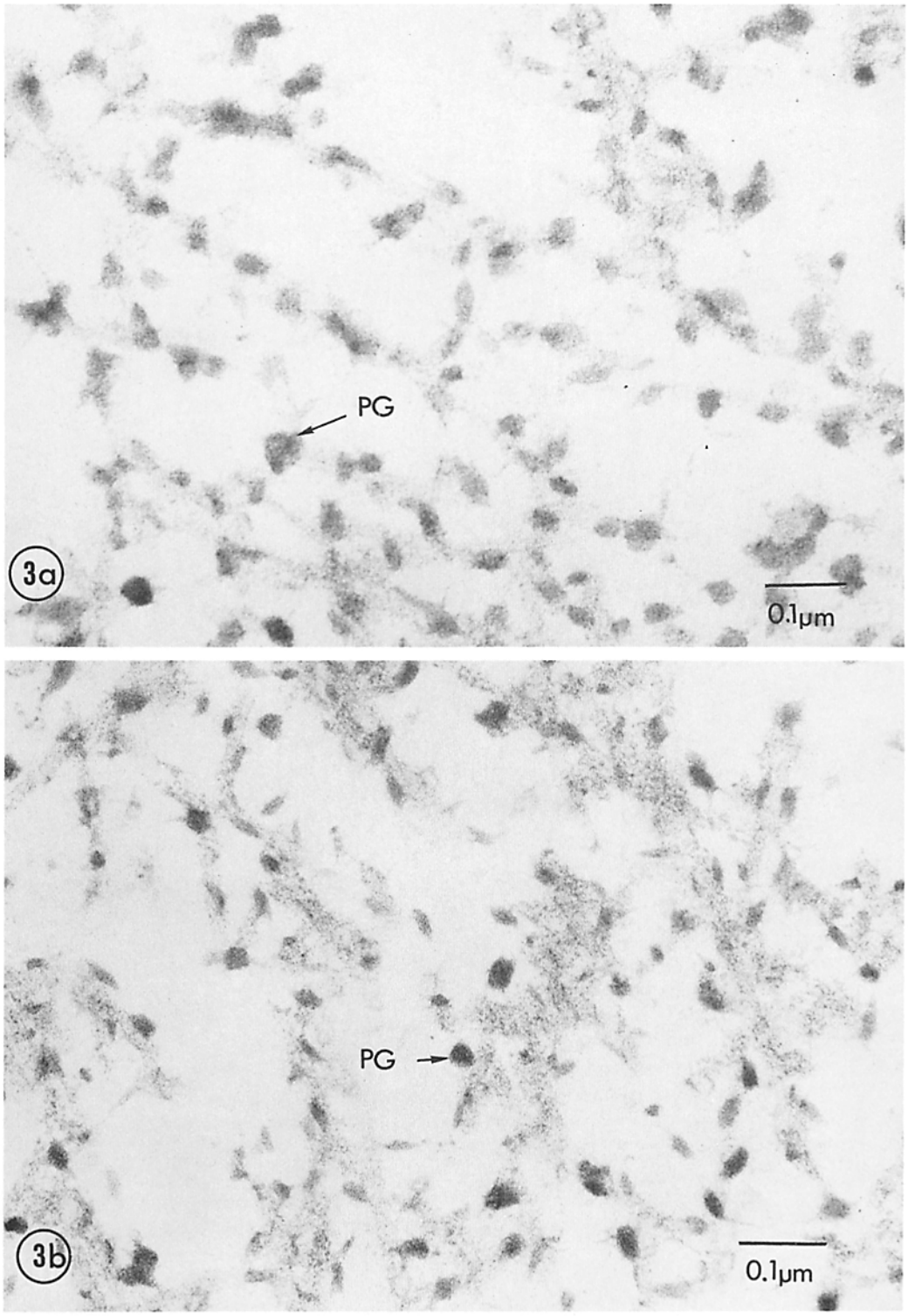

FIGURE 3 Electron micrograph of the extracellular matrix from the reserve zone of 5-day-old normal *C57Bl/6J* (*a*) and mutant *bm/bm* (*b*) epiphyseal growth plates. Both the mutant and normal matrices contain similar numbers of proteoglycan matrix granules (*PG*) (Table II) characteristic of cartilage. Mutant PG granules, however, are 40–50% smaller than normal. × 144,000.

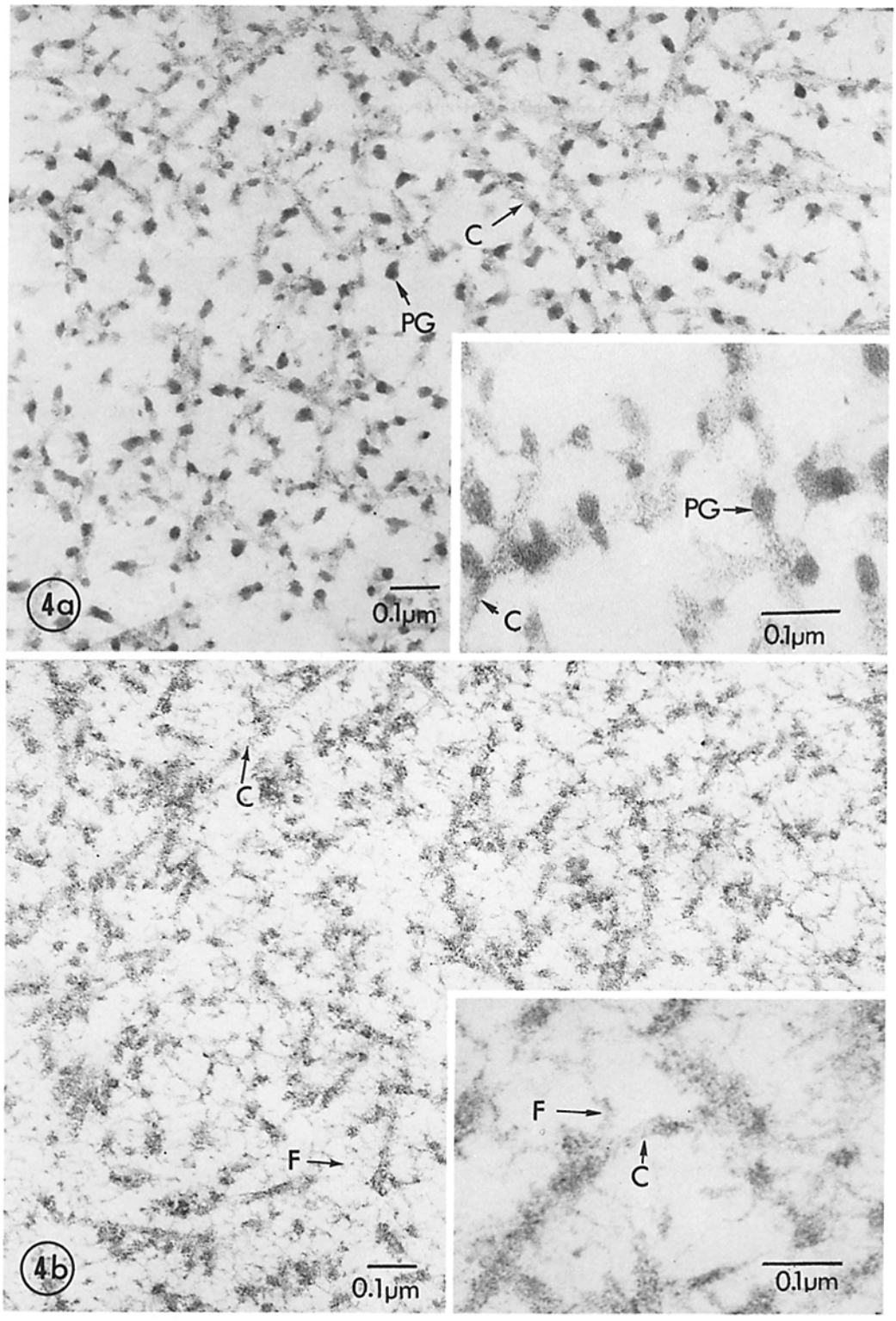

FIGURE 4 Electron micrograph of the extracellular matrix from the proliferative-columnar regions of 5-day-old normal *C57Bl/6J* (*a*) and mutant *bm/bm* (*b*) epiphyses. The *bm/bm* sample displays few proteoglycan matrix granules (*PG*). Unlike the extracellular matrix from normal proliferative-columnar zones in which PG granules are closely associated with collagen fibrils (*C*), collagen fibrils in the *bm/bm* extracellular matrix from this region are surrounded by a prominent fine filamentous material (*F*). The ultrastructure of the extracellular matrix of the hypertrophic zone resembles that shown here. × 60,000; *inset*: × 144,000.

teoglycan matrix granules, as compared with normals (Table II). However, in the mutant, these granules are reduced in size (mean granule size, 50–60% of normal). After treatment of *bm/bm* cartilage with ruthenium red-OsO_4, the constituents of the matrix in this region of the growth plate exhibit a somewhat more diffuse appearance. The matrix granules, in particular, are less distinct. Brachymorphic proliferative-columnar and hypertrophic zones exhibit only few polygonal matrix granules after routine fixation (Table II). The matrix of these lower zones appears to consist of the indistinctly banded collagen fibrils characteristic of normal cartilage type collagen, as well as a prominent network of thin filaments associated with the collagen fibrils (Fig. 4). Ruthenium red-OsO_4 postfixation of the lower regions of the growth plate in brachymorphic animals results in a marked alteration of the extracellular constituents into large deposits of stippled material. In addition, the interconnecting filamentous material appears coarser and more pronounced than after routine fixation and staining (Fig. 5*b*).

After extraction of the cartilagenous epiphyses with 4 M guanidine hydrochloride, the extracellular matrices of both normal and *bm/bm* tissues were devoid of proteoglycan matrix granules (Fig. 6*b* and *d*). The unextracted *bm/bm* cartilage matrix, however, while lacking polygonal granules in the lower zones of the growth plate, contains a prominent fine filamentous network of material morphologically similar to the filaments found in normal cartilage (20). This filamentous material, so apparent in the untreated brachymorphic cartilage matrix (Fig. 6*c*), was largely extracted by the 4 M guanidine hydrochloride treatment (Fig. 6*d*). This finding strengthens the interpretation that the fine filamentous network is composed of proteoglycans.

TABLE II

Distribution of Matrix Proteoglycan Granules in Brachymorphic (bm/bm) and Normal (C57Bl/6J) Epiphyseal Growth Plates

	5-Day *C57Bl/6J* epiphyses	5-Day *bm/bm* epiphyses
	No. of granules/2 μm²	
Resting zone	45.5 ± 5.5	59 ± 10.7
Columnar zone	105.7 ± 11.5	7.25 ± 3.9
Hypertrophic zone	107.3 ± 26.3	0.5 ± 0.5

Results are presented as the mean number of granules ± SEM.

DISCUSSION

Brachymorphia is due to a recessive gene which produces reduced growth of the long bones and some alterations in cranial-facial structures. In contrast to mice that have other defects affecting cartilage, brachymorphic *bm/bm* animals are vigorous, and breeding colonies are readily established. The studies reported here indicate that the epiphyseal growth plates in brachymorphic mice are reduced in size and that each zone of growth, reserve, proliferative-columnar, and hypertrophic, is shorter than normal, with the most pronounced reductions occurring in the hypertrophic zone of *bm/bm* epiphyseal cartilage by day 16. The extracellular matrix of cartilage from brachymorphic animals adsorbs less stain for sulfated proteoglycans than that of cartilage from normal mice. In normal samples the uptake of stain is similar in all three zones of growth. In contrast, the mutant cartilage is generally less intensely stained than normal, and its columnar and hypertrophic zones are less intensely stained than its reserve zone. These findings suggest the presence of altered sulfated proteoglycans in the mutant and suggest that the defect is most pronounced in the lower regions of the growth plate.

Electron microscope observations further localize the defect in the extracellular matrix of *bm/bm* cartilage to the precipitable form of the proteoglycan component. In routine preparations of uranyl acetate-lead citrate-stained materials, polygonal granules of precipitated proteoglycan aggregates are distributed throughout the matrix of normal epiphyseal cartilage and are distinguishable from cross sections through collagen fibrils. While the number of matrix granules per area in the matrix of the reserve zone of *bm/bm* epiphyseal cartilage is the same as in the normal, the granules are 40–50% smaller. Moreover, these granules are essentially absent from the brachymorphic columnar and hypertrophic zones of the growth plate. A number of studies has established that the polygonal matrix granules are composed, at least in part, of precipitated proteoglycan aggregates. These studies include the following criteria: Alcian blue stains localize the proteoglycan in these granules (2, 5, 30, 34); prior treatment of cartilage with testicular hyaluronidase or chondroitinase ABC reduces the size and number of these matrix granules (18, 34); extraction of cartilage with 4 M guanidine hydrochloride removes more than half

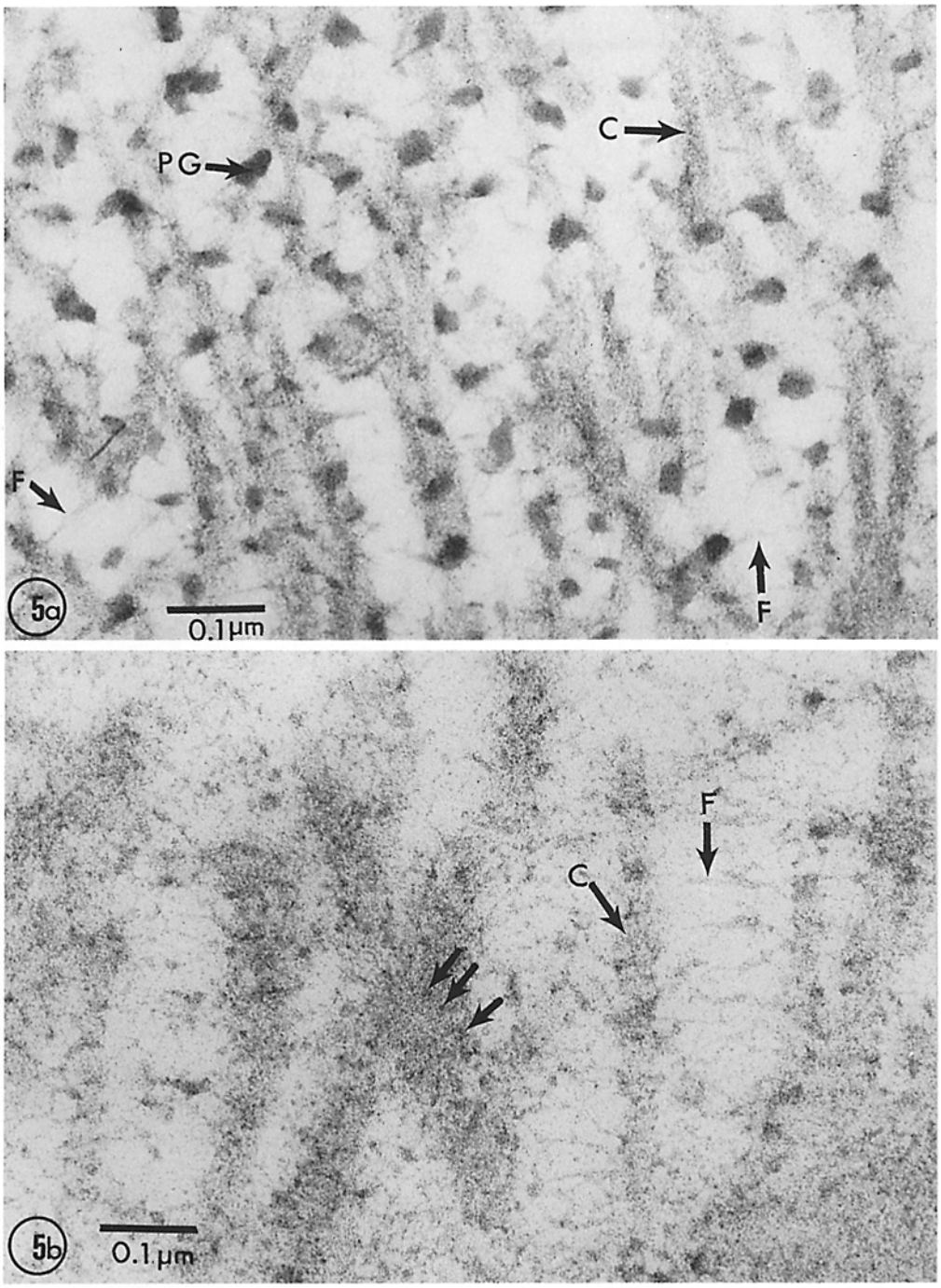

FIGURE 5 Electron micrographs comparing the ultrastructure of the cartilage matrix from normal *C57Bl/6J* (*a*) and *bm/bm* (*b*) epiphyses after postfixation in ruthenium red-osmium. These micrographs illustrate regions of the hypertrophic zone matrix. In the normals, the thin filamentous components (*F*) of the proteoglycans are enhanced. In the mutants, the filamentous network is coarser and more pronounced than in samples not treated with ruthenium red. In addition, the collagen fibrils (*C*) appear to be coated with proteoglycan deposits (arrows). *PG*: proteoglycan matrix granules. × 144,000.

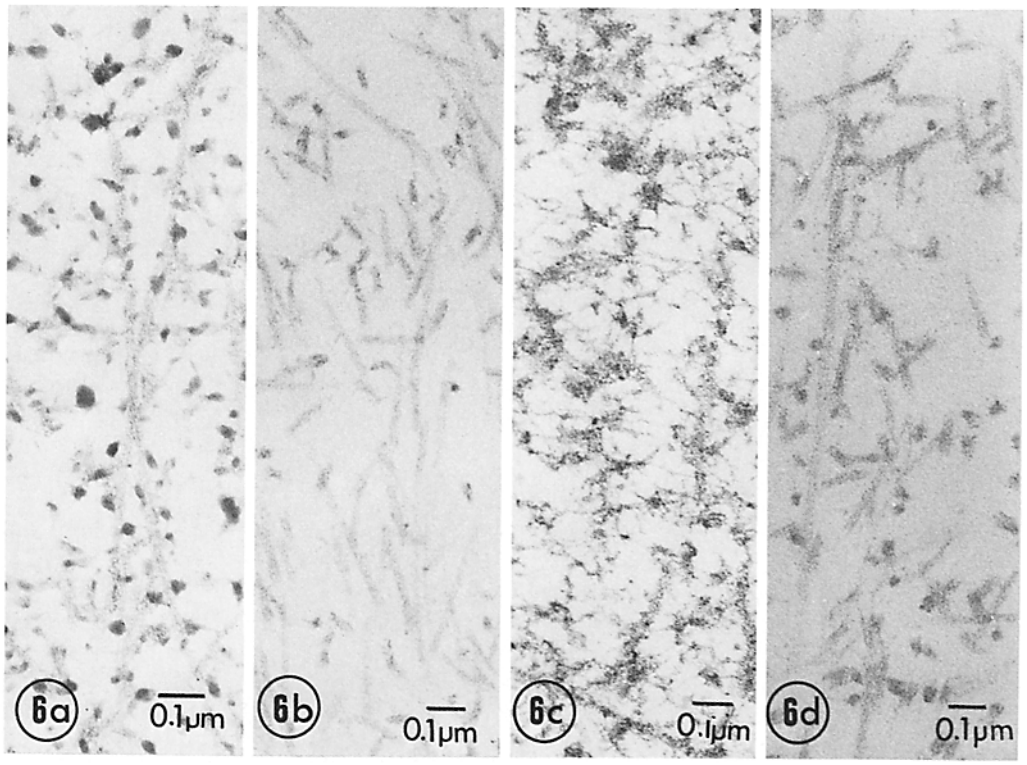

FIGURE 6 Electron micrographs comparing the ultrastructural appearance of the extracellular matrices from untreated normal *C57Bl/6J* (*a*) and *bm/bm* (*c*) epiphyseal cartilages with those extracted with 4 M guanidine hydrochloride. Extracellular matrix material from the proliferative-columnar zones shown here resembles that seen in the hypertrophic zones. The unextracted *bm/bm* cartilage (*c*) appears similar to the extracted normal cartilage (*b*), as well as to the extracted mutant cartilage (*d*), in that proteoglycan matrix granules are absent in all three samples. However, fine filamentous proteoglycans characteristically associated with cartilage collagen fibrils are prominent in the unextracted *bm/bm* cartilage (*c*). This further indicates that untreated *bm/bm* cartilage matrix of the proliferative-columnar and hypertrophic zones fails to display the characteristic cartilage proteoglycan matrix granules (PG) and that mutant cartilage collagen appears normal. × 60,000.

of the proteoglycan from cartilage, and the polygonal granules are no longer apparent (1, 35).

Ultrastructural histochemistry, with the use of ruthenium red treatment, and, in other experiments, extraction with guanidine hydrochloride have proven useful in further defining the defect in the *bm/bm* cartilage matrix. Postfixation of the tissue with ruthenium red-osmium tetroxide has further emphasized the morphological distinctions between normal and brachymorphic cartilage matrix. In control animals, this treatment emphasizes the presence of the fine filamentous form of the proteoglycan throughout the different zones of the growth plate. This filamentous form of the proteoglycan, however, is never so prominent as that observed in the matrix of *bm/bm* cartilage. The morphology of the extracellular matrix granules in normals remains unchanged by the ruthenium red treatment. Similar observations have been made on the proteoglycans present in the extracellular matrix of ruthenium red-treated arterial walls (39).

In contrast, ruthenium red treatment of *bm/bm* epiphyseal cartilage results in a fuzzy appearance of the matrix granules present in the reserve zone. Particularly striking, however, are the differences present in the lower regions of *bm/bm* growth plates treated with ruthenium red. Here, in the proliferative-columnar and hypertrophic zones of the brachymorphic growth plates, collagen fibrils appear coated with proteoglycan material, as judged by their thickened, stippled appearance. In addition, the network of thin, interconnecting filaments is now extremely pro-

nounced, appearing coarser than that found in nonruthenium red-treated *bm/bm* samples. Although ruthenium red is known to react with polyanions, its mechanism of action is not well understood (15). We presume that the altered appearance of the cartilage matrix in the mutant reflects the lack of sulfation, which we have biochemically demonstrated to occur in these animals (21), thereby rendering the *bm/bm* proteoglycans less polyanionic.

Treatment of both normal and *bm/bm* cartilages with guanidine hydrochloride extracts the proteoglycan component from the tissue. In normals, this procedure results in the removal of both polygonal granules and the relatively sparse quantities of fine filamentous proteoglycan normally associated with collagen fibrils (20). The resulting image is a matrix containing virtually only cartilage-type collagen fibrils. Guanidine hydrochloride treatment of mutant tissue also extracts the proteoglycan component. In this instance, however, in the lower regions of the brachymorphic growth plates (columnar to hypertrophic zones) the prominent fine filamentous material is removed. The resulting electron microscope image is similar to that of extracted normal cartilage, further indicating that the morphology of the collagen in the brachymorphic cartilage is normal.

Although the polygonal matrix granules are reduced in size and number in brachymorphic cartilage, biochemical studies establish that this cartilage contains relatively normal levels of proteoglycan (21). These studies further show that brachymorphic cartilage contains undersulfated chondroitin sulfate (21). The ultrastructural studies reported here support the biochemical findings and indicate that the majority of the proteoglycans in brachymorphic cartilage are visualized as a network of thin filaments associated with collagen (see reference 20), rather than being in the form of polygonal matrix granules. The change in configuration of the proteoglycans in the brachymorphic cartilage may be the result of a reduction in the net ionic charge of the proteoglycan due to the presence of undersulfated chondroitin sulfate. This apparent configurational change in the proteoglycan structure is most pronounced in the columnar and hypertrophic regions of the growth plate, suggesting that as the brachymorphic chondrocytes develop the configuration of the secreted proteoglycans changes from an aggregate polygonal granule to a thin filamentous form.

Although our observations indicate that the proteoglycan portion of the cartilage matrix rather than the collagen is altered in *bm/bm* mice and, in addition, that the growth of cartilage in this mutant is reduced, we do not know to what degree, if any, these abnormalities are causally related. The concept that extracellular matrix proteoglycans may affect cartilage growth is suggested by earlier studies of experimentally induced dwarfism in rabbits by intravenous injections of papain (9, 10, 38). In these studies, administration of papain to young rabbits was found to result in a rapid loss of metachromasia in epiphyseal cartilages, as well as a reduction in the size of the epiphyseal growth plates (38).

Considerable heterogeneity exists among the animal cartilage mutants, although in their gross morphology these mutants may resemble one another. Unlike the brachymorphic mice, mice homozygous for chondrodysplasia (*cho/cho*) (28) and for the "cartilage anomaly" (*can/can*) (11) die at or shortly after birth. Mice homozygous for achondroplasia (*cn/cn*) and so-called stubby mice (*stb/stb*) are viable but breed poorly (12). It should be noted that the *cn/cn* gene in mice is not analogous clinically, histologically, or genetically to the dominant achondroplasia gene which occurs in man.

The histological appearance of *bm/bm* epiphyseal cartilage differs considerably from that described for most other short limbed cartilage mutants including the *cho/cho* (28) and *can/can* (11) mice, nanomelic chicks (23), as well as such drug-induced micromelics as thallium- (7) or 6-aminonicotinamide- (29) treated chicks. In these other mutants, or drug-induced anomalies, chondrocytes in the epiphyseal growth zones are disordered and/or the ratio of matrix volume to cell volume is greatly reduced. The cellular disarray is likely to result from significantly altered structures of the cartilage extracellular matrix. These disorders apparently involve greater changes in the proteoglycan portion of the cartilage matrix than in the collagen, although collagen fibers are thicker than normal and are well banded in *cho/cho* mice (28). In the case of nanomelic chicks, chondrocytes synthesize greatly reduced levels of cartilage-specific proteoglycan monomer (22) which results in the production of only minimal amounts of extracellular matrix material. In contrast, the alteration in the matrix of brachymorphic cartilage does not appear to affect significantly the alignment of the epiphyseal chondrocytes.

Until recently, the classification of the human chondrodystrophies has been based predominently on clinical findings. These heritable disorders of cartilage which result in disproportionately short stature have now begun to be reclassified on a histological basis (26). Of the many types of human chondrodystrophies, the most common form is achondroplastic dwarfism (26). While human achondroplasia is a dominant genetic disorder, it is considerably similar to brachymorphia in mice at both the gross morphological and histological levels. The striking feature of the cartilage in both human achondroplasia and brachymorphia in mice is the relatively normal alignment of chondrocytes in the epiphyseal growth plates. In contrast, cartilage in other types of human chondrodystrophies, such as thanatophoric dwarfism and achondrogenesis, displays a cellular disarray (26) similar to that found in a number of the other animal cartilage mutants.

The existence of a mouse mutation (*bm/bm*) which interferes with normal chondrogenesis and which closely resembles a human disorder (achondroplasia) provides a probe for the study of normal and abnormal cartilage development. Biochemical studies reported (21) elsewhere corroborate the morphological findings and indicate a defect in the brachymorphic mutant proteoglycans.

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