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Disproportionate micromelia (*Dmm*): an incomplete dominant mouse dwarfism with abnormal cartilage matrix

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SUMMARY

This paper describes a new autosomal incomplete dominant dwarfism, disproportionate micromelia, which has been characterized genetically and phenotypically, and the cartilage of homozygotes, and heterozygotes has been examined by histochemical, immunofluorescence and biochemical methods. Homozygotes, which die at birth, are disproportionately short and have cleft palates. The heterozygotes appear normal at birth but beginning at 1 week of age dwarfism is apparent and increases during growth. Histochemical and biochemical analyses of the cartilage rudiments of homozygotes at day 18 of gestation demonstrate that the cartilage growth plate is disorganized and that the matrix components, collagen and proteoglycan, are altered. Total collagen synthesis is reduced by approximately 30% and the amount of type II collagen is greatly reduced. By immunofluorescence staining with collagen antibodies, it appears that type II collagen is located primarily near the cell surface of chondrocytes but is poorly distributed throughout the remainder of the matrix. The amount of proteoglycan in the cartilage matrix is reduced by approximately 70% as determined by chemical analysis of hexosamines and by [^{35}S]sulfate incorporation. Although the proteoglycans synthesized by the mutant are normal in size and in glycosaminoglycan composition, they were more easily extractable from the matrix than were normal cartilage proteoglycans. Heterozygotes had reduced cartilage matrix proteoglycan by histochemical methods, but the organization of the epiphyseal cartilage was not abnormal. These data suggest that a reduced or abnormal cartilage matrix is the cause of the dwarfism.

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INTRODUCTION

Normal growth of the endochondral skeleton depends on the deposition of cartilage matrix in the growth plate of the epiphysis. The extracellular matrix of normal cartilage has been well characterized. It contains a unique collagen (type II) (Miller & Matukas, 1969; Miller, 1971, 1973) and proteoglycan (Hascall & Heinegard, 1975), with a protein core with covalently attached chondroitin sulfate chains. The proteoglycan molecules interact with hyaluronic acid to form a large aggregate structure (Hardingham & Muir, 1972; Hascall & Heinegard, 1974).

A variety of genetic defects of cartilage matrix have been described which result in reduced skeletal growth. The cartilage matrix of the nanomelic chick (Landauer, 1965; Mathews, 1967; Palmoski & Goetinck, 1972; and Pennypacker & Goetinck, 1976) and of the cartilage matrix deficiency mouse (Kimata, Barrach, Brown & Pennypacker, 1979) do not contain the cartilage specific proteoglycan due to a failure in the synthesis of the core protein. In contrast, the cartilage matrix of the brachymorphic mouse (Lane & Dickie, 1968; Orkin, Pratt & Martin, 1976) contains proteoglycans with undersulfated chondroitin sulfate chains. Studies of these dwarf animals have demonstrated the importance of the matrix constitution for normal growth (Hall, 1978).

This report describes a new incomplete dominant dwarfism in the mouse called disproportionate micromelia (*Dmm*). In the homozygote, *Dmm/Dmm* is characterized by disproportionately reduced limbs, cleft palate, and neonatal death and in the heterozygote by a postnatal progressive dwarfism resulting in *Dmm/+* adults with short legs, blunt head and broad rump. The cartilage rudiments of the limbs of the homozygote are drastically reduced in length and are significantly wider than those of unaffected sibs. Histochemical and biochemical analyses of the homozygote show that both the collagen and proteoglycan of the growth plate cartilage are altered.

MATERIALS AND METHODS

The phenotype of the heterozygote *DMM/+* was first observed at Oak Ridge National Laboratory by Ehling (Ehling, 1966; Ehling, personal communication) in the offspring of a male (no. 42745) of strain 101 whose spermatogonia had been subjected to irradiation with 600R. The mother (no. 68385) was C3H strain and was unirradiated. The short legs, blunt head and broad rump in the adult was described as being the result of an autosomal gene with complete penetrance (Kelly, 1975). The initial stock was extensively outcrossed because of reduced fertility and has been outcrossed with selection for the dominant trait for over 15 years (K. Stelzner, personal communication). Our stock of 22 animals from several lines was originally obtained from Oak Ridge.

Description of phenotypes. At birth, animals with lethal cleft palate were of

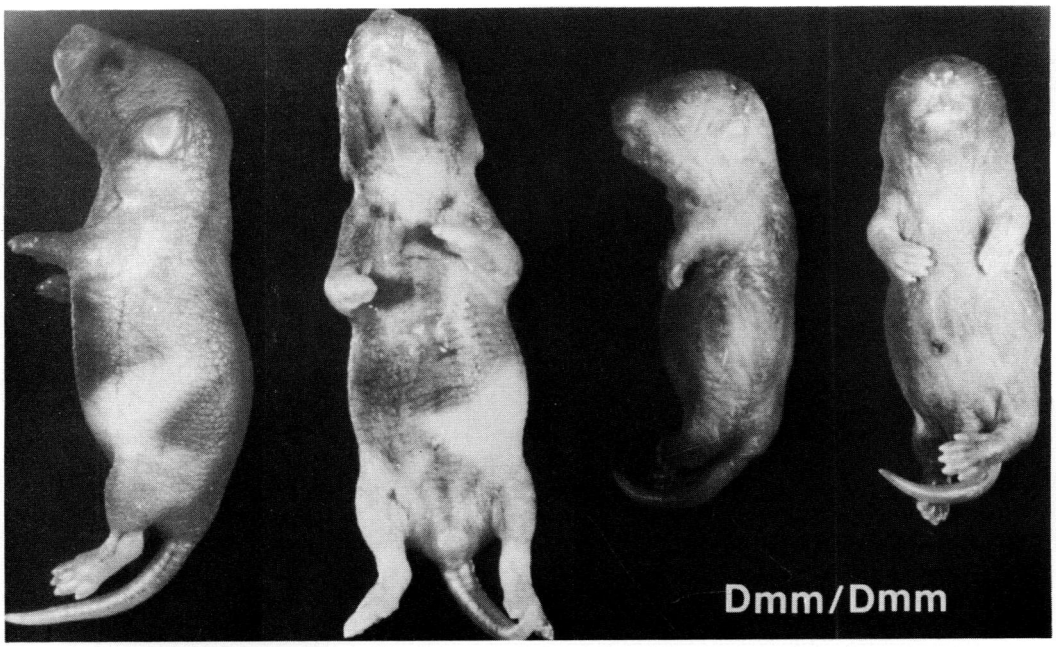

Fig. 1. Frontal and lateral photographs of normal and dwarf *Dmm/Dmm* newborn mice.

two classes, dwarf and normal size (Fig. 1). The viable newborn appeared normal but it was clear at 4 weeks of age that some of these animals were dwarfs. The subsequent viability and reproductive behavior of the normals and dwarfs were similar.

Genetics. Matings between normal and dwarf, between two dwarfs and between two normal animals were made. The offspring were classified at birth and at 4 weeks of age. Thirteen litters from two dwarf parents were killed on day 18 of gestation and the number of viable and dead young as well as the phenotypic classification were tabulated. Tissues from two different severely affected fetuses were cultured and cells examined by Dr Beverly White of Cytogenetics Section, National Institute of Arthritis, Metabolism and Digestive Diseases for cytogenetic abnormalities using the Giemsa-Trypsin banding technique.

Tissue preparation. There were no obvious differences in weight, size or shape of the major viscera from viable dwarfs and normal types of both sexes at 4 weeks. Differences of skeletal structures were examined by several methods. Newborn, 1-week- and 4-week-old mice were cleared in alkali and stained with Alizarin red. Stained bones from at least ten normal and abnormal mice at each age were measured with an ocular micrometer in a dissecting microscope. Histologic study was done on distal femoral epiphyses, promptly fixed in 10% neutral phosphate-buffered formalin with 0·5% cetylpyridinium chloride (CPC) (Pearse, 1968), processed using standard histologic technique (Luna, 1960), and stained with either hematoxylin and eosin, Alcian blue at pH 2·5 (Spicer, Horn

& Leppi, 1967), Safranin-O, Masson's Trichrome (Lillie, 1965; Rosenberg, 1971), or the periodic acid-Schiff (PAS) reaction (Lillie, 1965). Similar measurements and histologic preparations were made from day-18 fetuses of the severe short leg and normal types.

Distal femoral epiphyses of the severely affected and normal fetuses were dissected and either quickly frozen for immunofluorescence or were carefully freed of adhering tissues, lyophilized and weighed. Dissected specimens of cartilaginous distal femoral tissue were used for biochemical analyses of proteoglycan and collagen.

Proteoglycan analysis. Knee joints were incubated in 1 ml of F-12 medium containing 10% fetal calf serum, [^{35}S]sulfate (200 μCi/ml), [^{3}H]glucosamine (100 μCi/ml) and ascorbic acid (50 μg/ml). After labeling for 18 h, the medium was dialysed against two changes of a solution containing 25 mM Na_2SO_4 and 25 mM glucosamine, and then against two changes of distilled water. Radioactivity incorporated into macromolecules in the medium was then determined. The knee joints were rinsed in cold phosphate-buffered saline pH 7·4 (PBS), frozen and lyophilized. The lyophilized tissues were weighed and processed either for determination of intact proteoglycan content or for glycosaminoglycan composition.

Intact proteoglycans were extracted with 4·0 M-guanidine-HCl (GuHCl) containing protease inhibitors (0·1 M-6-aminohexanoic acid, 0·005 M-benzamidine hydrochloride, 0·01 M-disodium ethylenediamine-tetraacetate in 0·05 M-sodium acetate, pH 5·8) for 24 h in the cold (Oegema, Hascall & Dziewiatkowski, 1975*a*). The extract was clarified by centrifugation, dialyzed against 0·5 M-GuHCl containing the protease inhibitors, and centrifuged in an associative CsCl density gradient (0·9 gm CsCl/gm extract) at $1 \times 10^5 g$ for 48 h. The gradient was divided into three fractions with the bottom of the gradient, designated A_1, containing most of the proteoglycans (Oegema *et al.* 1975*a*). Each fraction of the gradient was dialyzed against 0·1 M-sodium acetate, pH 7·0, and an aliquot of each was counted. A portion of the A_1 fraction was chromatographed on a Sepharose 2B column (1 × 150 cm) in 0·5 M-sodium acetate, pH 7·0.

Alternatively, glycosaminoglycans (GAG) were extracted with 0·5 M-NaOH for 24 h at room temperature (Orkin *et al.* 1976). The extracts were then neutralized with HCl and precipitated by adding trichloroacetic acid to a final concentration of 10%. After centrifugation, the supernatant fraction was dialyzed against water and a sample was taken to determine total radioactivity. The GAG in the supernatant fraction were characterized by Sephadex G-200 column chromatography (0·8 cm × 100 cm) in the presence of 0·5 M-sodium acetate, pH 7·0, or by DEAE-cellulose column chromatography (1·7 × 7 cm) with a linear 0–1·0 M-NaCl gradient in 0·05 M-Tris-HCl, pH 7·2 (Orkin *et al.* 1976). Another portion of the GAG fraction was digested with chondroitinase ABC and analyzed by descending paper chromatography (Saito, Yamagata & Suzuki, 1968).

The hexosamine content of the 18-day fetal knee joints was determined on a Durrum autoanalyzer after hydrolysis in 4 N-HCl at 100 °C for 10 h. A sample of the 4·0 M-GuHCl extract, which was dialyzed against water and hydrolyzed in 6 N-HCl at 105 °C for 24 h, was used for amino acid analysis on a Beckman autoanalyzer.

Collagen. Knee joints were incubated for 18 h in Eagle's minimal essential medium containing [^{3}H]proline (50 μCi/ml), [^{3}H]glycine (50 μCi/ml) and ascorbic acid (50 μg/ml) with β-aminopropionitrile fumarate (50 μg/ml) to inhibit collagen crosslinking. The tissue was then rinsed in PBS, frozen, and lyophilized. The lyophilized knee joints were weighed and extracted for 24 h at 4 °C with 1 M-NaCl, 0·05 M-Tris, pH 7·4. The extracted material was then dialyzed against 0·2 M-NaCl, 0·05 M-Tris, pH 7·6, and chromatographed on a DEAE-cellulose column (1 × 20 cm) to remove proteoglycans (Miller, 1971). The purified collagen was characterized by chromatography under denaturing conditions on carboxymethyl (CM)-cellulose (Miller, 1971). Labeled collagen in the medium and in a pepsin extract of the residue after salt extraction (Miller, 1972) was processed in a similar manner and was chromatographed on CM-cellulose.

The location of type II collagen in the normal and mutant cartilage was determined by immunofluorescence microscopy. Rabbit anti-rat IgG raised against type II collagen from a rat chondrosarcoma (Smith *et al.* 1975) was purified by affinity column chromatography. These antibodies were provided by Dr L. Paglia (NIDR) and were used for immunofluorescence studies at a concentration of 125 μg/ml. *Dmm/Dmm* and normal control limbs from day-18 embryos were excised, immersed in optimal cutting temperature (OCT) compound, and immediately frozen in a mixture of acetone/dry ice. Cryostat sections (6 μm) were cut and air dried on glass slides. Sections were digested with 2% testicular hyaluronidase in PBS for 30 min at room temperature. After washing with PBS, sections were exposed to either rabbit anti-type II IgG or preimmune rabbit serum for 30 min at room temperature, rinsed for 30 min in four changes of PBS, and then incubated for 30 min with a 1 : 40 dilution in PBS of fluorescein-isothiocyanate-labeled goat anti-rabbit IgG. Excess antibody was washed off with PBS prior to mounting in glycerol: PBS (9:1), pH 9·0. The sections were examined for immunofluorescence using a Leitz Orthomat-W microscope equipped with epifluorescence optics.

RESULTS

Bone size and development. The gross appearance of the severely affected newborns was uniform. These dwarfs each had a small head with short snout, cleft palate and protruding tongue (Fig. 1). The lengths of the calcified long bones from severely affected newborns were greatly reduced and the diameter of the tubular bones was increased compared to normal littermates (Table 1).

Table 1. *Ratios of bone size between newborn mice with cleft palate* (Dmm/Dmm) *and normal sibs* (Dmm/+ *and* +/+)

Bone	Length	Width
Skull	0·86	0·99
Nasal	1·15	0·95
Frontal	1·09	1·18
S-F*	0·63	0·31
Mandible	0·87	1·01
Scapula	0·50	0·64
Humerus	0·51	1·01
Radius	0·62	1·28
Ulna	0·55	1·48
Femur	0·55	1·36
Tibia	0·58	1·36
Fibula	0·53	1·35

* Total skull length minus frontal bone length.

Day-18 fetuses were removed from *Dmm*/+ by *Dmm*/+ matings and bone sizes measured as described in Materials and Methods. At day 18 the *Dmm*/+ and +/+ mice could not be distinguished. Each value represents the ratio of means of ten measurements which did not differ by more than 1%.

In contrast, the nasal and frontal bones were normal. Total skull length of affected animals was shortened due to reduction of the basicranium as judged by the length of the total skull minus the length of facial bone. In the dwarf, cranial width was also disproportionately small compared to facial bone width. The shortening of the mandible was intermediate between that of basicranium and frontal bone in proportion to the total skull length.

Among the viable young a classification into dwarf and normal was possible by 1 week after birth based on reduced length of the nose, tail and limbs (Fig. 2). Viable animals classified as dwarfs showed a greater relative shortening of limbs at 4 weeks than at 1 week. The humerus and femur were most severely affected (Table 2). The growth of long bones in females was relatively more retarded than in males. The body and tail length of the 4-week-old dwarfs was reduced by 15% in females and by 10% in males. At 4 weeks, the females achieved 69% of normal body weight while males achieved 78%. The weight per length ratio was 0·81 for females and 0·86 for males.

Genetics. The distribution of various phenotypes found among the offspring of different matings is presented in Table 3. Matings of two normal parents from this stock never gave an offspring with short legs, demonstrating that the trait of disproportionate micromelia is inherited as a dominant. Since short males mated to normal females produced both short male and female offspring, the gene is autosomal. These results confirm the observations made at Oak Ridge. The gene has been designated *Dmm*.

Fig. 2. Photograph of mice at age 4 weeks with short leg (left) and normal phenotype (right).

Cytogenetic examination of two severely affected fetuses demonstrated that both were 40, XX females with normal chromosomes. The Giemsa-Trypsin banding pattern was also normal.

The average litter at birth contained 7·2 young if both parents were normal

Table 2. *Ratios of bone length between* Dmm/+ *and* +/+ *mice at 1 and 4 weeks after birth*

Bone	1 week	4 weeks
Scapula	0·87	0·87
Humerus	0·87	0·73
Radius	0·89	0·85
Ulna	0·88	0·85
Femur	0·88	0·77
Tibia	0·90	0·81
Fibula	0·90	0·83

Each value represents the ratio of means of ten measurements which did not differ by more than 1%.

Table 3. *Distribution of phenotypes in offspring*

		At birth			At weaning	
			Cleft palate			
Mating type	No. pairs	Normal*	Normal legs	Short legs	Normal	Short
normal × normal	18	432	1	0	343	0
short × normal	27	421	14	2	157	105
short × short	90	1096	70	196	222	386

* The heterozygotes at birth could not be distinguished from normals.

but only 5·2 if both were short. There was no evidence of maternal effect. In addition, the number of short young which reached weaning in the matings with one or two short parents was reduced, and the numbers of severely affected young at birth in litters with two short parents were reduced compared to genetic expectation (Table 3). These data suggest that *Dmm*/+ newborns have a 10% risk of cleft palate and about a 25% greater risk of death before weaning than +/+ littermates and that the *Dmm/Dmm* phenotype had significantly reduced intrauterine viability.

We observed a single example of apparent incomplete penetrance of the *Dmm* gene in a mating of a male classified as normal to a normal female. The offspring had a cleft palate but normal legs. The male parent subsequently produced several litters containing offspring with the short legs and cleft palate when mated with several short females. This mouse was probably a *Dmm*/+. We estimate about 95% penetrance by the mating test.

Histology. Histological comparison of the femoral epiphyseal growth plates

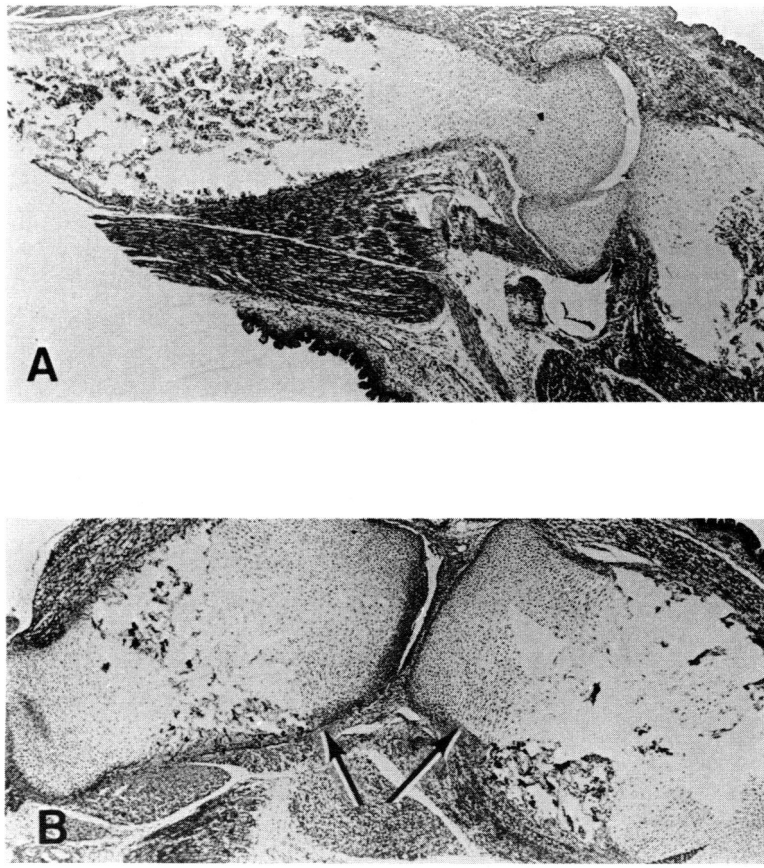

Fig. 3. Knee region of day-18 fetuses stained with Masson's Trichrome stain (× 35). A. normal. B. *Dmm/Dmm*. The shortened length of calcified metaphysis, and lack of organized proliferation zone are apparent. Columnar organization perpendicular to ossification groove is marked by arrows.

of day-18 fetuses demonstrated several differences between the *Dmm/Dmm* and normal appearing littermates (Figs. 3, 4). The very short calcified metaphyses of the *Dmm/Dmm* have an increased diameter (Fig. 3). The inner region of the cartilagenous epiphysis shows loss of structural integrity of the matrix resulting in a 'cystic' appearance. This is most marked centrally where large and rounded extracellular spaces have formed. These spaces may contribute to the fragile and liquid character of the growth plate observed during dissection. In the growth plate, the cells of the *Dmm/Dmm* were not arranged in the normal columnar pattern oriented toward the metaphysis, but were slightly compressed so that their axes were parallel to the long axis of the bone. The lack of normal cell columnation, in conjunction with decreased matrix and cell compression, resulted in a pattern of cell alignment oriented transversely across the growth plate, rather than perpendicular to it (Fig. 4B).

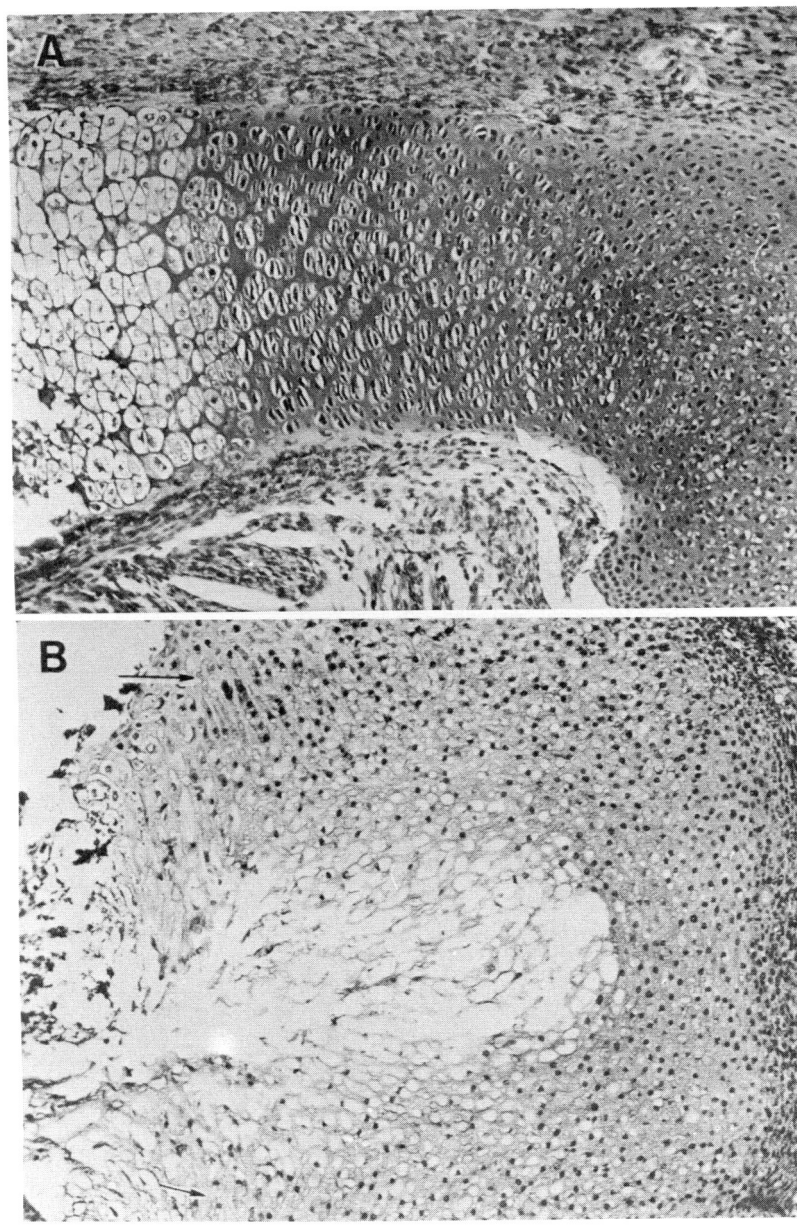

Fig. 4. Knee region of day-18 fetuses stained with Alcian blue (× 130) A. normal. B. *Dmm/Dmm*. The intensity of the stain is reduced in the cartilage matrix of the entire epiphyses, and cystic disorganization is present in the central region of the epiphysis in *Dmm/Dmm*. Arrows indicate columnar organization perpendicular to ossification groove as in Fig. 3.

Comparison of the histology of the distal femoral epiphyses of dwarf *Dmm/+* and normal mice at 1 and 4 weeks of age showed a reduction in the height of the proliferative and hypertrophic zones in the mutant but normal organization in

the growth plate. The matrix of the mutant stained less intensely with Alcian blue or Safranin-O (not shown).

All biochemical studies were done on *Dmm/Dmm* because of the uncertainty of diagnosis of *Dmm/+* in the fetus.

Proteoglycans. The intensity of matrix staining of hindlimb and forelimb bones of 18-day fetuses by Safranin-O (not shown) or Alcian blue was greatly reduced in the *Dmm/Dmm* growth plate (Fig. 4A, B). The reduction was observed throughout the resting, proliferative and hypertrophic zones.

Hexosamine determinations of 18-day fetal cartilages revealed that both control and *Dmm/Dmm* contained similar amounts of glucosamine per mg dry weight (control 1·52 p-moles/mg; *Dmm/Dmm* 1·70 p-moles/mg). However, the galactosamine content in the cartilage from the mutant fetuses was reduced (control 8·03 p-moles/mg; *Dmm/Dmm* 2·67 p-moles/mg). Decreased galactosamine content in the mutant cartilage correlated with a decrease in proteoglycan synthesis since [^{35}S]sulfate and [^{3}H]glucosamine incorporation per mg dry weight were reduced by 74 and 52 % respectively.

The distribution of total incorporated radioactivity into the 4·0 M-GuHCl extract, the medium, and the residue after extraction was measured in tissues from control and *Dmm/Dmm* mice (Table 4). There was little difference between the control and mutant cartilage in terms of extractable proteoglycans. However, ten-fold more incorporated radioactivity was present in the medium of the tissue from the *Dmm/Dmm* mice. Cartilage residues from *Dmm/Dmm* contained very little labeled material compared to normal, suggesting that the proteoglycans were poorly bound within the cartilage matrix.

The radioactive sulfate and glucosamine extracted in 4·0 M-GuHCl from cartilage of normal and *Dmm/Dmm* fetuses had similar distribution on a CsCl density gradient. Molecular sieve chromatography (Fig. 5) showed similar relative amounts of proteoglycan aggregate and monomer, which suggests that there is no difference in the structural organization of the proteoglycans. In addition, the component glycosaminoglycans were similar in size as determined by molecular sieve chromatography and type based on digestion with chondroitinase ABC.

Collagen. Immunofluorescence of the fetal growth plate labeled with anti-type II collagen antibody was reduced throughout the matrix of *Dmm/Dmm* animals when compared to normal tissue (Fig. 6). In the *Dmm/Dmm*, type II collagen had a pericellular distribution and there were large areas between cells which lacked type II collagen. When labeled with anti-type I collagen antibodies, *Dmm/Dmm* bone showed a pattern of fluorescence similar to the normal.

The amount and type of collagen was characterized from cartilage labeled with [^{3}H]proline. The distribution of labeled proteins in the salt extract, medium, and pepsin extract of the residue was similar for control and mutant cartilages. However, total incorporation per mg dry weight was reduced by 30 % in the cartilage from the *Dmm/Dmm* mice. After partial collagen purification, each

Table 4. *Relative incorporation of* [^{35}S]*sulphate and* [^{3}H]*glucosamine in tissue extract, residue and culture medium from unaffected and* Dmm/Dmm *cartilage*

	Unaffected				*Dmm/Dmm*			
	^{3}H		^{35}S		^{3}H		^{35}S	
	cpm × 10^4	%	cpm × 10^4	%	cpm × 10^4	%	cpm × 10^4	%
Tissue extract	158·5	61	292·4	65	49·2	64	55·4	74
Residue	93·7	36	151·2	33	8·4	11	4·2	5
Medium	7·5	3	9·3	2	19·7	25	15·4	20
Total	259·8	100	452·8	100	77·3	100	74·9	100

The results of a representation experiment using four to six dissected knee cartilages of day-18 fetuses of each type.

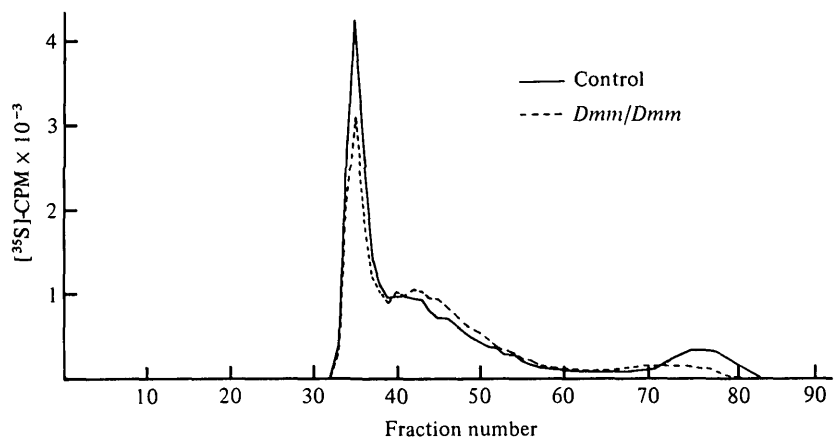

Fig. 5. Sepharose 2B column chromatography of intact proteoglycans ^{35}S in a portion of CsCl gradient from knee joint cartilage of normal control and *Dmm/Dmm* fetuses.

fraction was analyzed for collagen content by CM-cellulose column chromatography. The differences between the ratio of α_1:α_2 chains (control 4·6:1; *Dmm/Dmm* 2:1) on CM-cellulose (Fig. 7) suggest that both cartilages contain type I collagen but that the mutant cartilages contain less type II collagen. Similar profiles were observed from the medium and the pepsin extract indicating that there is no unusual pattern of collagen solubility.

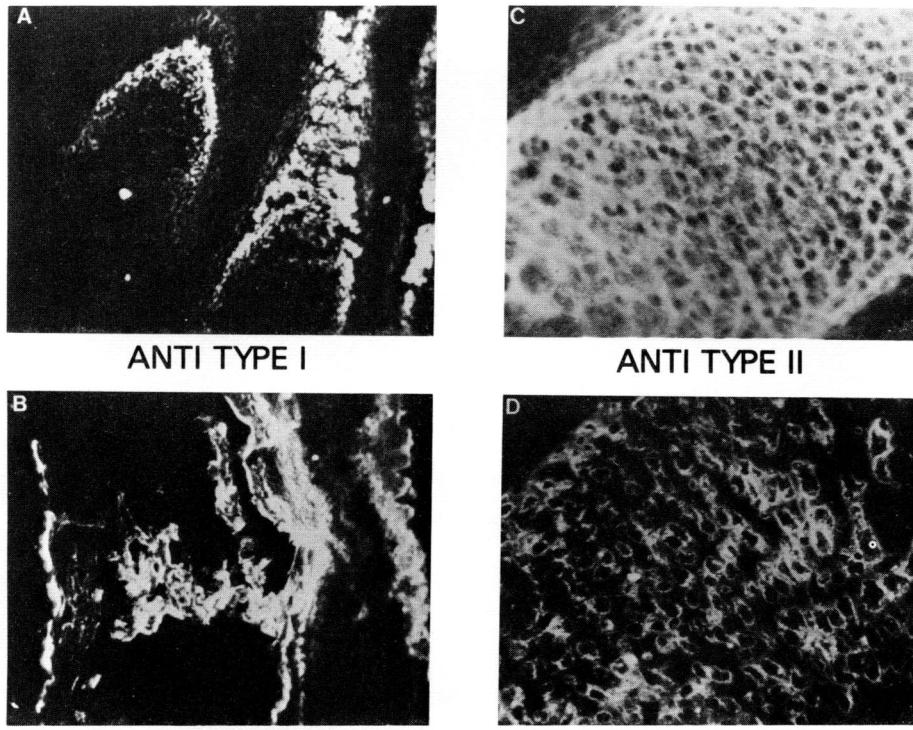

Fig. 6. Immunofluorescence with antibodies to type I and II collagen in long bones from normal and *Dmm/Dmm* fetal mouse cartilages. Type I collagen showed similar localizations in calcified regions of normal (A) and *Dmm/Dmm* (B) mice fetuses. Antibodies to type II showed reduced reaction in the matrix of *Dmm/Dmm* (D) compared to normal (C) with irregular intensity in matrix and a localized reaction close to the cells of *Dmm/Dmm* cartilage.

DISCUSSION

There seems to be some phenotypic overlap between *Dmm/Dmm* and *Dmm/+* in regard to palate and limb development *in utero* since a few animals with normal limbs but with cleft palate were produced in litters expected to give only *Dmm/+*, and one example of nonpenetrance of *Dmm* was observed. This suggests that the basic defect may be quantitative but that the gene dose effect is large.

Histochemical comparisons of the affected limbs in the various offspring indicated progressive changes associated with gene dosage. There was a decrease in intensity of both Alcian blue and Safranin-O staining from +/+ to *Dmm/+* to *Dmm/Dmm* indicating a progressive reduction in the proteoglycan content of the growth plates. In the heterozygote, the tissue organization was maintained in the growth plate despite reduced staining. In contrast, the growth plate of *Dmm/Dmm* showed not only a significant reduction in staining but a lack

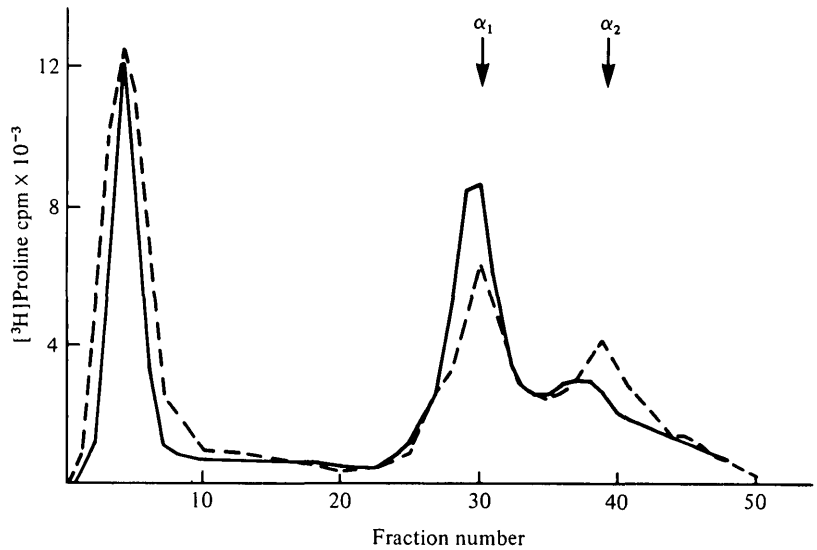

Fig. 7. Carboxylmethyl-cellulose column chromatography of salt extractable [^{3}H]proline-labeled material from control (——) and *Dmm/Dmm* (– – –) hind knee joints. After pepsin digestion equal amounts of radioactivity from control and *Dmm/Dmm* samples were applied to columns and run at 41 °C in the presence of 0·04 M-sodium acetate, pH 4·8. Fractions were eluted with a linear salt gradient of 0 to 0·12 M-NaCl over a total volume of 1600 ml. Fractions of 10 ml were collected. Similar profiles were obtained with pepsin digests of the media and tissue residue (not shown).

of normal organization. There was also increased metaphyseal cellular proliferation in the ossification groove. The diameter of bones in *Dmm/Dmm* was greater than normal, which may represent the effect of compensatory growth of bone formed intramembranously in the ossification groove.

The growth of the skull and face seem to reflect a reduction of endochondral growth in the basicranium with relatively normal membrane bone formation. Calvaria is short but broad, mandible is short, but nasal and frontal bones are normal length. This growth pattern and that of the limbs and spine suggest that the primordia of various bones are normal and that the size reduction is secondary to a growth defect of the epiphyses. Preliminary observations of day-14 embryos in these mice support this idea since abnormal embryos are hard to distinguish at that stage of development.

Both collagen and proteoglycan appear to be altered in the affected cartilage. Biochemical analysis demonstrated a reduced rate of synthesis of collagen and a relatively reduced synthesis of type II collagen compared to type I in the mutant. Type II collagen in the matrix of *Dmm/Dmm* appeared greatly reduced and was predominantly pericellular rather than uniform throughout the matrix. This suggests that reduction in synthesis, possibly associated with abnormal

processing, prevents the normal distribution of the collagen in the extracellular matrix.

The 67% reduction in proteoglycan content of the mutant cartilage, is in part due to decreased synthesis, since total incorporated [^{35}S]sulfate/mg dry weight was also decreased by at least 70%. These observations correlate with the decreased histochemical staining for proteoglycans and the reduced number of matrix granules seen ultrastructurally (G. Hascall, personal communication).

Increased solubility and extractability of proteoglycans could explain why areas in the mutant growth plate did not stain for proteoglycans. The areas devoid of stain could result from artifactual extraction of proteoglycan prior to staining. This has been observed in another mouse mutant, *chondrodysplasia* (*cho*) (Seegmiller, Fraser & Sheldon, 1971; Stevens & Seegmiller, 1976) where increased proteoglycan extractability resulted in decreased matrix staining. However, fixation of the 18-day-old fetuses in cetylpyridinium chloride should stabilize the proteoglycans. Alternatively, the observed reduction of proteoglycan may represent an abnormal localization or reduced synthesis secondary to a defect of some other component of cartilage matrix.

The abnormal pattern of rows of nuclei extending from the perichondrial region into the inner part of the *Dmm/Dmm* growth plate is similar to that described for *cho/cho*. Seegmiller *et al.* (1971) interpreted the absence of longitudinal columns in the proliferative and hypertrophic zones of *cho/cho* as the result of the inability of the unusually soft cartilage matrix to hold the chondrocytes in a column after mitosis so the disorganized mitotic activity resulted in a wider shorter bone. The *Dmm/Dmm* cartilage matrix is also extremely soft and a similar mechanism might apply in *Dmm/Dmm*.

However, increased disorder of the pattern of mitosis in the columns of the proliferative and hypertrophic zones as suggested by Seegmiller *et al.* (1971) does not seem to explain well organized rows of nuclei extending at right angles to the normal orientation. Since subperiosteal chondrocytes appear to arise in the ossification groove of Ranvier (Brighton, 1978), it seems more probable that these rows of cells are chondrocytes which are either hypertrophic or more widely dispersed than normal in both *Dmm/Dmm* and *cho/cho* due to lack of firm matrix texture.

The pericellular localization of type II collagen may also result from the abnormal texture of the *Dmm/Dmm* cartilage. Prior to antibody staining, cartilage sections are pretreated with hyaluronidase to remove the proteoglycans. Normally, this pre-treatment has no influence on collagen distribution. If the matrix contains a reduced amount of proteoglycan, it is conceivable that hyaluronidase treatment might cause the remaining matrix constituents including collagen to be deposited adjacent to the cells or cause matrix constituents from between the cells to be preferentially extracted. However, the nanomelic chick (Mathews, 1967; Palmoski & Goetinck, 1972; Pennypacker & Goetinck, 1976) and *cmd/cmd* mouse (Kimata *et al.* 1979; Rittenhouse *et al.* 1978) have a loss of

cartilage proteoglycan of greater than 80% without change in the distribution of collagen. This suggests that in *Dmm/Dmm* the primary abnormality may be in the collagen since there is both quantitative reduction and change of extracellular distribution of type II in the matrix.

The matrix constituents primarily responsible for maintaining the turgidity of cartilage are the proteoglycans and hyaluronic acid. Numerous proteoglycan molecules interact with a single hyaluronic acid molecule to form large aggregate structures, and this interaction is stabilized by a glycoprotein (Hascall & Heingard, 1974). Mutations resulting in either reduced amounts or abnormal organization of proteoglycans could decrease the turgidity of cartilage. In the *Dmm/Dmm*, the proteoglycans synthesized are similar to those from control animals in size, aggregation and GAG content but are present in a decreased amount. This suggests that the defect of the *Dmm/Dmm* cartilage does not result from the inability of proteoglycans to organize properly, but rather from the reduced quantity of normal proteoglycans.

The reduction in type II collagen could also affect the matrix. Studies on the interaction of proteoglycans and collagen *in vitro* have shown that proteoglycans bind to collagen (Mathews, 1965; Lee-Own & Anderson, 1975; Toole & Lowther, 1968) and, depending on whether the collagen is type I or type II, either enhance or inhibit fibrillogenesis (Oegema, Laidlaw, Hascall & Dziewiatkowski, 1975*b*); Toole, 1976). A change in type II collagen could produce an alteration in collagen-proteoglycan interactions which would be expected to affect the structural integrity of the tissue and lead to pericellular collagen deposition.

It is likely that initiation of chondrogenesis proceeds normally in *Dmm/Dmm* but the cartilage matrix function is abnormal. This interpretation is consistent with the quantitative nature of the cellular and biochemical findings and the observation that the cartilage components are all present in *Dmm/Dmm*. The abnormal localization and reduced quantity of type II collagen appear unique to this mutant.

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