

A NEW CHONDRODYSTROPHIC MUTANT IN MICE

Electron Microscopy of Normal and Abnormal Chondrogenesis

R. SEEGMILLER, F. C. FRASER, and H. SHELDON

From the Departments of Genetics and Pathology, McGill University, Montreal, P. Q., Canada

ABSTRACT

The occurrence of a new mutation affecting cartilage and bone in mice is reported. The gene is lethal, shows autosomal recessive inheritance, and has high penetrance. It is not allelic to *shorthead* and probably not to *phocomelia* or *achondroplasia*. It results in a foreshortened face, cleft palate, defective trachea, and shortened long bones with flared metaphyses. Chondrocytes of epiphyseal cartilage from the mutant are not aligned in columns, and there is a decrease in the usual staining of the cartilage matrix. Electron microscope observations show large, wide collagen fibrils with "native" banding in the matrix of mutant cartilage, which are not present in normal cartilage. Possible explanations for the expression of this genetic disorder of cartilage development are put forward.

INTRODUCTION

The occurrence of a recessive mutation that affects the skeletal system in mice provides a model for studying genetic control of growth and organization at the tissue, cell, and molecular levels. The new mutation, tentatively designated *chondrodysplasia*, has been tested for allelism (Seegmiller, 1970, unpublished data) and results indicate that it differs from the phenotypically similar mutants *phocomelia* (Gluecksohn-Waelsch et al., 1956; Siskin and Gluecksohn-Waelsch, 1959), *achondroplasia* (Dickie, 1961; Lane and Dickie, 1968), and *shorthead* (Fitch, 1961). Observations on the anatomy, histology, and fine structure of the epiphyseal apparatus and other cartilage provide the basis for an hypothesis to explain why the long bones are unusually short and wide in this mutation.

MATERIALS AND METHODS

Mice of the C57/BL/Fr strain which carry the recessive gene tentatively named *chondrodysplasia* (*cho*) were used for these studies. Where timing of gestation was required, the vaginal plug method was

used; the day of conception is referred to as day 0; day 1 begins 24 hr later. Embryos were removed from the uterus at various stages of pregnancy, or collected immediately after normal delivery which usually occurs on day 19. Observations of the gross anatomy and skeletal system were made on Bouin's-fixed or alizarin red S-stained and cleared specimens. A total of 219 mutant embryos from 106 litters have been used in this study; normal litter mates were used as controls. Electron microscopy was done on 17 embryos, 10 controls and 7 chondrodystrophic litter mates, taken from six litters.¹

For routine histological studies, embryos were either fixed in alcoholic Bouin's and embedded in paraffin, or fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3, 20°C), postfixated in 2% osmium tetroxide in 0.1 M phosphate buffer, and flat-embedded in Epon 812 (Luft, 1961). Histochemical techniques used were the Gomori test for alkaline phosphatase, periodic acid-Schiff (PAS) test for glycogen and mucopolysaccharides, von Kossa and

¹ Preliminary observations were presented at the Federation Proceedings 54th Annual Meeting, 15 April 1970.

alizarin red S for calcified matrix and bone, and toluidine blue and alcian blue for acid mucopolysaccharide (Pearse, 1961).

From the Epon blocks, thin sections or sections at 2 μ were cut with a diamond knife on a Porter-Blum MT-1 microtome. Staining of thick sections mounted on glass slides was done with 1% toluidine blue in borax for light microscopy; staining of thin sections, mounted on 200-mesh uncoated grids or formvar-coated slotted grids, was done with 2% uranyl acetate and Reynold's lead citrate for examination with an RCA EMU-3 or a Philips-300 electron microscope. For some tissues, en bloc staining with uranyl acetate was performed. Other fixatives used for electron microscopy were osmium tetroxide (without prefixing in glutaraldehyde), 3% glutaraldehyde in 30% ethanol, followed by fixation in osmium tetroxide, or 6% glutaraldehyde containing 0.5% cetylpyridinium chloride followed by osmium tetroxide fixation. Survey electron micrographs were taken with the Philips-300 electron microscope in the scanning position. Increased specimen contrast was achieved by inserting a diffraction aperture 100 μ in diameter. In some instances electron micrographs were taken of mutant and normal specimens which had been embedded in the same tissue block to facilitate comparison and obviate such sources of artifact as differing section thickness. Use of the photographic mask in the Philips-300 enabled us to photograph both specimens on the same photographic plate, nullifying differences from variations in photographic processing.

Light micrographs were made with a Zeiss photomicroscope II with phase contrast or bright-field illumination; a 16, 25, or 40 \times planapochromat lens, optovar lens at 1.25; a VG9 green and 0.5 gray filter; and Kodak panatomic-X or Iford Pan-F film developed in microdol-X at 72°F for 12 min. All photographic prints were made on Kodabromide single weight, grades II-V paper.

OBSERVATIONS

Gross Description

Fig. 1 portrays the *normal* newborn mouse of the C57/BL/Fr strain. The mouth is closed. The limbs are in proportion to the body. *Mutants* at birth have a short snout, short mandible, protruding tongue, cleft palate, and disproportionately short limbs (Fig. 2). The distal portion of the hind limbs is rotated externally. The mutant animals die immediately after birth probably of asphyxia, apparently because the cartilage in the trachea does not have sufficient mechanical strength to maintain an open airway. The mutant animals can be distinguished first by gross ex-

amination on day 15 by the reduction in length of limbs, shortening of the jaw, and the presence of a cleft palate.

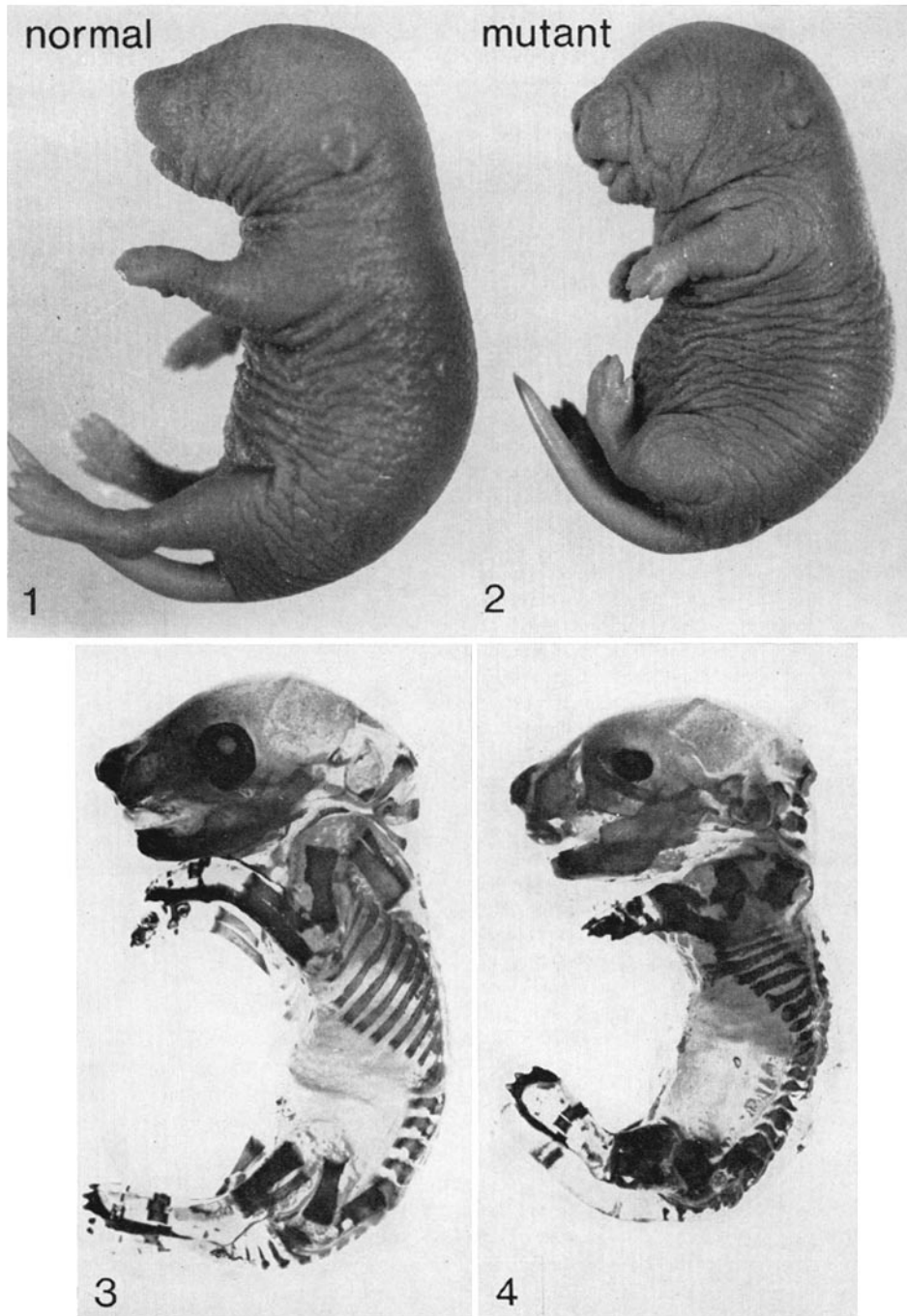
Skeletal System

The skeletal system of normal day-19 embryos comprises the bones of the axial skeleton (skull, vertebrae, ribs, and sternum) and the appendicular skeleton (limb bones, and pectoral and pelvic girdles). Alizarin staining of whole fetuses delineates the bony skeleton (Fig. 3). Bones of the limbs show diaphyseal ossification, and in the sternum six mineralized segments can be seen. In day-19 mutants endochondral long bones are shorter and wider than normal and have flared metaphyses (Fig. 4). Membrane bones, such as those of the skull and clavicle, appear normal. However, the reduced Meckel's cartilage apparently results in shortening of the mandible. Mineralization is reduced in cartilage of the sternum and phalanges as shown by alizarin staining. Unfixed epiphyseal cartilage from mutants was soft when touched with a probe.

Epiphyseal Cartilage

To facilitate comparison of mutant and normal epiphyseal cartilage, we define three zones of cells in the growth zone of developing long bones. The zone nearest the calcification front (nearest the metaphysis) will be designated the *hypertrophic zone*; the zone most removed from the calcification front is called the *reserve-cell zone*; and the area between, the *proliferation zone*.

The aggregates of mesenchymal cells and newly formed cartilage of the mutant appear normal in size up to day 13. As limb development proceeds, differences become apparent in sections for light microscopy, e.g., at day 17 the cartilage ends of the mutant femur are enlarged relative to the amount of newly formed diaphyseal bone, and 10 μ thick sections show large areas devoid of chondrocytes, mainly in the proliferation zone. The axial lengths of mutant and normal epiphyseal cartilage (measured from articular surface to metaphysis of day-19 fetuses) are similar, whereas the diameter of mutant cartilage is much greater than that of normal animals. Accurate measurements of the three zones of cartilage in the mutant could not be made because of lack of delineation between any two zones, particularly during the late stages of development.



FIGURES 1 and 2 Bouin-fixed normal (Fig. 1) and mutant (Fig. 2) day-19 fetuses. The mutant has disproportionately shortened limbs, a dorsoventrally shortened head, a protruding tongue, and a cleft palate.

FIGURES 3 and 4 Alizarin red S-stained and cleared specimens, day 19. Short, wide long-bones with flared metaphyses are seen in the mutant's limbs and ribs. Microphthalmia, shown in the mutant, is not part of the syndrome but occurs spontaneously in the C57/BL/Fr inbred strain of mice.

The long axes of *normal* cells near the perichondrium lie parallel to the long axis of the bone; the cells in the central area of the cartilage appear round. In the proliferation zone the cartilage cells are spindle- or wedge-shaped with their long axes lying perpendicular to the long axis of the bone. The polar axes of cells which undergo mitotic division in the proliferation zone are also oriented perpendicular to the long axis of the bone. *Mutant* chondrocytes in the reserve-cell zone of young limb bones appear normal in number, size, shape, and distribution (Fig. 6), but at later stages of development they become quite variable in size and shape; in many cases, they are not distinguishable from proliferating chondrocytes. Cells of the proliferation zone are flattened, irregular in shape, and generally arranged end-to-end (laterally) rather than aligned into vertical columns. The displacement of daughter cell nuclei at telophase occurs in a direction perpendicular to the long axis of the bone, which is the same for normal. Proliferating and hypertrophic chondrocytes show an accumulation of PAS-positive, diastase-removable material, as well as alkaline-phosphatase activity.

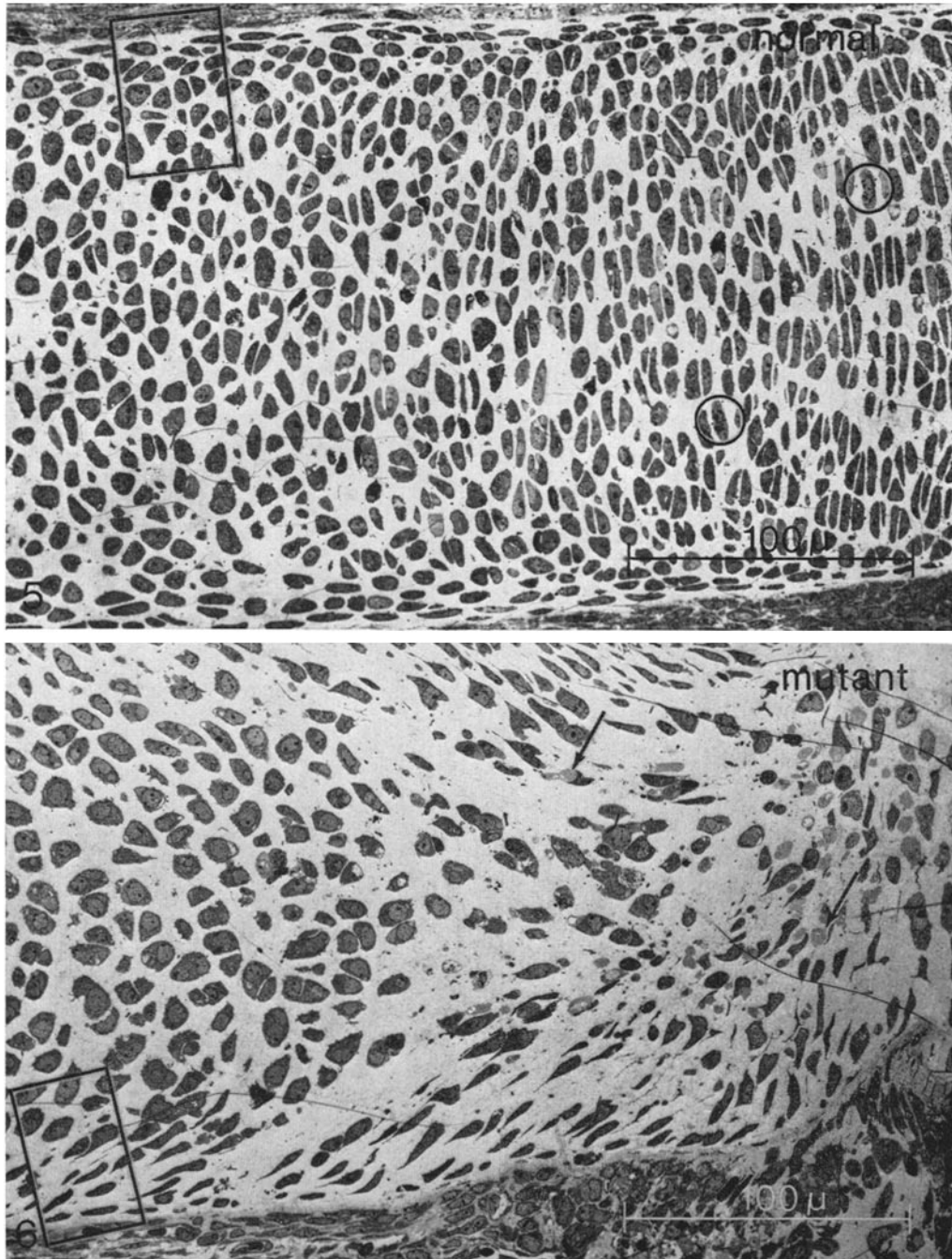
Normal matrix of all hyaline cartilage appears blue after staining with hematoxylin, is metachromatic after staining with toluidine blue, and is pink after PAS staining. The matrix has a uniform ground-glass appearance except in the immediate vicinity of the chondrocytes where the lacunae do not stain. Little difference in staining was observed between tissues fixed in formalin and tissues fixed in formalin-alcohol. The matrix of all hyaline cartilage from day 13–19 *mutant* mice is heterogeneous, vesicular, and stains lighter than the normal with toluidine blue, alcian blue, and PAS (both before and after glycogen digestion with diastase) when formalin fixatives are used. These differences in staining remain, but are less pronounced, when alcohol-containing fixatives are used.

Trabeculae, which stain with alizarin or von Kossa's stain, extend from the lower hypertrophic zone into the metaphysis of *normal* long bones. In the *mutant*, calcified bone surrounds the lower part of the epiphysis and extends up the sides of this bulbous mass of cartilage. In the diaphysis, there is an abundance of calcified bone with little formation of a marrow cavity. The trabeculae are much wider in diameter than normal and show a considerable amount of fusion. Apparently most bone formation occurs at the periosteum, with

relatively little being contributed by matrix calcification.

Electron Microscope Observations of Rib Cartilage at the Costochondral Junction

The three zones of cartilage can be identified at the costochondral junction in normal day-17 mouse ribs. A survey electron micrograph through the reserve-cell zone and the proliferation zone of a normal rib is shown in Fig. 5. Chondrocytes of the reserve-cell zone are generally round but vary in size, shape, and intensity of staining. Chondrocytes of the proliferation zone are usually flattened wedge-shaped and are arranged into longitudinal columns. Mitotic cells with laterally oriented centrioles were frequently observed (Fig. 5). Chondrocytes at the periphery, adjacent to the perichondrium, are oriented with their long axes parallel to the long axis of the bone. In mutant ribs, the reserve-cell zone is readily distinguishable from the proliferation and hypertrophic zones, but a clear distinction between the latter two zones cannot be made. A survey electron micrograph of the reserve-cell and proliferation zones of mutant costal cartilage is shown in Fig. 6. Chondrocytes of the reserve-cell zone from mutant mouse ribs do not differ in number, size, shape, or distribution from those in equivalent areas in the normal. By inspection, it is seen that the amount of matrix between cells in this zone does not differ in the normal and mutant cartilage, so the density of cells in the tissue appears the same. Mutant reserve-cell chondrocytes possess normal-appearing cytoplasmic and nuclear components such as rough endoplasmic reticulum, Golgi complexes, mitochondria, and nucleoli (Figs. 7, 8). In contrast to their column formation at the costochondral junction in the normal, chondrocytes in mutants are not aligned in vertical columns. Instead, oblique arrays of chondrocytes appear in the proliferation zone, and there is a paucity of chondrocytes in mutant compared to normal cartilage. Cells in this zone are not spindle-shaped, but ameboid or fibroblastic in appearance; several cells are in contact with each other through long cytoplasmic processes. In an area comparable to the normal hypertrophic zone, characterized by the swelling of chondrocytes, there is a paucity of chondrocytes, and cellular debris is scattered throughout the matrix. Darkly stained, pycnotic cells sometimes occur in clusters, and were observed in all three zones of mutant cartilage.



FIGURES 5 and 6 Survey electron micrographs of day 17 normal (Fig. 5) and mutant rib cartilage (Fig. 6) at the costochondral junction, reserve-cell zone (left) and proliferation zone (right). The most noticeable difference occurs at the proliferation zone where there is an absence of columns in the mutant. Laterally dividing chondrocytes are circled in the normal proliferation zone. Each micrograph is a montage prepared from two photographic plates taken with the Philips-300 electron microscope in the scanning position. The *inset* squares represent areas from which following electron micrographs were taken. $\times 400$.

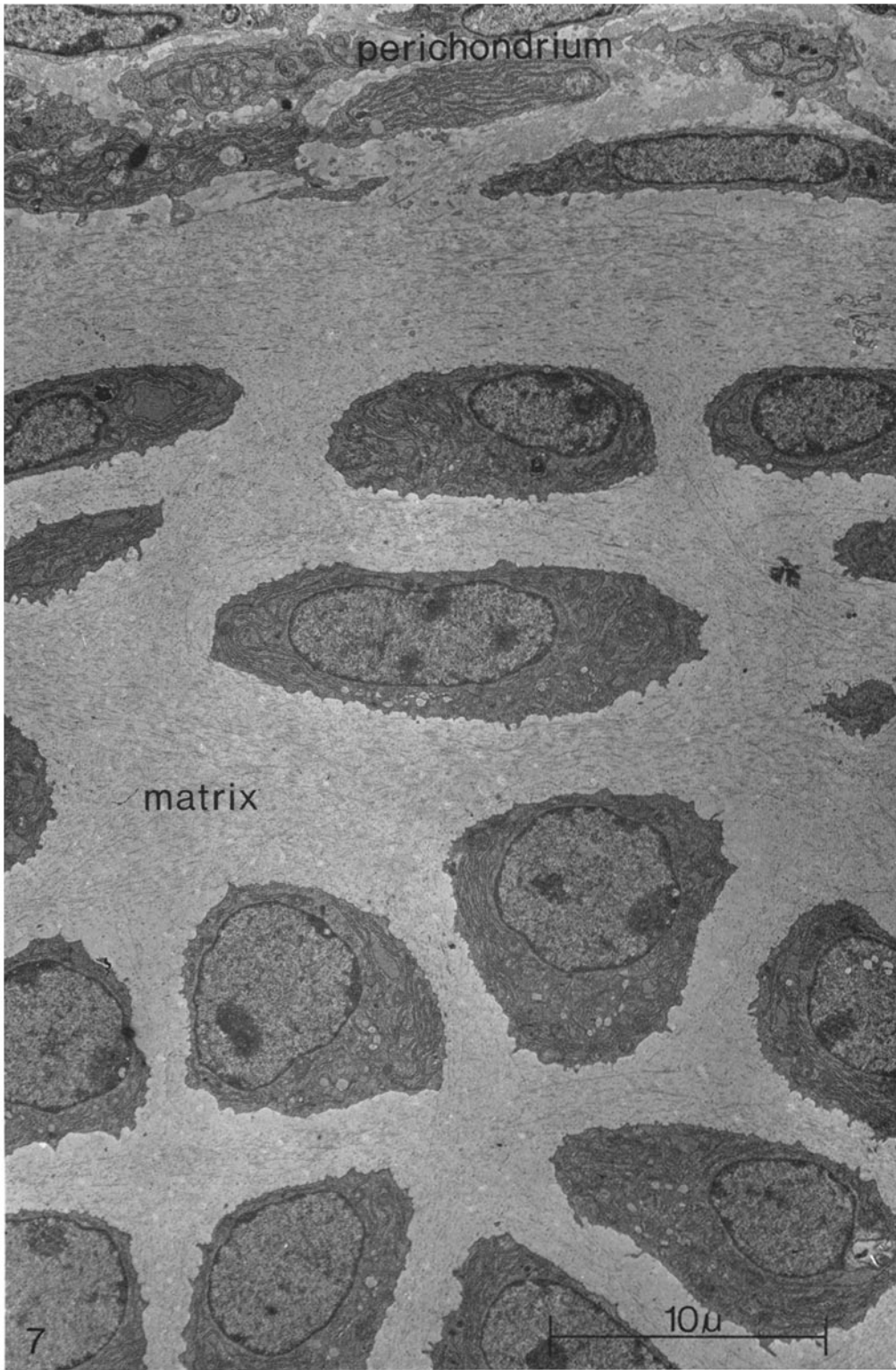


FIGURE 7 Electron micrograph of normal rib cartilage, sectioned through the reserve-cell zone. Chondrocytes are surrounded by numerous fine collagen fibrils which are embedded in an amorphous ground substance. $\times 4000$.

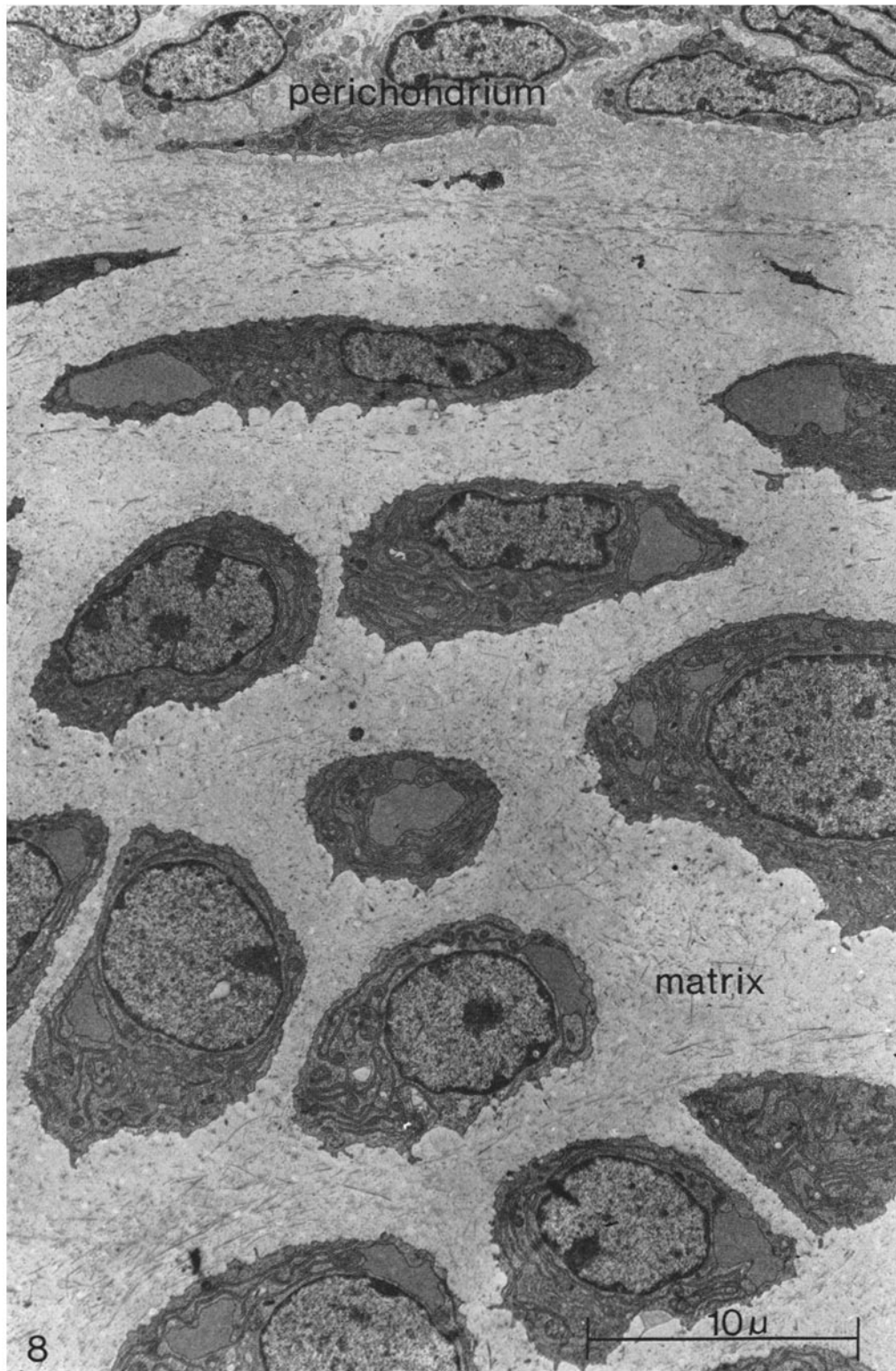


FIGURE 8 Electron micrograph of mutant rib cartilage sectioned through the reserve-cell zone shows normal-appearing chondrocytes. The matrix lacks the fine feltwork appearance observed in normal matrix. $\times 4000$.

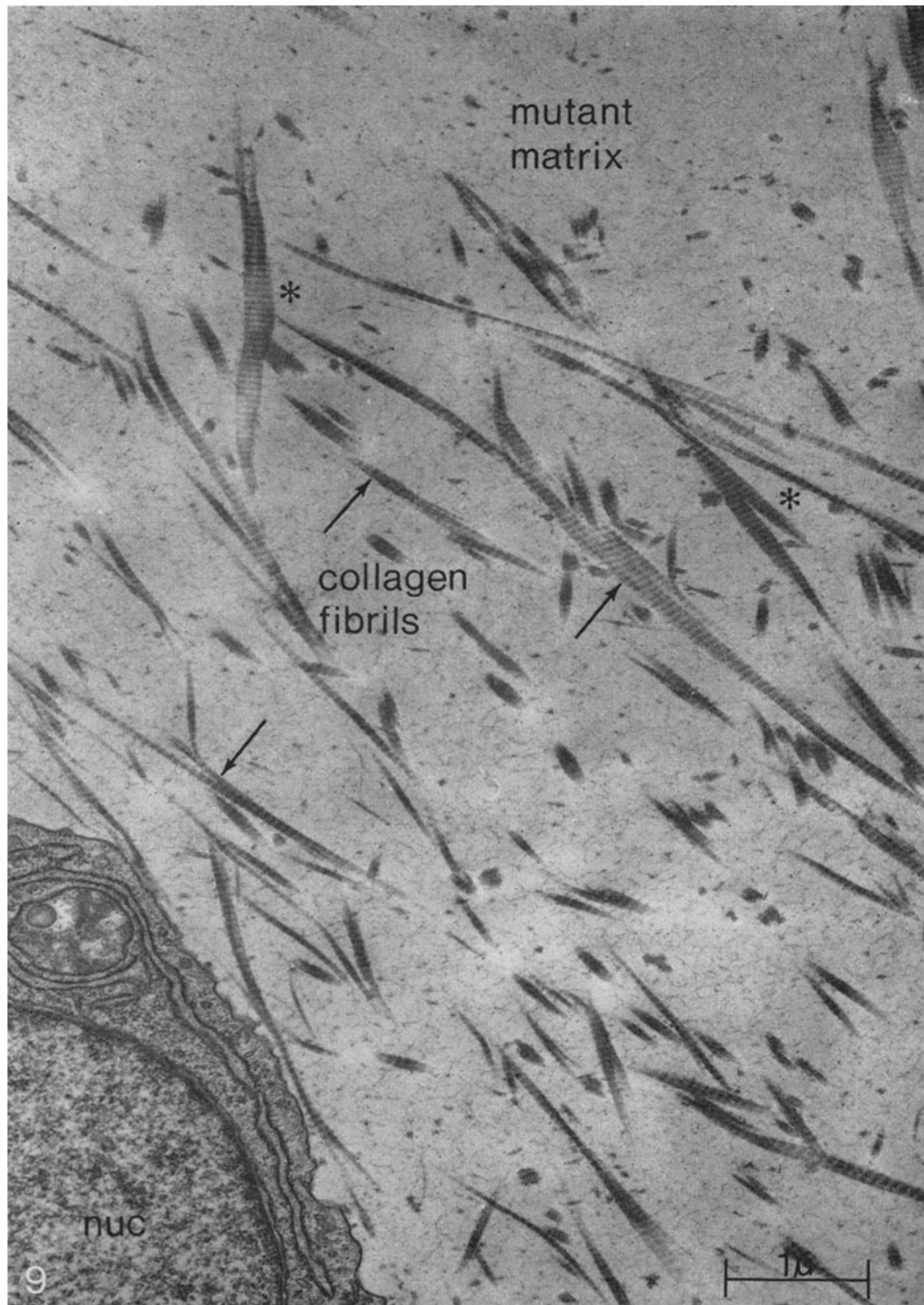


FIGURE 9 Electron micrograph of mutant costal cartilage sectioned through the proliferation zone. A chondrocyte is surrounded by unusually large collagen fibrils (up to 2000 Å in diameter) which possess the native banded structure (arrows). Asterisks point out places where collagen fibrils have anastomosed in register. $\times 20,000$.

Normal cartilage matrix in the reserve-cell zone viewed with the electron microscope shows many fibrils of small and uniform diameter (250 A); the fibrils seldom show any repeating transverse banded structure (Fig 7). The fine feltwork appearance of the matrix in the reserve-cell zone can be seen even in the area of degenerating hypertrophic chondrocytes. Mutant matrix from rib cartilage of the reserve-cell zone shows a paucity of 250 A fibrils, but many large collagen fibrils are present and have a well defined transverse banded structure (Figs. 8, 9). Unlike the normal cartilage, which shows a uniform matrix in all zones, mutant cartilage has collagen fibrils up to 2000 A in diameter in its matrix in the proliferation zone (Fig. 9). At higher magnification, collagen fibrils with "native" banding are shown.

Summary of Observations

The mutant gene *chondrodysplasia* in the mouse, which causes short limbs, cleft palate, and death by asphyxia, appears to act primarily on chondrogenesis and endochondral bone formation. Differences in the array and disposition of cells are apparent in hyaline cartilage. Generally speaking, in the growth zone of mutants there is an absence of longitudinal columns in the proliferation and hypertrophic zones. Instead, several flattened chondrocytes are shown in arrays oblique to or perpendicular to the long axis of the bone. Matrix of all types of mutant cartilage studied showed reduced metachromatic staining and collagen fibrils of wide diameter which taper or anastomose and have a 640 A periodicity.

DISCUSSION

Chondrodystrophy in mice provides a model system for the study of mechanisms which affect the development of cartilage. The fact that the mutant gene is recessive suggests that it may act by altering an enzyme necessary for normal differentiation of cartilage. The observations presented here demonstrate differences between the matrices of normal and mutant cartilage. This discussion attempts to relate these differences to what is already known about chondrocytes and cartilage matrix, and to suggest mechanisms whereby the mutant gene may modify the development of cartilage. In addition, observations on mutant cartilage shed new light on the interrelationship

between collagen fibril formation and the presence of mucopolysaccharide.

The fine structure of normal and experimentally altered cartilage has been the subject of numerous reports (Robinson and Cameron, 1956; Sheldon and Robinson, 1958, 1960; Godman and Porter, 1960; Sheldon and Kimball, 1962; Revel and Hay, 1963; Fewer et al., 1964; Glauert et al., 1969).

It is believed that long unbranched chains of disaccharides are secreted by the cartilage cells but that they do not lie freely in the extracellular space. Rather, they are covalently linked to protein. While the characterization of the protein remains incomplete, some data are available on the nature of the polysaccharide-protein links (Muir, 1969). It is believed that the protein-polysaccharide (i.e., polymers composed of acid mucopolysaccharides [AMPS] and protein) complex assumes a very large molecular weight (Mathews and Lozaityte, 1958; Luscombe and Phelps, 1967). Recent studies with the electron microscope suggest that, after appropriate fixation, large noncollagenous particles in the cartilage matrix contain protein-polysaccharide complexes (Anderson, 1967). The possible effect of the gene *chondrodysplasia* on other carbohydrate-rich polymers such as polysaccharides and neutral muco-substances (Spicer et al., 1965) will not be discussed here.

It has been shown repeatedly that collagen in cartilage, unlike collagen in most connective tissues, does not aggregate into native fibrils with an average diameter of about 700 A nor does it usually show the uniform 640 A banded pattern. In a variety of species, cartilage matrix from several sites has been shown to appear as a fine feltwork of fibrils embedded in an amorphous background when examined in thin sections after routine preparation for electron microscopy (Robinson and Cameron, 1956; Sheldon, 1964).

In the present study, cartilage of unaffected litter mates demonstrates these delicate filaments which clearly differ from the large fibrils of other connective tissues (Fig. 9). The explanations which have been given for this appearance usually invoke the role played in cartilage by the presence of high molecular weight protein-polysaccharides containing mainly chondroitin 4- and 6-sulfates. The metachromasia seen in the light microscope with appropriate stains is caused by the presence of acidic, high-molecular weight compounds. Recent studies on the interrelationship of the

chondroitin-sulfate proteins and collagen in cartilage have led to the conclusion that the assembly and growth of collagen fibrils in vitro and in vivo are altered by the presence of AMPS (Mathews and Decker, 1968; Toole and Lowther, 1968; Wasteson and Obrink, 1968; Jackson and Bentley, 1968). The initiation of collagen nucleation is enhanced by the presence of AMPS, but subsequent growth of the fibrils is inhibited by their presence. It is likely that electrostatic binding of acidic, negatively charged protein-polysaccharide to collagen plays an important role in the structure and function of connective-tissue matrix. One function of the organic component of cartilage matrix is that of regulating mineralization at the hypertrophic zone of growth cartilage (Sheldon and Robinson, 1961; Hirschman and Dziewiatkowski, 1966; Bonucci, 1967; Matukas and Krikos, 1968; Bowness, 1968; Hirschman and Silverstein, 1968; and Woodward and Davidson, 1968).

The present study has demonstrated in the mutant a reduction in metachromasia and a lack of staining with PAS, alcian blue, and von Kossa's of the cartilage matrix. In addition, electron microscopy has demonstrated the presence of unusually large, native collagen fibrils in the matrix (Fig. 9). These fibrils exceed in diameter the typical collagen fibril found in skin or tendon, (Cox and Grant, 1969; Grant, Cox, and Horne, 1965), and were observed in aggregates measuring up to 2000 Å in diameter. These findings lead to the conclusion that there is a paucity in the mutant matrix of those substances, particularly AMPS, which are deemed responsible for the appearance of normal cartilage matrix. If this is true, it follows that extra sites on the tropocollagen polymer would be available for binding to other tropocollagen units. This would allow growth into large anastomosing fibrils. The absence of mineralization at the hypertrophic zone of mutants further suggests that the matrix of mutants is deficient in AMPS.

What are some of the possible explanations for a lack of AMPS in the mutant matrix? First, the AMPS moiety of cartilage matrix may have been removed at some time during the process of preparing the tissue for examination (Engfeldt and Hjertquist, 1967; Korhonen and Lindholm, 1969). This explanation for the observations cannot be excluded despite the use of different methods for fixation in the present study. Should this be the case, the basic defect in the mutant matrix could be attributed to a different bonding of AMPS to

protein than occurs in normal tissue or to a different solubility of the mutant AMPS.

A second possibility is that there is quantitatively less, but still normal, polysaccharide in the mutant matrix. Possible defects for which to look are: (a) reduced synthesis of the polysaccharides either in the embryo as a whole or in the chondrocytes in particular, (b) defects in the secretion of polysaccharides by the chondrocyte, or, (c) an increased rate of degradation of the polysaccharide as soon as it has been synthesized and/or secreted. Instances of these possibilities have been described. For example, Thomas (1956) showed that papain hydrolyzes the chondroitin-sulfate-protein bonds in cartilage, and that chondroitin sulfate is excreted in the urine while the cartilage collapses and loses its metachromasia. Follis (1958) reported on the removal of chondroitin sulfate by separate treatment with CaCl_2 , hyaluronidase, trypsin, and papain. Decreased metachromasia has been reported in human achondroplasia (Morrii et al., 1967), and in other chondrodystrophies (Bona et al., 1967; Mathews, 1967; Ford et al., 1968), and can be produced in cell and organ culture (Caplan et al., 1968; McCallum and Arbuthnott, 1969; Levenson, 1969). Lysosomal cathepsin D is known to degrade organic matrix of cartilage and release polysaccharides (Weston et al., 1969).

Possible Effects of Defects in Matrix on the Dynamics of Development

We have observed that column formation does not occur in the mutant proliferation zone. We postulate that the matrix is not structurally firm enough to promote the alignment of daughter cells after mitosis; rather, we suggest that mutant matrix permits daughter cells to move in any direction. This suggestion arises from the histological observations and from the observation that unfixed cartilage is unusually soft. Histologically, mitotic figures were usually observed with their polar axes oriented in the direction of the cellular arrays. That is, mitotic division took place laterally if the cells were arranged into lateral arrays, and obliquely if the cells were arrayed obliquely. Cell division and separation in the mutant are schematically represented in comparison with normal column formation (Fig. 10). Failure of column formation in the growth zone of mutants results in decreased linear growth of long bones (Fig. 11). Appositional growth at the perichondrium and periosteum along with reduced inter-

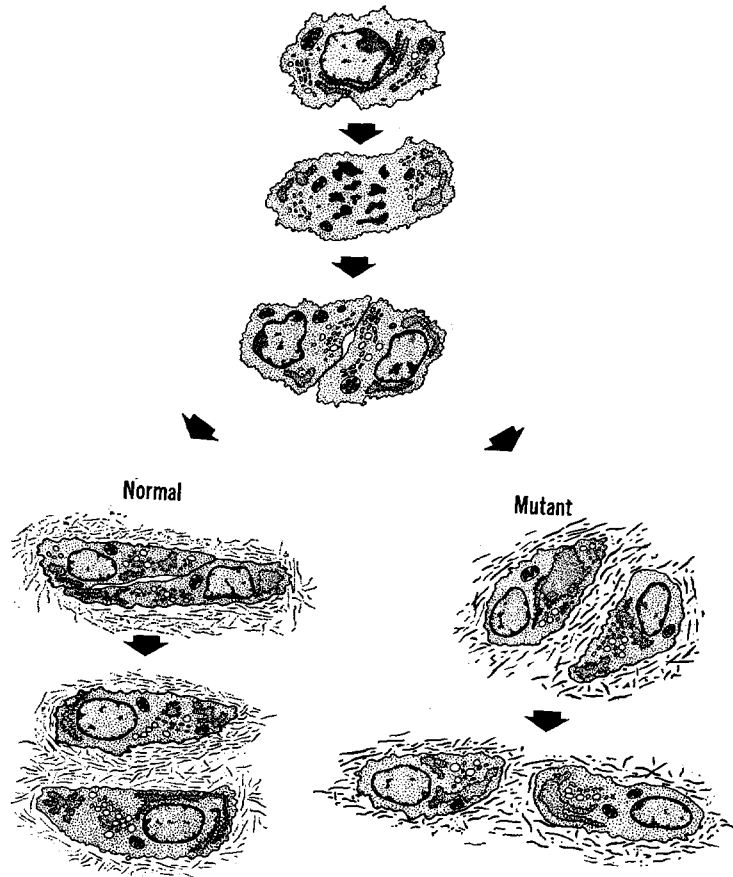


FIGURE 10 This drawing shows dividing chondrocytes of the proliferation zone. Normally, chondrocytes separate into longitudinal columns. Mutant chondrocytes are portrayed separating laterally after mitosis.

stitial growth results in short, wide bones. A similar growth pattern has been postulated (Rubin, 1964) in human diastrophic dwarfism (Lamy and Maroteaux, 1960; Amuso, 1968) and other forms of chondrodystrophy (Rang, 1969).

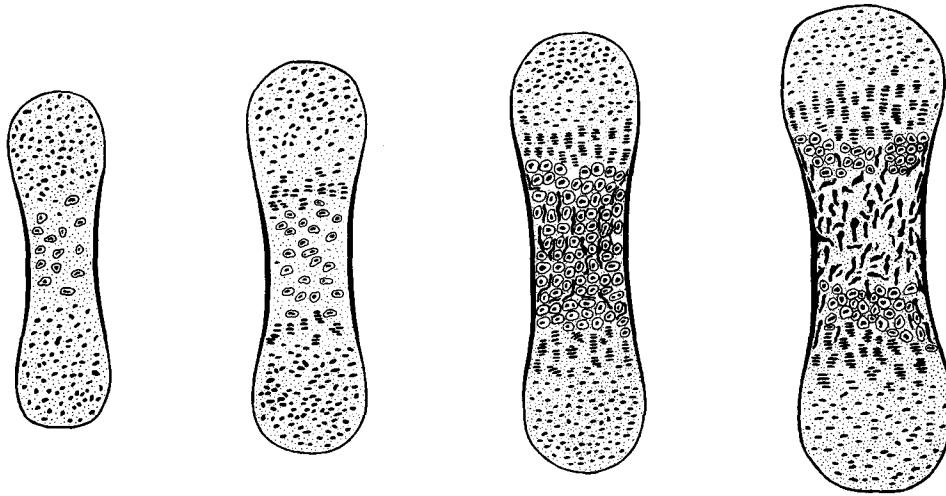
We propose that a disturbance in the matrix of mutant cartilage interferes with interstitial growth of bones of the endochondral skeleton. The matrix in the reserve-cell zone of costal cartilage appears to be deficient in ground substance despite the fact that chondrocytes of this zone appear normal. That there is a deficiency in the "proliferation zone" is suggested by the reduction in staining of the matrix of this zone with toluidine blue and other stains. In vitro studies have shown that chondrocytes which lose their normal shape and become migratory and ameboid do not synthesize proteins and other products of the matrix (Holtzer and

Abbott, 1968; Chacko et al., 1969). A paucity of chondrocytes in this zone also suggests that there is reduced matrix production. The reduction in number of chondrocytes in this zone, noted particularly in ribs, could result from reduced cell division or from increased cell death, or both.

It is postulated that, in addition to impairment of growth of long bones, the occurrence of other anomalies also results from abnormal development of cartilage. Failure of palate closure may result, as suggested by Fraser (1969), from reduction in forward growth of the mandible due to retarded growth of Meckel's cartilage. Asphyxia probably results from collapse of the defective tracheal cartilage.

The occurrence of a mutation in mice which affects chondrogenesis provides a model which adds new insight into the biology of normal

Normal



Mutant

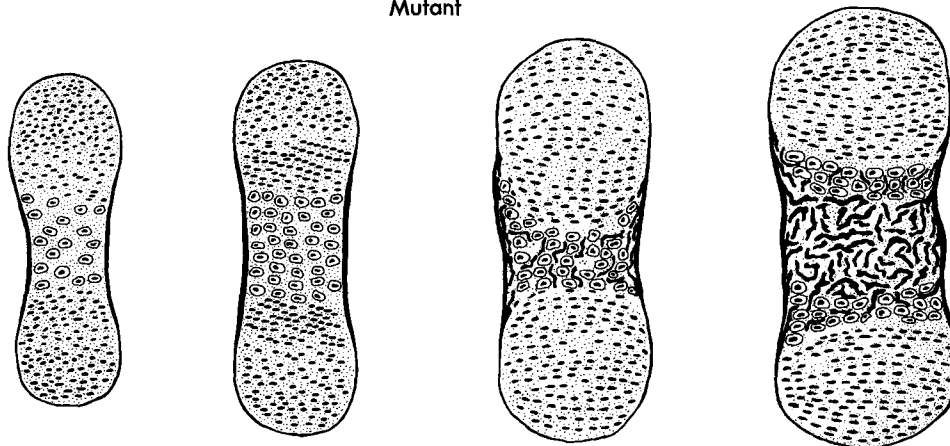


FIGURE 11 Schematic representation of growth of normal and mutant long bones (gestational days 13-18). At day 18 the mutation results in long bones which are shorter and wider than normal. Column formation, shown in the normal at early developmental stages, does not occur in the mutant proliferation and hypertrophic zones.

cartilage and bone. Further studies on the nature of the defect in cartilage matrix and on the intermediary metabolism of the chondrocytes may define the locus of action of the gene *cho*.

The authors are grateful to Miss C. Claudia Ferguson for her help in many phases of the electron microscopy and thank Brian Snow for the drawings.

Supported by National Institutes of Health Training Grant No. GM00837 and Fellowship GM421201 and in part by Medical Research Council of Canada Grant Nos. MA3296 and MT1584.

Received for publication 8 June 1970, and in revised form 24 August 1970.

BIBLIOGRAPHY

- AMUSO, S. J. 1968. Diastrophic dwarfism. *J. Bone Joint Surg.* 50 A(1):113.
- ANDERSON, H. C. 1967. Electron microscopic studies of induced cartilage development and calcification. *J. C. Biol.* 35:81.
- BONA, C., V. STANESCU, and D. STREJA. 1967. Differential regional distribution of mucopolysaccharides in the human epiphyseal cartilage matrix in normal and pathologic conditions. *Virchows Arch. Pathol. Anat. Physiol.* 342:274.
- BONUCCI, E. 1967. Fine structure of early cartilage calcification. *J. Ultrastruct. Res.* 20:33.

- BOWNESS, J. M. 1968. Present concepts of the role of ground substance in calcification. *Clin. Orthop.* 59:233.
- CAPLAN, A. I., E. ZWILLING, and N. O. KAPLAN. 3-Acetyl-pyridine: Effects *in vitro* related to teratogenic activity in chicken embryos. *Science (Washington)*. 160:1009.
- CHACKO, S., S. HOLTZER, and H. HOLTZER. 1969. Suppression of chondrogenic expression in mixtures of normal chondrocytes and BUDR-altered chondrocytes grown *in vitro*. *Biochem. Biophys. Res. Commun.* 34:183.
- COX, R. W., and R. A. GRANT. 1969. The structure of the collagen fibril. *Clin. Orthop.* 67:172.
- DICKIE, M. M. 1961. *Mouse News Letter*. 25:36.
- ENGELFELD, B., and S. O. HJERTQVIST. 1967. The effect of various fixatives on the preservation of acid glycosaminoglycans in tissues. *Acta Pathol. Microbiol. Scand.* 71:219.
- FEWER, D., J. THREADGOLD, and H. SHELDON. 1964. Studies on cartilage. V. EM observations on the autoradiographic localization of S^{35} in cells and matrix. *J. Ultrastruct. Res.* 11:166.
- FITCH, N. 1961. A mutation in mice producing dwarfism, brachycephaly, cleft and micromelia. *J. Morphol.* 109:141.
- FOLLIS, R. H., JR. 1958. Observations on the structure and metabolism of epiphyseal cartilage. *Bull. N.Y. Acad. Med.* 34:689.
- FORD, J. K., E. J. EYRING, and C. E. ANDERSON. 1968. Thallium chondrodystrophy in chick embryos. An histological and biochemical investigation. *J. Bone Joint Surg.* 50 A:687.
- FRASER, F. C. 1969. Gene-environment interactions in production of cleft palate. In *Methods for Teratological Studies in Experimental Animals and Man*. H. Nishimura and J. R. Miller, editors. Igaku Shoin Ltd., Tokyo.
- GLAUERT, A. M., H. B. FELL, and J. T. DINGLE. 1969. Endocytosis of sugars in embryonic skeletal tissues in organ culture. II. Effect of sucrose on cellular fine structure. *J. Cell Sci.* 4(1):105.
- GLUECKSOHN-WAELSCH, S., D. HAGEDORN, and B. SISKEN. 1956. Genetics and morphology of a recessive mutant in the house mouse affecting head and limb skeleton. *J. Morphol.* 99(3):465.
- GODMAN, G. C., and K. R. PORTER. 1960. Chondrogenesis studied with the electron microscope. *J. Biophys. Biochem. Cytol.* 8:719.
- GRANT, R. A., R. W. COX, and R. W. HORNE. 1965. The structure and assembly of collagen fibrils. II. An electron microscope study of cross-linked collagen. *J. Roy. Microsc. Soc.* 87:143.
- HIRSCHMAN, A., and D. D. DZIEWIATKOWSKI. 1966. Utilizing fluorescence-labeled antibodies to protein polysaccharide complex from rat calf cartilage. *Science (Washington)*. 154:393.
- HIRSCHMAN, A., and D. SILVERSTEIN. 1968. The effect of proteolytic enzymes and hyaluronidase *in vitro* on the calcification mechanism of epiphyseal cartilage. *Proc. Soc. Exp. Biol. Med.* 129:675.
- HOLTZER, H., and J. ABBOTT. 1968. Oscillations of the chondrogenic phenotype *in vitro*. In *The Stability of the Differential State*. H. Ursprung, editor. Springer-Verlag New York Inc., New York.
- JACKSON, D. S., and J. P. BENTLEY. 1968. Collagen-glycosaminoglycan interactions. In *Treatise on Collagen, Biology of Collagen*. B. S. Gould, editor. Academic Press Inc. Ltd., London. 2 A:189.
- KORHONEN, L. K., and K. LINDHOLM. 1969. Effect of histological fixation on carbohydrate-rich tissue compounds and their periodate consumption. *Histochemie*. 18:87.
- LAMY, M., and P. MAROTEAUX. 1960. Le Nanisme Diastrophique. *La Presse Medicale*. 68 (52):1977.
- LANE, P. W., and M. M. DICKIE. 1968. Three recessive mutations producing disproportionate dwarfing in mice. *J. Hered.* 59(5):300.
- LEVENSON, G. E. 1969. The effect of ascorbic acid on monolayer cultures of three types of chondrocytes. *Exp. Cell Res.* 55:225.
- LUFT, J. H. 1961. Improvements in epoxy resin embedding methods. *J. Biophys. Biochem. Cytol.* 9:409.
- LUSCOMBE, M., and C. F. PHELPS. 1967. Action of degradative enzymes on the light fraction of bovine septa polysaccharide. *Biochem. J.* 103:103.
- MATHEWS, M. B. 1967. Chondroitin sulfate and collagen in inherited skeletal defects of chickens. *Nature (London)*. 213:1255.
- MATHEWS, M. B., and L. DECKER. 1968. The effect of acid mucopolysaccharides and mucopolysaccharide proteins on fibril formation from collagen solutions. *Biochem. J.* 109:517.
- MATHEWS, M. B., and I. LOZAITYTE. 1958. Sodium chondroitin sulfate-protein complexes of cartilage. I. Molecular weight and shape. *Arch. Biochem.* 74:158.
- MATUKAS, V. J., and G. A. KRIKOS. 1968. Evidence for changes in protein polysaccharide associated with the onset of calcification in cartilage. *J. Cell Biol.* 39(1):43.
- MCCALLUM, H. M., and J. P. ARBUTHNOTT. 1969. The inhibition of cartilage matrix production by glutamyl-aminoacetonitrile *in vitro*. *Exp. Mol. Pathol.* 11:232.
- MORRIS, S., R. TAKADA, T. KANKO, and K. KAIDA. 1967. An autopsy case of achondroplasia in one of binovular twins of different sex. *Acta. Pathol. Jap.* 17(2):127.
- MUIR, H. 1969. The structure and metabolism of mucopolysaccharides (glycosaminoglycans) and the problem of mucopolysaccharidoses. *Amer. J. Med.* 47:673.

- PEARSE, A. G. E. 1961. Histochemistry. Theoretical and Applied. J. and A. Churchill Ltd., London.
- RANG, M. 1969. The growth plate and its disorders. E. and S. Livingstone Ltd., Edinburgh.
- REVEL, J. P., and E. D. HAY. 1963. An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. *Z. Zellforsch. Mikrosk. Anat.* 61:110.
- ROBINSON, R. A., and D. A. CAMERON. 1956. Electron microscopy of cartilage and bone matrix at the distal epiphyseal line of the femur in the newborn infant. *J. Biophys. Biochem. Cytol.* 2(4):253.
- RUBIN, P. 1964. Dynamic classification of bone dysplasias. Year Book Medical Publishers Inc., Chicago.
- SHELDON, H. 1964. Cartilage. In Electron Microscope. S. Kurtz, anatomy editor. Academic Press Inc., New York.
- SHELDON, H., and F. B. KIMBALL. 1962. Studies on cartilage. III. The occurrence of collagen within vacuoles of the Golgi apparatus. *J. Cell Biol.* 12:399.
- SHELDON, H., and R. A. ROBINSON. 1958. Studies on cartilage: Electron microscope observations on normal rabbit ear cartilage. *J. Biophys. Biochem. Cytol.* 4:401.
- SHELDON, H., and R. A. ROBINSON. 1960. Studies on cartilage. II. Electron microscope observations on rabbit ear cartilage following the administration of papain. *J. Biophys. Biochem. Cytol.* 8:151.
- SHELDON, H., and R. A. ROBINSON. 1961. Studies on rickets. I. The fine structure of uncalcified bone matrix in experimental rickets. *Z. Zellforsch. Mikrosk. Anat.* 53:671.
- SISKEN, B., and S. GLUECKSOHN-WAELSCH. 1959. A developmental study of the mutation "phocomelia" in the mouse. *J. Exp. Zool.* 142:623.
- SPICER, S. S., T. J. LEPPi, and P. J. STOWARD. 1965. Suggestions for a histochemical terminology of carbohydrate-rich tissue components. *J. Histochem. Cytochem.* 13:599.
- THOMAS, L. 1956. Reversible collapse of rabbit ears after recovery by cortisone. *J. Exp. Med.* 104:245.
- TOOLE, B. P., and D. A. LOWTHER. 1968. The effect of chondroitin sulphate-protein on the formation of collagen fibrils *in vitro*. *Biochem. J.* 109:857.
- WASTESON, A., and B. OBRINK. 1968. Demonstration of an interaction between collagen and chondroitin sulphate. *Biochim. Biophys. Acta.* 170:201.
- WESTON, P. D., A. J. BARRETT, and J. T. DINGLE. 1969. Specific inhibition of cartilage breakdown. *Nature (London).* 222:285.
- WOODWARD, C., and E. A. DAVIDSON. 1968. Structure-function relationships of protein polysaccharide complexes specific ion-binding properties. *Proc. Nat. Acad. Sci. U.S.A.* 60:201.