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Nonexpression of Cartilage Type II Collagen in a Case of Langer-Saldino Achondrogenesis

DAVID R. EYRE,[1,2] MELISSA P. UPTON,[3,4] FREDERIC D. SHAPIRO,[1] ROBERT H. WILKINSON,[5] AND GORDON F. VAWTER[3]

SUMMARY

A lethal short-limbed dwarfism was diagnosed at autopsy as the Langer-Saldino variant of achondrogenesis by radiological, histological, and gross pathological criteria. Cartilage was obtained for biochemical and ultrastructural analyses from the ends of long bones, from ribs and from a scapula of the newborn infant. At all sites, it had an abnormal gelatinous texture and translucent appearance. Biochemical analyses of the cartilages to identify pepsin-solubilized collagen α-chains and collagen-specific CNBr-peptides failed to detect type II collagen at any site where it would normally be the main constituent. Instead, type I was the predominant collagen present. However, three cartilage-specific minor collagen chains identified as 1α, 2α, and 3α chains by their electrophoretic mobility were present at about 10% of the total collagen. Cartilage-specific proteoglycans also appeared to be abundant in the tissue judging by its high hexosamine content and high ratio of galactosamine to glucosamine. The findings indicate that a chondrocyte phenotype had differentiated but without the expression of type II collagen. In addition to the skeletal abnormalities, the severe pulmonary hypoplasia was also felt to be directly related to the underlying pathology in collagen expression. The term chondrogenesis imperfecta rather than achondrogenesis should be considered a more accurate description of this and related conditions.

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[1] Laboratory for Skeletal Disorders, Harvard Medical School and Children's Hospital, Boston, MA 02115.

[2] Present address: Orthopaedic Research Laboratory, RK-10, University of Washington, School of Medicine, Seattle, WA 98195. Address from which to request reprints.

[3] Department of Pathology, Harvard Medical School and Children's Hospital.

[4] Present address: National Cancer Center Research Institute, Tsukiji 5-1-1, Chuo-ku, Tokyo 104, Japan.

[5] Department of Radiology, Harvard Medical School and Children's Hospital.

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INTRODUCTION

A hundred or more different kinds of inborn skeletal dysplasia can be defined clinically, a large proportion of which are chondrodystrophies [1–3]. None of these has yet been traced to a specific defect in a structural macromolecule of cartilage matrix, although such defects have long been suspected. In osteogenesis imperfecta, a heterogeneous group of genetic mutations that affect the structure or expression of the proα chains of type I collagen have now been identified and are probably the primary basis of this inborn disease of connective tissue [4, 5]. Mutations in genes coding for type I and III collagens or for enzymes involved in their posttranslational biosynthesis also lie behind several of the subtypes of Ehlers-Danlos syndrome [4, 5]. As yet genetic mutations that specifically affect the expression of type II collagen of cartilage have not been discovered. They are anticipated among the large collection of inborn chondrodystrophies.

Achondrogenesis is a lethal newborn dwarfing syndrome that has been classified into two main subtypes, achondrogenesis type I or Ia (Parenti-Fraccaro variant [2, 6–8]) and type II or Ib (Langer-Saldino variant [2, 9–11]). The former includes cases that exhibit defective ossification of endochondral and membrane bones. In the latter, membrane bone formation appears to be normal. In both types, infants are often premature, are stillborn, or die soon after birth and demonstrate pulmonary hypoplasia and diminutive thoracic volume [12, 13]. These cases are distinct from the nonlethal Grebe-Quelce-Salgado short-limbed dysplasia, reported for a large Brazilian kinship [14, 15] and termed achondrogenesis II in some reviews [16]; hence, the variable nomenclature. The Langer-Saldino variant of achondrogenesis is an extremely rare form of short-limbed dwarfism characterized by ossification defects of vertebral bodies, ilia, ischium, and pubis, and by metaphyseal flaring and cupping of long bones. Its diagnosis must also be based on an histological examination of the cartilage, bearing in mind though that the morphology may be heterogeneous [2].

We had the opportunity to examine a typically affected infant by complete autopsy and to carry out biochemical analyses of the cartilage. The results are reported here.

MATERIALS AND METHODS

Clinical Summary

The parents were of English/Scots and Canadian Irish extraction, aged 30 and 31. At 30 weeks gestation, the mother had noted no fetal movements, but uterine fundus measured 38 cm. Uterine ultrasound demonstrated a markedly abnormal fetus, without evident extremities and with marked polyhydramnios as well as enlarged, rounded, sonolucent, and fluid-filled soft-tissues at the occiput. This infant girl was born at 30 weeks gestation after spontaneous delivery. She demonstrated gasping respirations at birth and a markedly depressed heart rate, had no movements or muscle tone, and died 1 hr later. A sister and brother are normal. There is no family history of consanguinity or of other childhood deaths or abnormalities.

Tissue samples were obtained at autopsy for biochemical analysis and stored frozen at −20°C before use. Tissue was fixed in 2.5% glutaraldehyde for transmission electron-

microscopy and processed using conventional techniques. Tissue sections were stained with lead citrate and uranyl acetate prior to examination on a JEOL 100C transmission electron microscope [17]. Neonatal human rib cartilage was also obtained from infants who had died of causes other than connective tissue disease.

Tissue Dissection and Biochemical Analysis

Cartilaginous ribs were carefully dissected free of perichondrium and cut into short (5-mm) segments. The central gelatinous portion was separated from an outer, more opaque rim. Desiccated portions were weighed for assorted biochemical analyses. One portion (~10 mg dry weight) was digested with papain at 60°C for 48 hrs (in 1 ml 0.2 M Na acetate, 0.02 M cysteine, 0.004 M EDTA, pH 6.0), and aliquots of the digest were sampled for assay of glucosamine, galactosamine, and hydroxyproline. For hexosamine analysis, tissue was hydrolyzed in 6 M HCl at 100°C for 5 hrs. The hydrolysate was diluted, neutralized with NaOH, then buffered to 0.2 M Na citrate, pH 2.0, with 1 M citric acid. Glucosamine and galactosamine were resolved and quantified on a Glenco modular amino acid analyzer, eluting the column (30 cm × 3 mm) at 70°C with a single buffer, 0.35 N Na citrate, pH 5.28, and using a ninhydrin detection system. These conditions of hydrolysis and analysis give quantitative recoveries of the hexosamines. Hydroxyproline was measured after hydrolysis at 110°C for 24 hrs in 6 M HCl, using a colorimetric procedure [18] adapted to the Technicon Autoanalyzer II. Full amino acid analysis was carried out on a similar hydrolysate using a Beckman 121MB instrument. The hydroxylysyl pyridinoline and lysyl pyridinoline cross-linking amino acids of collagen were quantified in the same acid hydrolysate of whole tissue using a direct procedure by fluorescence detection on HPLC [19].

Collagen Solubilization

Collagen was extracted from homogenized samples (about 10 mg wet wt.) of the gelatinous scapula cartilage core, a cross-section of the whole scapula cartilage (perichondrium removed), rib cartilage, and humeral head cartilage using pepsin (0.3 mg) in 5 ml of 3% acetic acid at 4°C for 24 hrs. Individual digests were centrifuged at low speed for 10 min in a bench centrifuge to remove any gross debris, and the supernatants were freeze-dried ready for slab-gel electrophoresis [20]. Effectively, therefore, total solubilized collagen was analyzed on the gel. Samples of neonatal rib cartilage from an infant that died of causes other than connective tissue disease were processed similarly, but, in addition, the neutralized pepsin digest was salt fractionated into 2.5 M and 4.0 M NaCl precipitates in order to increase the yield of soluble collagen and partially purify the $1\alpha 2\alpha 3\alpha$ collagen.

Similar-sized portions of tissue were dried and digested with CNBr (1 : 10 of tissue by dry wt.) in 70% formic acid at 25°C for 16 hrs. The digest was diluted 10-fold with water and freeze-dried for slab-gel electrophoretic analysis of collagen CNBr-peptides on SDS-10% polyacrylamide [20].

RESULTS

Pathologic and Radiologic Anatomy

Autopsy began 5 hrs after death and included postmortem radiographs of whole body, thorax, and extremities. This severely malformed baby girl had classical features of achondrogenesis II by gross morphologic, radiologic, and histological examination with extreme short-limbed dwarfism, large head for trunk size, and hydrops (figs. 1–3).

The infant weighed 1,403 g with 22-cm crown-to-rump length (84% of normal) and 25-cm crown-to-heel length (64% of normal). Head circumference mea-

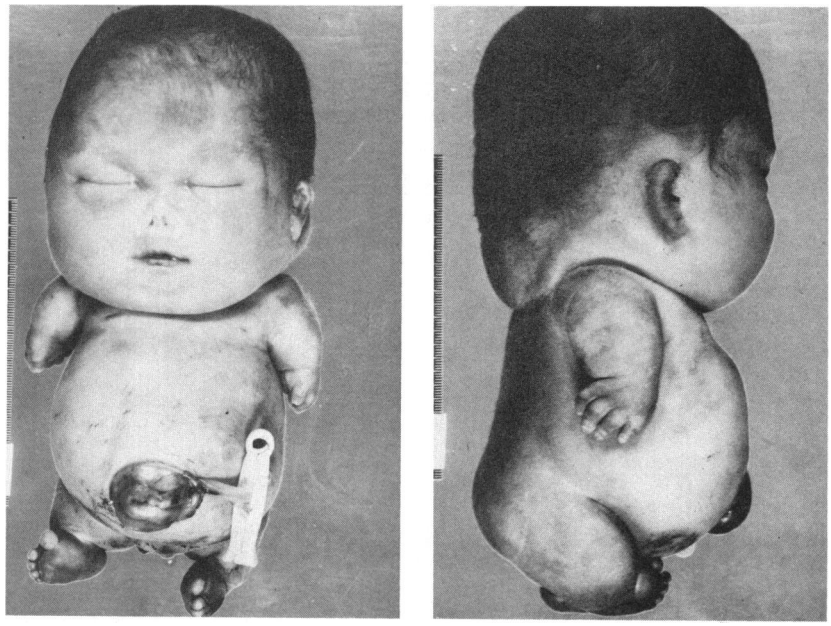

FIG. 1.—Photograph of infant at autopsy. *A* (*left*), Anterior. *B* (*right*), Lateral. Note the severe short-limbed dysplasia, generalized soft tissue swelling, distended abdomen, and small trunk relative to head size.

sured 32 cm (normal), and abdominal girth was 27.5 cm. Additional gross pathologic findings included: (1) triangular shaped face, (2) foreshortened nasal bridge, (3) microstomia, (4) total absence of palate, (5) three hair whorls: posterior-occipital, temporo-parietal, and frontal (of lanugo hair over forehead), (6) a midline dimple in posterior nasopharynx without pituitary contents, (7) extensive lymphangioma of nuchal region, and (10) an omphalocele 3.5 cm in diameter.

The fixed brain weighed 169.9 g (normal 180 ± 53). Lateral and third ventricles were slightly dilated. The cerebral aqueduct and fourth ventricle were normal. Scattered mineralized foci were seen in germinal matrix and around the blood vessels of cerebral white matter. Large areas of the lateral ventricles were devoid of ependymal lining cells, with a reactive underlying germinal matrix and occasional buried ependymal tubules.

The cerebral cortex, brainstem, cerebellum, hypothalamus, and nuclei were normal except for (1) a small lipoma within subarachnoid space of the interpeduncular fossa and (2) slightly increased folding of inferior olives and some flattening and thinning of pyramids.

Visceral abnormalities included: (1) marked pulmonary hypoplasia (lungs 10% of normal weight), (2) common mesentery with nonattachment of colon, (3) bilobed cervical lobe of thymus, and (4) bifid cardiac apex. Other organs were congested but structurally normal, as were the reproductive and urinary tracts. Organ weights were normal for gestational age except for: liver (89%

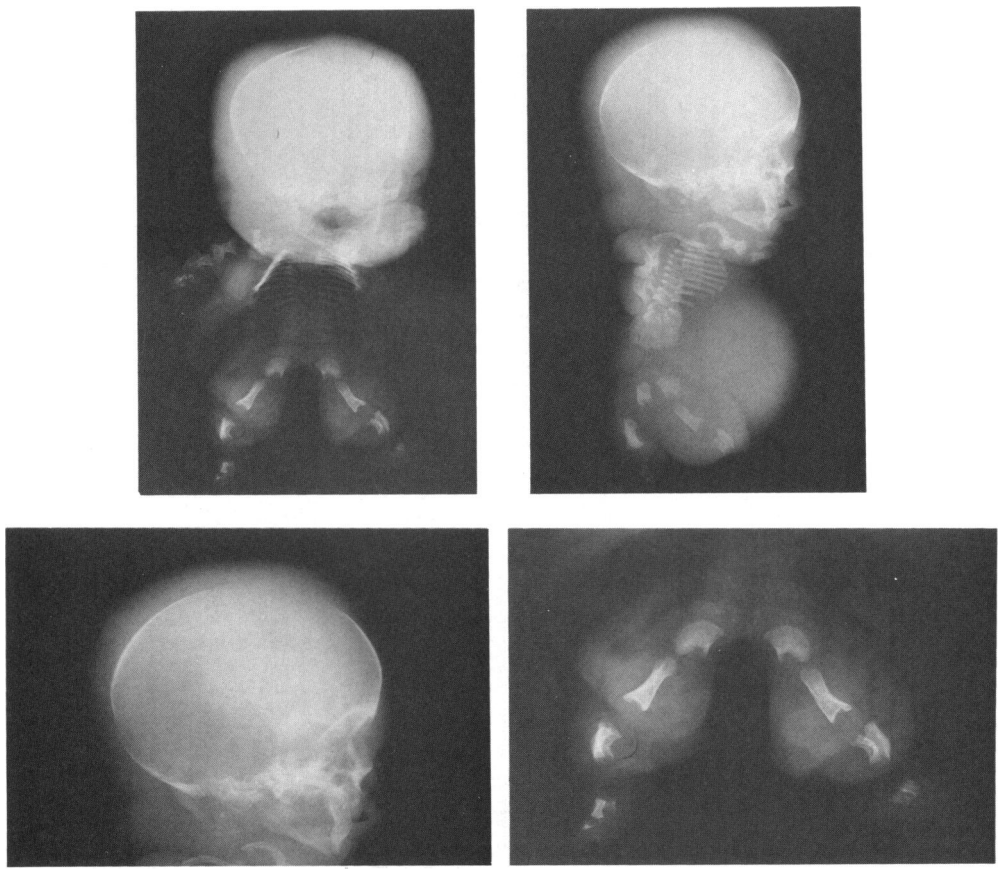

FIG. 2.—Radiographs of infant at autopsy. *A (top, left)*, Anterioposterior. *B (top, right)*, Lateral. *C (bottom, left)*, Cranium, lateral. *D (bottom, right)*, Sacrum and lower extremities (anterioposterior). Note the underossification of lumbar vertebrae and pelvic bones.

of normal), kidneys (61%), and adrenals (56%) [21]. Liver and spleen demonstrated generous extramedullary hematopoiesis. The pituitary was small (0.035 g) but histologically unremarkable.

The radiographs (fig. 2) were also typical of achondrogenesis II. Clavicles and bones of the cranial vault were normal while the ribs were slightly short and without fractures. The maxilla and other facial bones were diminutive; the mandible was more nearly normal but still slender and pointed at the symphysis. The tubular bones were extremely short, appearing somewhat cupped at their ends, but the intrinsic trabecular pattern was normal. The extremities were extremely short; the hands and feet were very small with short bones and very little ossification of the middle and distal phalanges.

The bones of the spine consisted of tiny ossification centers, paired in the thoracic and lumbar area, and somewhat larger in the upper cervical region, but no ossification centers were visible in the sacrum or sternum. The iliac bones

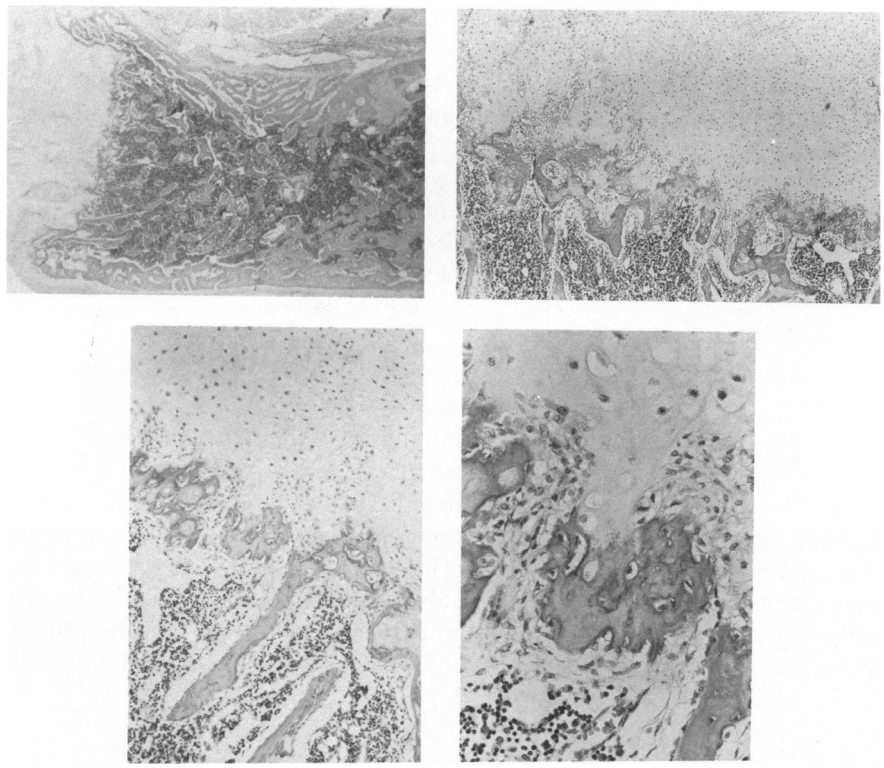

FIG. 3.—Light microscopy of the growth zone of the proximal humerus. *A* (*top, left*), Low power. *B* (*top, right*), Growth zone. *C* (*bottom, left*), Higher power of disordered ossification. *D* (*bottom, right*), Close-up from *C*.

were small and crescent-shaped with a convex border superiorly and a concave border inferiorly. It was difficult to identify acetabulae on radiographs as there was no ossification of pubis or ischial bones; however, the hips were not dislocated.

Bone and Cartilage Morphology

Examination of the skull demonstrated patent sutures. The supraoccipital bone was smaller than normal due to the absence of its superior portion with a consequent flattening of the superior border and enlargement of the posterior fontanelle. The anterior clinoids were increased in size, and the posterior clinoids and diaphragma sellae were absent. The vertebral canal was narrow with spinal cord fitted snugly within it.

Long bones and ribs had grossly thinner growth zones than normal and were fragile and brittle with the cartilage portion separating readily from the underlying metaphysis. Microscopy confirmed marked disorganization of the growth zone, with cupping of subchondral bone and lipping at the periphery seen in the humeral head in figure 3*A*. The normal parallel configuration of the hyper-

trophic and proliferative columns was absent (fig. 3*B*). Short transitional columns were occasionally seen but were of haphazard orientation, often interspersed with primitive mineralizing cartilage (fig. 3*B* and *C*). Primary osteoid seams were present in some places adjacent to poorly formed and shortened transitional columns (fig. 3*C* and *D*). The resting cartilage in all sites was markedly abnormal, being abundantly cellular and composed of large and ballooned chondrocytes with little intercellular matrix. It was arranged in large nodular masses interspersed with abnormally abundant blood vessels. Membrane bone and bone marrow appeared to be normal.

Lung Morphology

The lungs were prepared for morphometric analysis by established techniques [22]. Lung volumes were 1 cc preinflation and 3 cc at maximal inflation. Radiographic and microscopic examination (fig. 4) showed well-filled large arteries that were less branched than normal and extended close to the pleural surface (fig. 4*A*). There were fewer intraacinar arteries and decreased bronchial branching for gestational age. The parenchyma was collapsed. The interstitial septa were wide and of varying cellularity and many potential air spaces were filled with degenerating cells, presumably of epithelium (fig. 4*B*). Fetal mesenchyme was abundant in the interstitium. Of particular note was the primitive appearance of the airway cartilage with large ballooned cells and minimal matrix (fig. 4*B* and *C*).

Cartilage Chemistry

At all sites, hyaline cartilage had a watery, gelatinous texture and translucent appearance in contrast to the stiff, opaque properties of control neonatal cartilages.

Composition

The collagen content of the gelatinous cartilage core from ribs and scapula was only half that of control human neonatal rib cartilage (table 1). The hexosamine content was in the high range expected for hyaline cartilage, and the high ratio of galactosamine to glucosamine was typical for proteoglycans of hyaline cartilage and similar to that of control human neonatal rib cartilage (table 1). The hydroxylysine content of the cartilage relative to hydroxyproline was significantly lower than that of neonatal control cartilage, consistent with type I collagen rather than type II collagen predominating. The concentration of mature, hydroxypyridinium cross-links in the total cartilage collagen was only 15% of that of a control neonate. Since type II collagen is the richest known source of these stable cross-linking residues, this also was consistent with the other biochemical findings.

Collagen Types

Electrophoresis of the total pepsin-solubilized collagen from gelatinous scapula cartilage showed a pattern typical of type I collagen rather than the type II collagen that dominates in normal neonatal human cartilage (fig. 5) and

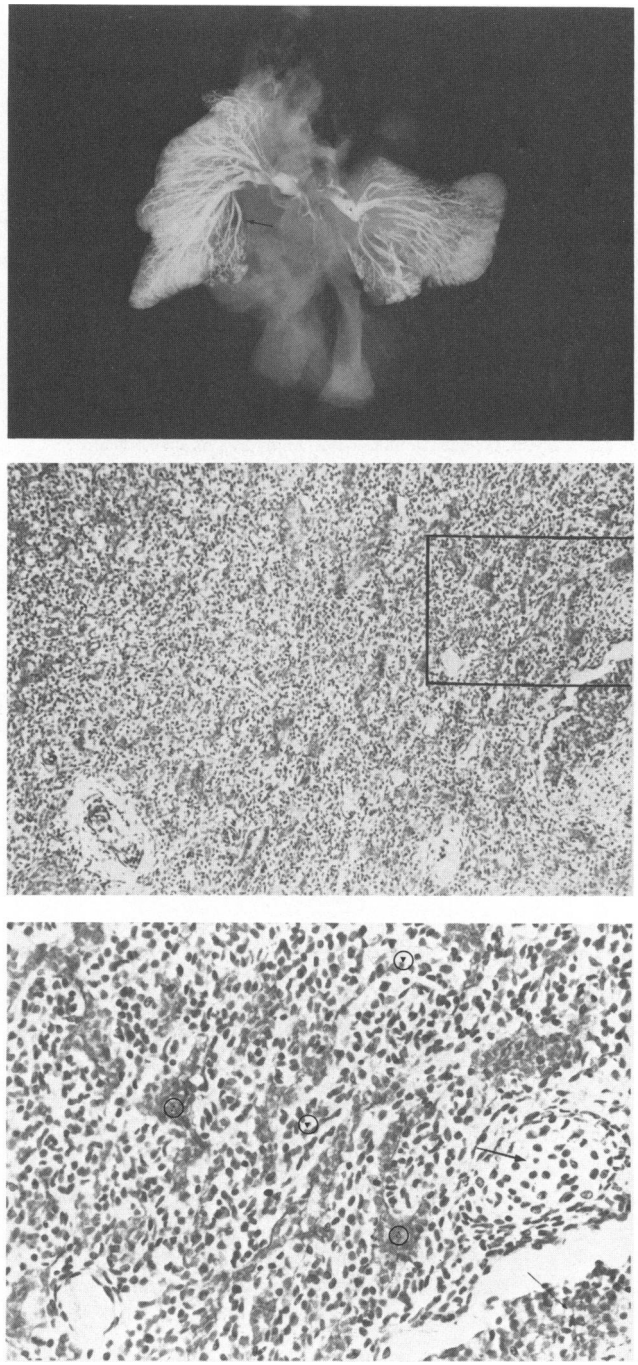

FIG. 4.—Pathology of the lung. *A* (*top*), Postmortem angiogram of lungs. Note the persistence to the periphery of large vessels (*at arrow*) with minimal branching. *B* (*middle*), Low-power light microscopy of parenchyma (*H* and *E*). *C* (*bottom*), Higher power of insert from *B*. *Heavy arrow* indicates cartilage core; *light arrow* indicates bronchial mucosa. *Closed arrowheads* (*encircled*) indicate expanded interstitium, and *open arrowheads* (*encircled*), epithelial clusters of cells in gland-like spaces.

TABLE 1

COLLAGEN AND HEXOSAMINE COMPOSITION OF HYALINE CARTILAGE FROM THE ACHONDROGENESIS INFANT AND A NEONATAL CONTROL

	HEXOSAMINES			COLLAGEN		
	% of dry wt. GalN	GlcN	Molar ratio GalN/GlcN	% of dry wt.	Hyl/ 100 Hyp	HP (mol/mol)
Achondrogenesis	5.4	1.0	5.4	17.4*	13.3	0.09
Control	8.8	1.8	5.0	28.7	18.6	0.62

NOTE: Control analyses on rib cartilage; achondrogenesis analyses on rib and scapula cartilages. HP, hydroxylysylpyridinoline cross-linking residues.

* Gelatinous inner core of rib; collagen content based on hydroxyproline content × 7.5.

in all mammalian hyaline cartilages that have been examined by this technique [23]. However, a characteristic pattern of three equally spaced bands were prominent that ran identically with the 1α, 2α, and 3α minor collagen chains previously shown to be specific for hyaline cartilages [24], in addition to the α1(I) and α2(I) chains of type I collagen (fig. 5). We have not seen this combination of 1α, 2α, and 3α chains with type I collagen in any other tissue, including neonatal human control cartilage specimens, assorted hyaline cartilages from animals, and various cartilage specimens from other forms of human lethal chondrodystrophy including two cases of spondyloepiphyseal dysplasia and a typical thanatophoric dwarfism [3]. The identity of the protein band running

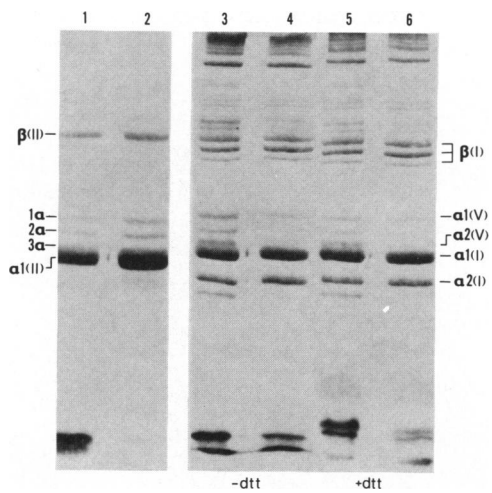

FIG. 5.—Slab-gel electrophoresis (sodium dodecyl sulfate-5% polyacrylamide) of pepsin-solubilized collagen prepared from control human neonatal cartilage and from the achondrogenesis infant's cartilage. *Lane 1*, control—2.5 M NaCl precipitate at pH 7.5 (type II plus 1α2α3α). *Lane 2*, control—4.0 M NaCl precipitate at pH 7.5 (type II enriched in 1α2α3α). *Lane 3*, Achondrogenesis scapula core cartilage—total pepsin soluble. *Lane 4*, Achondrogenesis scapula cartilage (core plus outer fibrous rim)—total pepsin soluble. *Lanes 5* and *6*, same as *3* and *4*, respectively, run after dithiothreitol reduction.

ahead of α2(I) in lanes 3 and 5 (fig. 5) is unknown. It may be an overdigestion product of α2(I) derived by pepsin, sometimes seen in pepsin digests of type I collagen. The opaque and more fibrous peripheral regions of the scapula and rib cartilages showed a higher proportion of what appeared to be α1(V) and α2(V) chains, as well as the 1α, 2α, and 3α chains, but again with type I collagen chains as the main molecular species. This tissue region by light microscopy appears to be a fibrous ingrowth from the perichondrium being populated more by flattened fibroblastic cells than by rounded chondrocytes.

Electrophoretic analyses of CNBr-digests of the core cartilage also showed a peptide pattern typical of type I collagen (fig. 6). No type II collagen peptides were recovered in detectable amounts from any region of cartilage including the gelatinous cores of rib, scapula (fig. 6), and humeral head (not shown). The fainter peptide bands in lane 3 compared with the other lanes of figure 6 reflect the low content of collagen in the gelatinous cartilage of the affected infant (equal loads were applied to the gel by tissue dry wt.). The profile is clearly that of type I collagen as expected from the pepsin results. Type II peptides [notably α1(II)CB10] would have been readily apparent down to a level of 5% of the total collagen. Type 3α collagen chains, which give a similar CNBr peptide map to α1(II) chains [24], would not be detectable beyond faint background bands in the amount indicated by the analyses of pepsin-solubilized whole chains (fig. 5).

Unfortunately, insufficient tissue was available to prepare individual CNBr-peptide maps for the isolated 1α, 2α, 3α collagen and its component chains.

Electronmicroscopy of Cartilage

In cross-sections of cartilaginous rib, two zones were apparent, an outer opaque and more fibrous zone and an inner translucent, gelatinous zone (fig.

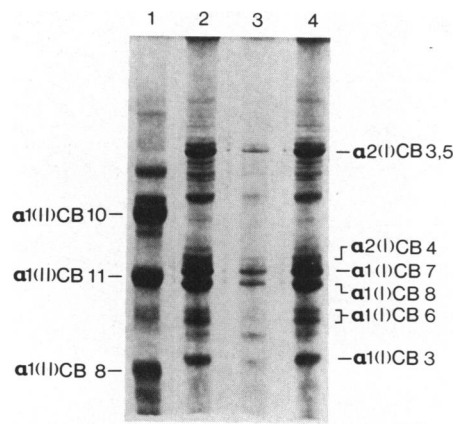

FIG. 6.—Slab-gel electrophoresis (sodium dodecyl sulfate-10% polyacrylamide) of collagen CNBr-peptides in digests of whole cartilage from the achondrogenesis infant and from a control. *Lane 1*, Control human neonatal rib cartilage. *Lane 2*, Achondrogenesis—scapula cartilage outer rim plus core. *Lane 3*, Achondrogenesis—rib, gelatinous cartilage core. *Lane 4*, Achondrogenesis—rib cartilage, outer rim.

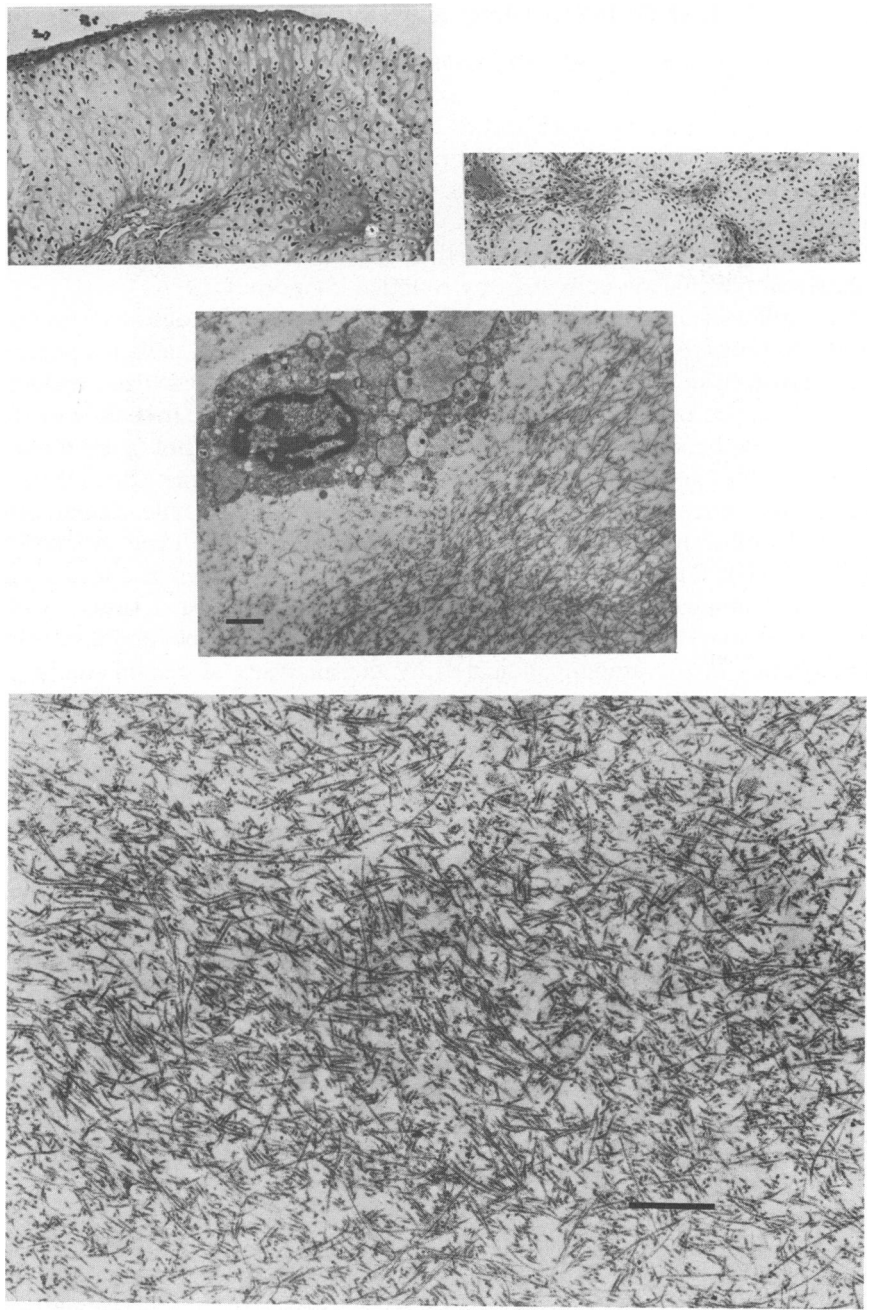

FIG. 7.—See figure legend on p. 63.

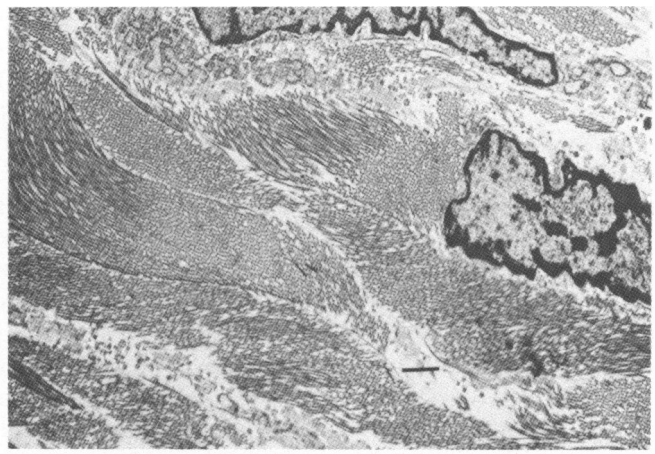

FIG. 7.—Morphology of the achondrogenesis infant's cartilage. *A* (*top, left, p. 62*), Light microscopy of cartilage from the abnormal rib showing obvious cartilaginous domains interspersed by fibrous septae (hematoxylin and eosin). *B* (*top, right, p. 62*), Light microscopy of proximal humerus showing whorls of cartilage tissue interspersed by fibrous septae (cf. fig. 4*C* of lung). *C* (*middle, p. 62*), Transmission electronmicroscopy of gelatinous rib cartilage showing a chondrocyte filled with lakes of amorphous material enclosed by dilated rough endoplasmic reticulum. *Bar* = 1 micron. *D* (*bottom, p. 62*), Transmission electronmicroscopy of gelatinous cartilage matrix showing the collagen fibril morphology. *Bar* = 1 micron. *E* (*above, p. 63*), Transmission electronmicroscopy showing an interspersed fibrous septum. *Bar* = 1 micron.

7*A*). Sections of humeral head cartilage showed similar cartilaginous whorls and interspersed fibrous septae (fig. 7*B*). Electronmicroscopy of the chondrocytes in the gelatinous region showed abnormal accumulations of amorphous material in dilated lakes of rough endoplasmic reticulum (fig. 7*C*). Electronmicroscopy of the extracellular matrix in the gelatinous region showed a random feltwork of well-spaced, uniformly thin (15–20 nm diameter) fibrils typical of young, growing hyaline cartilages (fig. 7*D*) [25]. Perichondral regions, however, showed the parallel arrays of densely packed, much thicker fibrils that are more typical of tissues rich in type I collagen (fig. 7*E*). Although the appearance of the collagen in the gelatinous central regions of cartilage gave the impression of young type II collagen, the biochemical results clearly showed that type II was absent and the bulk of the collagen was type I together with about 10% of type $1\alpha 2\alpha 3\alpha$.

DISCUSSION

The infant had all the clinical, radiological, and histological hallmarks of achondrogenesis type II. The resting cartilage was notable for its paucity of extracellular matrix surrounding the large, dilated chondrocytes. The low collagen content is consistent with this observation and apparently results from a failed expression of type II collagen by the chondrocytes. Type I collagen, which was the main type of collagen present in all cartilage samples including

the gelatinous rib core, apparently, in part, replaces type II collagen in this sparse matrix. It is not clear from the present results whether the type I is concentrated in fibrous pockets or uniformly codistributed with the $1\alpha2\alpha3\alpha$ collagen.

The abundant hexosamine, specifically galactosamine, suggests a normal expression of chondroitin sulfate-rich, cartilage-specific proteoglycans by the chondrocytes, and the presence of $1\alpha2\alpha3\alpha$ collagen suggests that at least one component of the cartilage-specific collagen phenotype had been expressed.

The collagen composed of 1α, 2α, and 3α chains seems to be specific to hyaline cartilages [24] and normally accounts for about 3% of total tissue collagen [26]. The chains, 1α and 2α, show some chemical similarities to the $\alpha1(V)$ and $\alpha2(V)$ chains of type V collagen but are believed to be genetically distinct species. The 3α chain is virtually identical to the $\alpha1(II)$ chain in its CNBr-peptide profile, but it is still unclear whether it is expressed from the same gene [27]. All evidence suggests that 3α is present in combination with 1α and 2α chains in heterotrimeric native molecules [27]. Type $1\alpha2\alpha3\alpha$ has until now been found only in those cartilages in which type II collagen predominates. In the present disorder, it would be important to establish that the 3α chain was expressed and used in native molecules in combination with 1α and 2α chains without any coexpression of type II collagen. Such mutants could help decide whether 3α is a genetically distinct species of collagen chain or a post-translational variant of $\alpha1(II)$.

It would also be important to know whether other cartilage-specific minor types of collagen such as type IX [28] and type X, the latter normally restricted to the calcifying zone of the growth plate [29], were expressed in the absence of type II collagen in the abnormal cartilage. However, insufficient tissue was available from the present case to identify these minor collagens biochemically using present techniques (see NOTE ADDED IN PROOF below). Using immunohistochemical methods and antibodies specific for the different collagen types, a reduction in the amount of type II collagen and appearance of type I collagen in fibrous domains was indicated in other cases of achondrogenesis [30, 31].

Collagen fibrils in the central, gelatinous region of the rib had the characteristic appearance of those in young cartilage matrix with thin, uniform diameters and a well-spaced random organization [25], despite type I collagen predominating instead of type II. This finding has important implications about the mechanism of regulation of fibril diameters and fibril organization, as it suggests that type I collagen produced in the presence of otherwise cartilage-specific macromolecules assumes a polymeric form and architecture typical of cartilage. It implies that the genetic type of fibrillar collagen in itself has little or no control of fibril diameter and organization. It seems likely, therefore, that ingredients of the cartilage extracellular matrix other than the principal collagen type are primarily responsible for regulating collagen fibril size and architecture. A comparison of inborn disorders of cartilage expression may help understand such control mechanisms. For instance in nanomelia, a lethal dwarfing syndrome in a strain of chicks [32] and in the cmd/cmd mouse [33],

synthesis of cartilage-specific proteoglycans is totally blocked, yet the type II collagen fibril architecture is basically unaffected [32]. This implies that cartilage-specific proteoglycans are not the primary regulators of fibril organization. One might speculate that one of the minor types of cartilage collagen such as the type $1\alpha 2\alpha 3\alpha$ collagen, which is expressed in the present achondrogenesis case, and presumably also in nanomelic chickens, serves to regulate the fibril organization of the bulk collagen phase. Type IX collagen would be another possibility, since it appears to occur in all hyaline cartilages, and its abundance relative to type II collagen seems highest in developing tissue when fibril diameters are narrowest (unpublished observations).

The polyhydramnios, hydrops fetalis, and mild erythroblastosis in this condition remain unexplained. Pulmonary hypoplasia is often associated with fetal hydrops, renal hypoplasia, and thoracic dystrophy, all present here, but causal mechanisms are unknown. Neonatal asphyxia and pulmonary hypoplasia in achondrogenesis and in other skeletal dysplasias have been attributed to secondary mechanical effects on lung development of a restricted thoracic cavity [21]. However, given the diminished bronchial branching seen in the present case, and the fact that type II collagen is a normal constituent of respiratory tract cartilages [23], it seems reasonable to suspect that the lack of type II collagen and defective cartilage growth had contributed directly to the abnormal lung growth. In addition, one might speculate that in the absence of type II collagen, most of the abundant proteoglycans made by chondrocytes have no matrix to become embedded in and so pass directly into body fluids where they would increase the osmotic swelling pressure.

In summary, the findings imply that the present disorder is based on a genetic defect, possibly monogenic, that prevents type II collagen expression. Since there appears to be only one copy of the type II collagen gene in humans [34, 35], located on the long arm of chromosome 12 [36, 37], we presume that all tissues that normally produce type II collagen are affected. Unfortunately, tissue was unavailable to test for a lack of type II collagen in vitreous humor. No obvious eye defects were noted, but no specialized eye exam was performed.

The term chondrogenesis imperfecta would appear more appropriate than achondrogenesis, since cartilage had clearly differentiated as a gross tissue, albeit of abnormal structure and composition. Indeed, chondrogenesis imperfecta would seem a useful collective term to describe those chondrodystrophies in which cartilage matrix proves to be structurally defective, by analogy to osteogenesis imperfecta. There seems little doubt that a large collection of heterogeneous mutations that affect production of type II collagen will emerge that are analogous to those affecting type I collagen in subtypes of the osteogenesis imperfecta syndrome.

Inborn disorders in which type II collagen synthesis is affected may give unique insights on the possible role of type II as a signal for tissue differentiation during embryonic development and on specific structural functions of type II collagen in cartilage matrix.

NOTE ADDED IN PROOF: By committing all remaining tissue to pepsin digestion and salt fractionation of collagen types, we recently identified type IX collagen clearly in the 1.8 M NaCl precipitate by SDS-polyacrylamide electrophoresis. Therefore, both minor types of cartilage collagen, type $1\alpha 2\alpha 3\alpha$ and type IX, were apparently expressed by the abnormal chondrocytes in the absence of type II collagen.

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