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Glycosaminoglycan-free Small Proteoglycan Core Protein Is Secreted by Fibroblasts from a Patient with a Syndrome Resembling Progeroid

HANS KRESSE,* STEEN ROSTHØJ,† EDELGARD QUENTIN,* JÜRGEN HOLLMANN,* JOSEF GLÖSSL,*[,1] SHINTARO OKADA,*[,2] AND TØNNE TØNNESEN‡

*Institute of Physiological Chemistry, University of Münster, Münster, Federal Republic of Germany; †Department of Pediatrics, Aalborg Sygehus, Aalborg, Denmark; and ‡John F. Kennedy Institute, Glostrup, Denmark

SUMMARY

A male patient, 4 years 9 mo old and having progeroidal appearance, exhibited delayed mental development and multiple abnormalities of connective tissues including growth failure, osteopenia of all and dysplasia of some bones, defective deciduous teeth, loose but elastic skin, delayed wound healing with formation of thin atrophic scars, scanty scalp hair, hypotonic muscles, and hypermobile joints. Skin fibroblasts of the patient converted only about half of the core protein of the small proteodermatan sulfate to a mature glycosaminoglycan chain–bearing proteoglycan. The remaining core protein, which contained complex-type asparagine-bound oligosaccharides, was secreted with almost normal kinetics. Xylosyltransferase activity and the synthesis of other proteoglycan types were normal. Normal induction of glycosaminoglycan synthesis occurred in the presence of 1 mM, but there was very little induction in the presence of 0.01 mM *p*-nitrophenyl-β-xyloside. An antibody against an N-terminal pentadecapeptide of the core protein recognized the glycosaminoglycan-free core protein from the patient less well than the chain-bearing protein treated with chondroitin ABC lyase. Though these results do not

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Address for correspondence and reprints: Dr. Hans Kresse, Institute of Physiological Chemistry, University of Münster, Waldeyerstrasse 15, D-4400 Münster, Federal Republic of Germany.

1. Present address: Department of Applied Genetics, University of Agriculture and Forestry, Gregor-Mendel-Strasse 33, A-1180 Vienna, Austria.

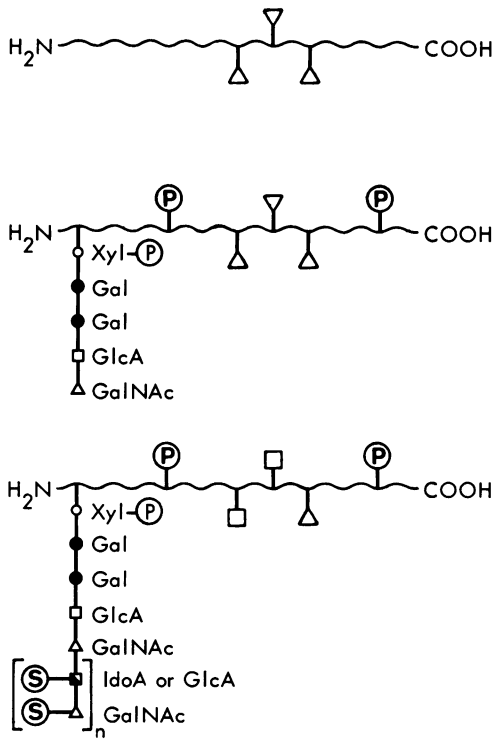
2. Present address: Department of Pediatrics, Osaka University Medical School, Fukushima-Ku, Osaka, Japan.

define the basic defect unambiguously, they provide the first report of a disorder being due to an abnormality in small proteoglycan biosynthesis.

INTRODUCTION

Fibrous proteins—e.g., collagen and fibronectin—and proteoglycans are important constituents of the extracellular matrix. Several genetic defects of expression, structure, and posttranslational modification of the collagens have been described that have allowed the biochemical characterization of various types of osteogenesis imperfecta and of Ehlers-Danlos syndrome (for reviews, see Pinnell and Murad 1983; Prockop and Kivirikko 1984; Cheak 1985). On the other hand, there are only very few reports on inborn abnormalities of proteoglycan biosynthesis that may lead to inherited disorders in human beings. Defective processing of keratan sulfate, possibly due to a deficient sulfotransferase, results in macular corneal dystrophy (Hassell et al. 1980; Nakazawa et al. 1984). A form of spondyloepiphyseal dysplasia has been considered to result from a deficiency of a chondroitin sulfate sulfotransferase (Mourao et al. 1981). Undersulfation of glycosaminoglycans in patients with Lowe syndrome has been explained in terms of an elevated level of nucleotide pyrophosphatase, which hydrolyses 3′-phosphoadenylyl sulfate, the common substrate of all sulfotransferases (Yamashina et al. 1983), but this contention has not been supported by other studies (Donnelly et al. 1984). In pseudoachondroplasia one population of proteoglycans could not be detected electrophoretically, but detailed studies on biosynthesis and processing of the respective proteoglycans have not been performed (Stanescu and Maroteaux 1975).

We wish to describe a patient who represents a progeroid variant with signs of the Ehlers-Danlos syndrome. We consider his disease to be the result of a mutation that leads to a defective biosynthesis of a small proteodermatan sulfate (PDS). This proteoglycan is a major secretory product of cultured skin fibroblasts. Its core protein (of M_r = 36,319), the primary structure of which has been deduced from cloned cDNA (Krusius and Ruoslahti 1986), is linked with only one dermatan sulfate chain and, additionally, with two or three asparagine-bound oligosaccharides (Glössl et al. 1984). In analogy to a similar proteoglycan from bovine skin, it is assumed that the single glycosaminoglycan chain is bound to the serine residue at the fourth position from the N-terminal end (Chopra et al. 1985). Major steps required for the biosynthesis of this PDS are schematically depicted in figure 1. The presence of the small PDS is not restricted to dermal tissue. By use of an antibody against the small PDS core protein, cross-reactive material was detected in all tissues examined, i.e., in the wall of blood vessels, in cartilage, lung, liver, muscle, and brain (Voss et al. 1986). Though the fine structure of the glycosaminoglycan chains of the small proteoglycan may vary in different tissues, it must be considered to be an abundant and widely distributed proteoglycan species.



Synthesis of pre-pro form of core protein; addition of 2 or 3 precursor oligosaccharides (△) to asparagine residues; removal of presequence

Phosphorylation of serine residues; synthesis of the polysaccharide protein linkage region by the sequential action of xylosyltransferase, galactosyltransferase I, galactosyltransferase II, glucuronyltransferase I, and N-acetylgalactosaminyltransferase I; phosphorylation of the xylose residue

Polymerisation, modification, and sulfation of the glycosaminoglycan chain by glucuronyltransferase II, N-acetylgalactosaminyltransferase II, D-glucuronyl 5-epimerase, and several sulfotransferases; conversion of high mannose-type into complex-type oligosaccharides (△)

FIG. 1.—Sequence of major events during small-PDS biosynthesis. Xyl = xylose; Gal = galactose; GlcA = glucuronic acid; GalNAc = *N*-acetylgalactosamine; and IdoA = L-iduronic acid residues. Ⓟ and Ⓢ = phosphate and sulfate esters, respectively. The removal of the propeptide (14 amino acids) cannot yet be arranged into this sequence of events.

MATERIAL AND METHODS

Fibroblast cultures were grown from skin biopsies obtained at two different occasions and maintained in culture as described elsewhere (Cantz et al. 1972); they were used for biochemical analysis after the second and the tenth passage, respectively.

PDS and its core protein were metabolically labeled, immune precipitated, and analyzed by polyacrylamide-gel electrophoresis (PAGE) exactly as described elsewhere (Glössl et al. 1984, 1986). In brief, confluent cultures were preincubated for 1 h with leucine-free Waymouth MAB 87/3 medium (as formulated in the catalogue of GIBCO) supplemented with 4% (v/v) dialyzed fetal calf serum, penicillin, and streptomycin; final volume was 2 ml/25-cm^2 Falcon plastic flask. Medium was then replaced by the same volume of medium containing, additionally, 20–80 μCi/ml of L-[4,5-^{3}H] leucine (specific radioactivity 40–60 Ci/mmol; New England Nuclear). Labeling was terminated by either harvesting or feeding the cultures with complete tissue-culture medium. For isolation of PDS and its core protein, medium was made 70% saturated with $(NH_4)_2SO_4$. After centrifugation, the precipitate was dissolved in 400 μl/25-cm^2 flask of 0.5% sodium deoxycholate, 0.5% Triton X-100, 1.0 M NaCl, 0.1 M

6-aminohexanoic acid, 5 mM benzamidine hydrochloride, 10 mM *N*-ethylmaleimide, and 10 mM ethylenediaminetetraacetate (EDTA) in 20 mM Tris/HCl buffer, pH 7.4 (buffer A). The solution was sequentially treated with control-IgG-coated protein A–Sepharose (Sigma) and with protein A–Sepharose coated with IgG from a monospecific antiserum against the small PDS core protein. Portions of adsorbed proteoglycan were treated with either 20 mU of chondroitin ABC lyase (Seikagaku Kogyo) or buffer alone (Saito et al. 1968) in the presence of the protease inhibitors mentioned above and were then processed for sodium dodecyl sulfate (SDS)-PAGE (Hasilik and Neufeld 1980) followed by fluorography (Bonner and Laskey 1974). Cell-associated immunoreactive material was isolated analogously after extraction of the cells for 90 min at 22 C with 400 μl/25-cm^2 flask of buffer A. In some experiments the immune complexes were digested with endo-β-*N*-acetylglucosaminidase H (Seikagaku Kogyo) to remove high-mannose-type asparagine-bound oligosaccharides. In this case, the immune complexes were washed twice with 10 mM sodium phosphate, pH 6.0, containing 0.1 M NaCl and were then suspended in 30 μl of this buffer containing 4 mU of enzyme, 10 mM EDTA, 10 mM *N*-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and 0.36 mM pepstatin. Incubation was for 20 h at 37 C. To study the immunoreactivity of PDS and free core protein, fibroblasts were incubated for 6 h in the presence of [^{3}H]leucine (40 μCi/ml). The medium was then chromatographed on a DEAE-Trisacryl column (LKB Instrument) as described elsewhere (Glössl et al. 1984). Core protein did not adsorb to the ion-exchange matrix under these conditions. It was precipitated with $(NH_4)_2SO_4$ (70% saturation) prior to immune precipitation. Proteoglycans were desorbed by 1.0 M NaCl, dialyzed against 0.1% Triton X-100, concentrated, and immune precipitated. The reactivities toward the antiserum against the intact core protein and toward a rabbit antiserum against a synthetic N-terminal pentadecapeptide of human core protein were compared. The latter serum was a gift of Drs. T. Krusius and E. Ruoslahti (Cancer Research Center, La Jolla).

Total [^{35}S]sulfate-labeled macromolecules from fibroblast secretions were obtained on incubation of confluent cultures in modified Eagle's minimum essential medium (Cantz et al. 1972) containing 5–10 μCi/ml of [^{35}S]sulfate (carrier free; Amersham-Buchler) and 10% (v/v) dialyzed fetal calf serum. Secreted products were collected by ethanol precipitation (final concentration 75% [v/v]). Induced glycosaminoglycan chains were obtained as follows: Fibroblasts were incubated in the presence of [^{35}S]-sulfate and *p*-nitrophenyl-β-D-xyloside (Sigma) for up to 6 h. Medium was made 70% saturated with $(NH_4)_2SO_4$, and the supernatant obtained after centrifugation was made 0.1% with Triton X-100 and dialyzed against 0.15 M NaCl. The dialysis residue was applied to a 1-ml column of Dowex AG 1 × 2 (200–400 mesh; Serva) equilibrated with 0.15 M NaCl in 0.1 Triton X-100. The column was washed with 0.5 M NaCl/0.1% Triton X-100 before glycosaminoglycan chains were desorbed by applying 2 M NaCl/0.1% Triton X-100.

For the determination of xylosyltransferase activity, fibroblasts grown to confluency in a 25-cm^2 Falcon plastic flask were suspended in 200 μl 10 mM

Tris/HCl containing 1% Triton X-100, pH 7.5, and homogenized by ultrasonication. The reaction mixture for the enzyme assay contained, in a final volume of 80 μl, 30–60 μg cell protein, 50 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5, 5 mM $MnCl_2$, 5 mM $MgCl_2$, 50 mM KCl, 20 mM CDP-choline, 1 μCi UDP-xylose (xylose-1-^{3}H; New England Nuclear; specific radioactivity 9.9 Ci/mmol), and 50 μg HF-treated proteoglycan core protein from bovine nasal cartilage as acceptor protein (Olson et al. 1985). After incubation for 1 h at 37 C, xylosylated core protein was precipitated with trichloroacetic acid (10% final concentration) and quantitated by liquid scintillation counting. Cell protein (Lowry et al. 1951) and urinary mucopolysaccharides (Nielsen et al. 1986) were determined as described elsewhere.

CASE REPORT

The patient, a boy of Danish ancestry, was born on July 26, 1981, to healthy, unrelated parents, aged 27 and 28, who had two healthy older children. An uncle had Downs syndrome; otherwise the family history was unremarkable. Pregnancy had been uneventful, and the patient had been delivered at term without complications (birth weight 3.03 kg, length 51 cm, head circumference 35.5 cm). Soon after birth, a chromosomal abnormality was suspected: transverse palmar creases were noted, as well as a broad, flat forehead; broad nasal bridge; small mouth; and long, slender fingers and toes. In addition, there were a palpable prominence on the right clavicle and defective extension and rotation of the left elbow. Radiographs showed a broad-based clavicular exostosis, proximal radioulnar synostosis, and distal radioulnar luxation. Chromosome analysis showed a $46,XYq^+,22s^+$ karyotype, considered a normal variant, and no specific diagnosis was made.

Subsequently, the boy failed to thrive, with poor weight gain and growth velocity, and in the second half of the first year of life he was investigated more extensively. His appearance remained conspicuous, the head triangular with a relatively small face, prominent eyes, and a very small mouth and the chest narrow with prominent sternum; and additional further features now noted included an arched palate, a hypoplastic bifid uvula, hypoplastic earlobes, second toes overlapping the first, and a peculiarly loose, but smooth and elastic, skin. Motor development was delayed, with inability to sit unsupported, and the patient displayed generalized hypotonia, hypotrophic muscles, and hypermobile joints. Otherwise, he appeared in good health, and clinical examination was unremarkable: liver and spleen were not enlarged, hernias not present, and ocular abnormalities not demonstrable. Radiographs of chest, skull, and long bones revealed rarefaction of bone structure. A computed-tomography scan showed a somewhat dilated, asymmetrical ventricular system. An intravenous pyelogram performed following a urinary-tract infection was normal. Serum levels of T_4, thyroid-stimulating hormone, and muscle enzymes were normal. The amino acid pattern in serum and urine was normal. Mucopolysaccharide excretion was 7.5 mg hexuronic acid/g creatinine, the normal reference value being 20 ± 10 mg/g. However, a predominance of heparan sulfate (80%) over chondroitin sulfate (20%) was found. Normally,

heparan sulfate accounts for 5%–10% of excreted mucopolysaccharides (Nielsen et al. 1986).

Up to his third year of life, in addition to the distinctive appearance described, the following four main problems were recognized:

1. *Growth Failure*

Growth velocity has been markedly reduced, the height falling increasingly below the third percentile, being 65.5 cm at age 12 mo and 75 cm at age 2 years 10 mo. Weight gain has paralleled height gain. There has been no evidence of malnutrition or malabsorption. The bone age has been slightly advanced, and a clonidine test has shown normal growth hormone release.

2. *Developmental Delay*

The boy was able to sit at age 15 mo, to walk without assistance at age 3½ years, and to form short sentences at age 4 years. Apart from hypotonia, neurological examination was unremarkable, and there have been no convulsions. Tests of vision and hearing have been normal. Head circumference, unlike height and weight, has increased normally for age, and a repeat computed-tomography scan at age 2 years 11 mo showed an indistinct cortical pattern as the only abnormality. In a kindergarten for handicapped children, the patient has been found to be quiet and cautious, lagging equally in cognitive, emotional, and social development.

3. *Dermal Abnormalities*

The boy has a very light complexion. The skin is loose but elastic without folds or wrinkles; it is not thin or atrophic, and palms and soles are somewhat thickened with many creases. Wound healing has been defective, with formation of thin atrophic scars. The boy sweats normally. Growth of scalp hair has been scant, and the eyebrows have been very thin. Apart from thick, brown nails on the second toes, the nails are normal. The deciduous teeth have had defective, brownish, and crumbling enamel, and several have been extracted owing to severe decay.

4. *Skeletal Abnormalities*

Body proportions are normal, and there are no gross deformities; but mobility in the left elbow remains restricted, and flat feet are to be noted. In addition to earlier mentioned findings, radiographs have shown rather short clavicles with broad medial ends, anterior splaying of the ribs, and coxa valga. Bone structure was normal at birth, but progressive generalized osteopenia became manifest. Clinical fracture episodes have not occurred, but possible rib infractions have been noted radiographically, and there is some height reduction of several midthoracic and of the second lumbar vertebral bodies. Also, the fourth lumbar interspace is narrowed. Serum calcium, phosphate, and alkaline phosphatase have been normal.

A further round of metabolic studies done at the age of 3 years confirmed the predominance of heparan sulfate excretion. Fibroblasts from a skin biopsy

showed normal incorporation of [^{35}S]sulfate and of ^{34}Cu, and enzyme activity determinations ruled out subtypes A, B, C, and D of the Sanfilippo syndrome. High-resolution chromosomal analysis revealed a 46,XY,var(22)(p13,QFQ34) karyotype, with a normal Y chromosome.

Apart from the problems listed, the boy has been in good health. He has not been prone to infections. The nutritional status has been satisfactory, and gastrointestinal symptoms are absent. He is not anemic, and hematological abnormalities have not been found. There has been no bleeding tendency, and the Rumpel-Leede's test has been negative. There is no clinical evidence of cardiac or vascular abnormalities; blood pressure has been normal, as has ECG. Renal function remains normal. Incompletely descended testes are palpable in the neck of the scrotum. Development of ocular abnormalities has been ruled out by means of ophthalmoscopy and slit-lamp examination.

Currently, at the age of 4 years 9 mo, the boy remains dwarfed, with a conspicuous appearance (fig. 2). Since age 3 years, however, he has grown at a normal rate of 4–5 cm/year, and he is now 84 cm tall, weighting 9.5 kg and having a head circumference 51.5 cm. Fine, silky, blond, scalp hair has developed. Also, his mental development has begun to progress satisfactorily: he communicates effectively by spoken language; toilet training has been completed; and he has been successfully integrated into a kindergarten for normal children.

RESULTS

Secretion of PDS Core Protein

Biosynthesis and secretion of the small PDS by human skin fibroblasts were studied by metabolically labeling the core protein and following its fate during a chase period. Figure 3 shows that during a pulse period of 10 min fibroblasts of the patient incorporated approximately the same amount of [^{3}H]leucine into the core protein of the small PDS as did a control cell line. The two core-protein bands were of normal size (M_r = 46,000 and M_r = 44,000, respectively), indicating the attachment of either two or three asparagine-bound oligosaccharides (Glössl et al. 1984). During the subsequent chase most of the core protein was converted to mature PDS in the control cell line, and approximately two-thirds of the radioactive proteoglycan became secreted during a chase period of 90 min. Treatment with chondroitin ABC lyase resulted in the formation of proteins of M_r = 48,000 and M_r = 45,000, respectively, which are of somewhat larger size than the intracellular core proteins because of the enzyme resistance of the polysaccharide-protein linkage region. The patient's fibroblasts, in contrast, were partially deficient in synthesizing a glycosaminoglycan chain–bearing proteoglycan and secreted glycosaminoglycan-free core protein in addition to mature PDS (fig. 4). Similar kinetics for the disappearance of immunoreactive material from the cells and for the appearance of labeled material in the culture medium were found in the control and in the patient's culture (fig. 5).

In more than 40 different fibroblast cultures studied in our laboratory, we found, at the most, traces of secretory products migrating as did glycosamino-

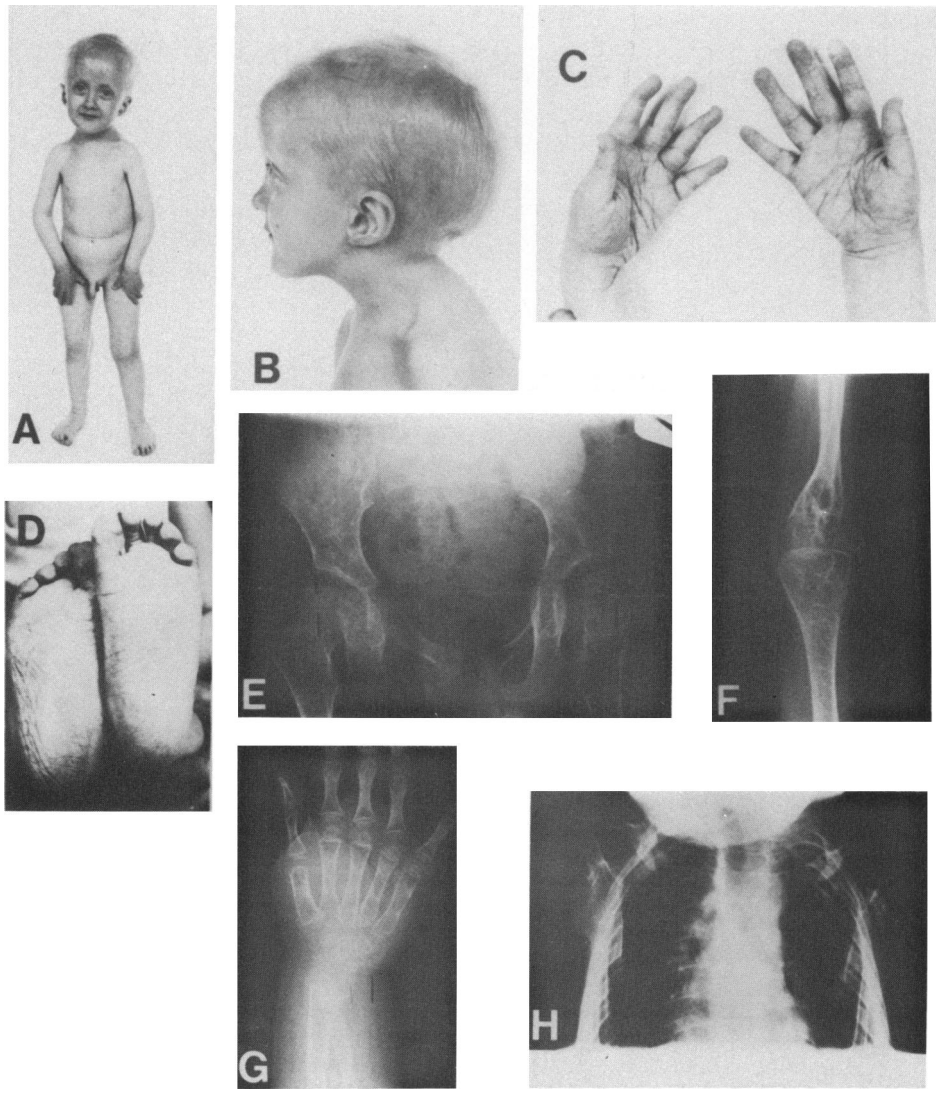


FIG. 2.—*A–D:* Appearance of patient at age 4 years 10 mo. Thin hair, abnormal scarring in the forehead, prominent eyes, small mouth, absent ear lobes, narrow chest, flat feet, and cutis gyrata in palms and soles are apparent. *E*, *F*, and *H:* Radiographs taken at age 2 years 11 mo, showing generalized osteopenia, abnormally shaped clavicles and ribs, coxa valga, and left proximal radioulnar synostosis. *G:* Radiograph of left hand at age 3 years 10 mo, showing distal radioulnar dislocation, osteopenia, and slightly advanced bone age.

glycan-free core protein during SDS/PAGE. Fibroblasts from the mother of the patient also did not show any abnormality in biosynthesis and secretion of PDS. In contrast, secretion of chain-free core protein by the patient's fibroblasts has invariably been found and was observed in cultures grown from two different skin biopsies. The proportion of free core protein in the total amount of immunoreactive material, however, varied between different experiments.

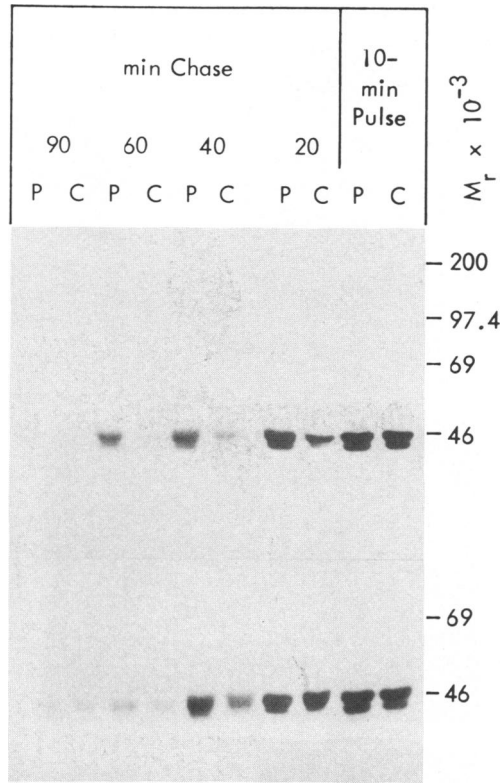


FIG. 3.—Formation of the small PDS by fibroblasts. Fibroblasts from the patient (P) and from an age-matched control person (C) were labeled with [^{3}H]leucine (~80 μCi/ml) for 10 min and subjected to chase periods for as long as 90 min. The same radioactive medium was used for sequential labeling of five flasks. Core proteins and mature proteoglycan were immune precipitated from the cell layer and treated with chondroitin ABC lyase (lower panel) or buffer alone (upper panel) prior to SDS-PAGE. The acrylamide concentration of the separation gel was 12.5%. The position of ^{14}C-methylated molecular standards (New England Nuclear) is shown on the right margin.

Densitometric scanning of the fluorograms gave values between 25% and 80% for [^{3}H]leucine-labeled core protein. The highest proportions were found after pulse or pulse-chase periods of <2 h. Decreased stability (Glössl et al. 1984) and/or increased endocytosis (Glössl et al. 1983) of free core protein could account, at least partially, for the experimental variability.

It is evident from figure 4 that the secreted chain-free core protein exhibits a slightly faster mobility than does the core protein obtained from normal cells by degradation with chondroitin ABC lyase. The former protein exhibits the same mobility as the normal intracellular protein synthesized during a short-term pulse period. Such electrophoretic behavior could therefore be expected for a protein devoid of the polysaccharide-protein linkage region.

It had been shown previously that, concomitant with the synthesis of glycosaminoglycan chains, asparagine-bound oligosaccharides of the core protein are processed to complex-type oligosaccharides (Hoppe et al. 1985). Secreted

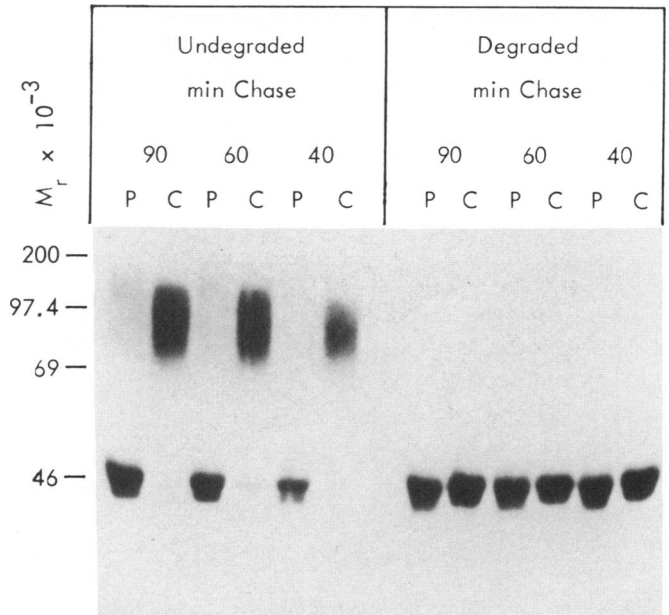


FIG. 4.—Secretion of the small PDS and of its core protein by fibroblasts. Fibroblast secretions from the patient (P) and a control (C) were obtained and further processed as described in the legend of fig. 3.

chain-free core protein from the patient showed limited sensitivity toward β-endoglucosaminidase H when three oligosaccharides were present but enzyme resistance when only two oligosaccharides were attached. A similar pattern of enzyme susceptibility was obtained with chondroitin ABC lyase–treated normal core protein (fig. 6). Thus, the core protein from the patient's fibroblasts must have been passed through the trans-cisternae of the Golgi complex, where endoglucosaminidase-H resistance is acquired (Hickman et al. 1984). Treatment of fibroblasts with 10 mM NH_4Cl did not alter the ratio of mature proteoglycans to secreted free core protein in the patient's culture; nor did it result in the secretion of free core protein in control cultures (results not shown).

As stated above, glycosaminoglycan chains are bound to serine-4 of the core protein. Antisera against intact core protein and against a synthetic pentadecapeptide representing the N-terminal sequence of the core protein were therefore used to study comparatively the immune reactivity of the chain-free and the chain-bearing core protein of the patient. Core protein and PDS were separated from each other by ion-exchange chromatography. Table 1 shows that the peptide antiserum had the same capacity for binding enzymatically prepared core protein, regardless of whether the latter was from the patient's intact proteoglycan or from a normal proteoglycan. In contrast, the peptide antiserum's capacity for binding the patient's glycosaminoglycan-free core protein was reduced.

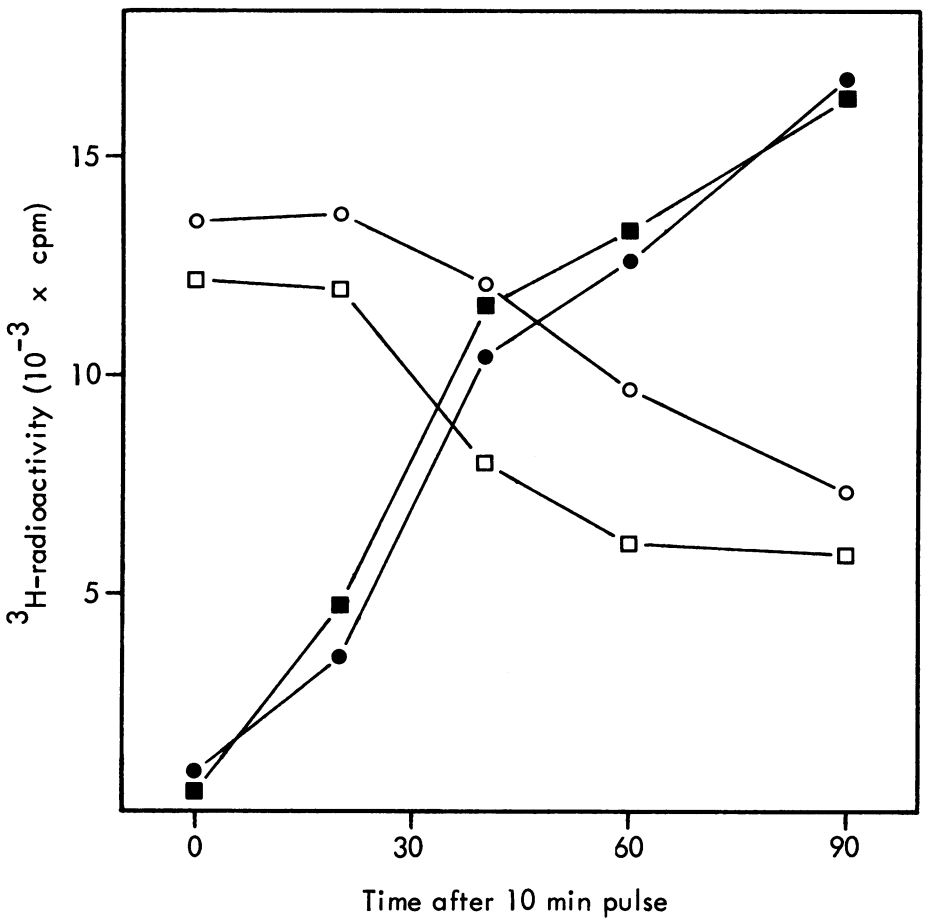


FIG. 5.—Kinetics of formation and secretion of small PDS and of its core protein. The amount of immune reactive material in the cell layer (open symbols) and in the culture medium (filled symbols) was determined in fibroblast cultures from the patient (○,●) and from a control person (□,■) after labeling as described in the legend of fig. 3.

Glycosaminoglycan Chain–synthesizing Capacity

The biosynthesis of a dermatan sulfate chain on a core protein requires the sequential action of several glycosyl transferases, of a D-glucuronyl 5-epimerase, and of sulfotransferases (fig. 1; see Rodén 1980 for review). Reduced activity of xylosyltransferase, the first enzyme of this series, could possibly lead to an impaired glycosaminoglycan biosynthesis. The patient's fibroblasts, however, exhibited even an increased xylosyltransferase activity when cartilage proteoglycan core protein was used as xylose acceptor (table 2). At least in Chinese hamster ovary cells there exists only a single xylosyltransferase, which is responsible for the initiation of chondroitin sulfate/dermatan sulfate and heparan sulfate biosynthesis (Esko et al. 1985). Faulty biosynthesis of UDP-xylose, the substrate of this reaction, can be excluded as the primary

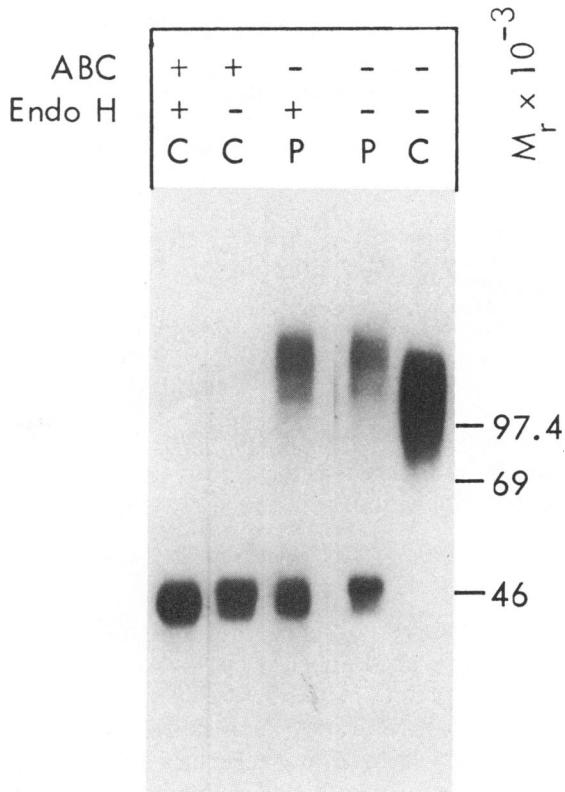


FIG. 6.—Sensitivity of [^{3}H]leucine-labeled core protein to endo-β-*N*-acetylglucosaminidase H. The small PDS and its core protein were immune precipitated from the culture media of the patient's (P) and of control (C) fibroblasts after labeling with [^{3}H]leucine (20 μCi/ml) for 3 h. The immune complex was treated with chondroitin ABC lyase (ABC) and/or endo-β-*N*-acetylglucosaminidase H (Endo H).

defect because the cells secreted normal amounts of the large PDS and of proteoheparan sulfate (fig. 7), and the amount of cell-associated proteoheparan sulfate was also normal (results not shown). Both proteoglycans contain a polysaccharide-protein linkage region identical to that of the small PDS. Lack of UDP-xylose should, therefore, concern all these proteoglycans similarly. Figure 7 demonstrates that, additionally, the fibroblasts of the patient do not secrete glycosaminoglycan peptides. The activity of the other chain-polymerizing enzymes was determined by an indirect procedure only. In the presence of exogenously added *p*-nitrophenyl-β-xyloside, glycosaminoglycan synthesis can be stimulated because (1) the amount of xylosylated core protein appears to be rate-limiting in PDS biosynthesis and (2) glycosaminoglycan chains can be synthesized on the artificial xyloside. The data shown in table 3 demonstrate that the patient's fibroblasts respond to the treatment with 1 mM *p*-nitrophenyl-β-xyloside with an excessive production of protein-free glycosaminoglycan chains. However, compared with the control cell line, very little induction of

TABLE 1

XYLOSYLTRANSFERASE ACTIVITY IN CULTURED HUMAN SKIN FIBROBLASTS

Enzyme Source	Xylosyltransferase Activity (μU/mg cell protein)
Patient:	
First biopsy	1.2
Second biopsy	1.4
Controls (N = 5):	
Mean	0.96
SD	0.15

glycosaminoglycan biosynthesis occurred in the presence of 0.01 mM *p*-nitrophenyl-β-xyloside and there was apparently also less competition for glycosaminoglycan-chain elongation between the xyloside and the xylosylated core protein.

DISCUSSION

The boy described in the present report presents a clinical picture suggestive of progeria, with dwarfing, aged appearance, craniofacial dysproportion, and generalized osteopenia. He does lack, however, several features characteristic of true Hutchinson-Gilford syndrome, such as diminished subcutaneous fat, prominent scalp veins, generalized alopecia, and joint contractures (DeBusk 1972). Neither does he conform with well-defined progeroid syndromes, since he is lacking the severe mental deficiency and neurological abnormalities of de Barsy syndrome (Kunze et al. 1985), the deafness and photosensitivity of Cockayne syndrome, and the ocular abnormalities of Hallermann-Streif syndrome. Thus, as with similar (but not identical) progeroid variants (Wiedemann 1969), diagnostic classification is difficult.

The skeletal changes—i.e., generalized osteopenia, abnormal clavicles, and coxa valga—seem compatible with those described in progeria. Even though a

TABLE 2

IMMUNE REACTIVITY OF PDS AND ITS CORE PROTEIN TOWARD ANTIBODIES AGAINST COMPLETE CORE PROTEIN (A) AND AGAINST AN N-TERMINAL PENTADECAPEPTIDE (B)

Source	B/A[a]
Control:	
PDS	0.36
PDS treated with chondroitin ABC lyase	0.83
Patient:	
PDS	0.32
PDS treated with chondroitin ABC lyase	0.86
Free core protein	0.65

[a] Data are expressed as ratio of activities that are obtained by immune precipitation with the respective antibodies.

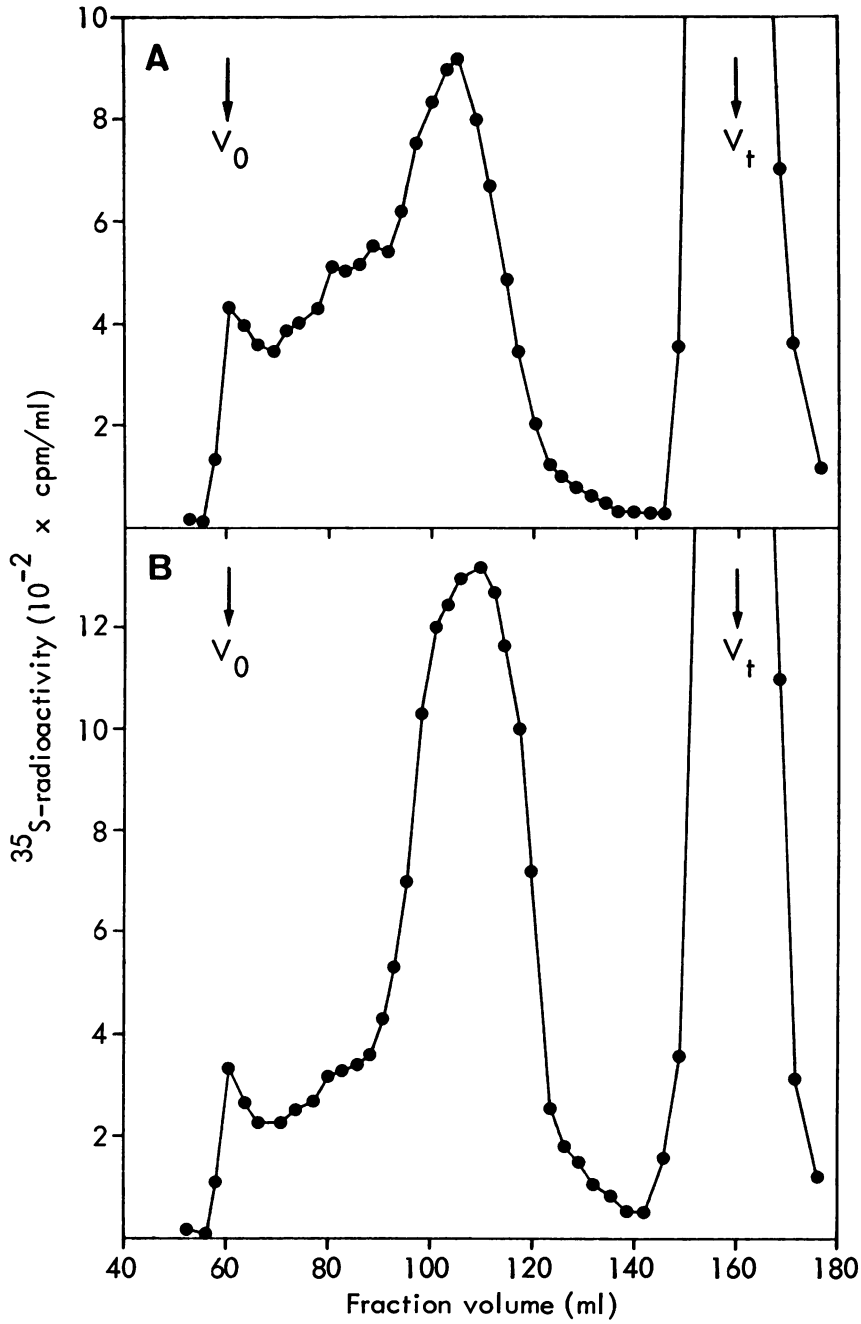


FIG. 7.—Chromatography on Sepharose CL-4B of [^{35}S]sulfate-labeled fibroblast secretions. Fibroblasts from the patient (*A*) and from a control (*B*) were incubated in modified and supplemented Eagle's minimum essential medium containing [^{35}S]sulfate (5 μCi/ml) for 90 h. Two milliliters of medium were mixed with 6 ml of ethanol. The precipitate was washed five times with ethanol and dissolved in 50 mM sodium acetate buffer, pH 5.8, containing 4 M guanidinium chloride, 0.5% Triton X-100, 0.1 M 6-aminohexanoic acid, 10 mM EDTA, 10 mM *N*-ethylmaleimide, and 5 mM benzamidine hydrochloride prior to chromatography on a Sepharose CL-4B column (1 cm × 151 cm) equilibrated with the same buffer.

TABLE 3

INFLUENCE OF *p*-NITROPHENYL-β-XYLOSIDE ON GLYCOSAMINOGLYCAN BIOSYNTHESIS

XYLOSIDE CONCENTRATION (mM)	PATIENT[a]		CONTROL[a]	
	PDS	*p*NP	PDS	*p*NP
0	96	...	254	...
0.01	85	13	173	276
0.1	77	108	90	382
1.0	54	300	67	389

NOTE.—Fibroblasts from the patient and from a control were preincubated with xyloside for 4 h and then incubated with [^{35}S]sulfate (10 μCi/ml) for an additional 4 h in the continuous presence of the xyloside before secreted PDS and *p*-nitrophenyl-β-xyloside-induced glycosaminoglycan chains were quantitated. *p*NP = *p*-nitrophenyl-β-xyloside-induced glycosaminoglycan chains.

[a] Data are expressed as $10^{-3} \cdot$ cpm · mg cell protein^{-1}.

primary radioulnar malformation is present, an osteodysplastic syndrome is not suggested. Osteogenesis imperfecta (which could account for imperfect teeth and hyperextensible joints) could be considered, but definite fractures have not occurred.

On the other hand, the presence of elastic skin with defective wound healing concomitant with hypermobile joints, hypotonic muscles, pedes plana, and cutis gyrata of palms and soles seems compatible with a mild type of Ehlers-Danlos syndrome. A female patient combining symptoms of progeroid and Ehlers-Danlos syndrome has been described previously (Bommer et al. 1961), but, in contrast to our patient, this girl exhibited a bleeding tendency and was not dwarfed. In summary, then, this case—although so far defying unambiguous diagnostic labeling—could be described as a progeroid variant with signs of Ehlers-Danlos syndrome, indicating a multisystem connective-tissue defect.

Despite the diagnostic difficulties, the fibroblasts of the patient exhibited a hitherto unknown biochemical abnormality resulting in a partial deficiency in the ability to synthesize the mature small PDS. The following several possible explanations of this abnormality have been considered:

1. There is an abnormal intracellular trafficking whereby a certain proportion of the core protein bypasses those Golgi cisternae responsible for glycosaminoglycan synthesis. Free core protein of a large chrondroitin sulfate–rich proteoglycan was found on the cell surface of cultured human melanoma cells, and it was postulated that in glycosaminoglycan-chain biosynthesis a compartment is involved that is sensitive to NH_4Cl (Harper et al. 1985). Treatment with NH_4Cl, however, did not influence the posttranslational processing of PDS in normal fibroblasts and did not affect core-protein secretion in the cells of the patient. Furthermore, processing of the oligosaccharides on the glycosaminoglycan-free core protein was similar to that of oligosaccharides on the normal proteoglycan. Secretion of β-*N*-acetylhexosaminidase by the patient's

fibroblasts was also quantitatively normal. All these results argue against abnormal intracellular transport.

2. Abnormal proteolysis occurs near the amino terminus of the core protein, where the dermatan sulfate chain is attached. A slight size reduction of the core protein would escape electrophoretic detection. If abnormal proteolysis takes place, it must occur prior to glycosaminoglycan-chain synthesis, since dermatan sulfate peptides were not secreted into the culture medium. It should also be stressed that no evidence for an increased lability of the intracellular-located core protein was obtained during pulse-chase experiments.

3. The abnormality could be due to altered properties of an enzyme involved in the synthesis of the polysaccharide-protein linkage region. The limited induction of glycosaminoglycan biosynthesis in the presence of low concentrations of *p*-nitrophenyl-β-xyloside is compatible with the existence of a K_m mutant, e.g., of galactosyltransferase I. This explanation, however, must be considered in light of normal synthesis of other proteoglycan types that contain the same linkage region. Thus, the mutant enzyme would have to be selective among the different potential substrates. On the other hand, it seems possible that the glycosaminoglycan-free core protein of the patient's fibroblasts interfered with linkage region–synthesizing enzymes in the presence of low xyloside concentrations. This assumption gains some support from the observation that, in the patient vis-à-vis the control, there was less competition between xyloside and core protein for glycosaminoglycan chain elongation.

4. An alternative explanation could be based on the assumption that the product of a mutant allele for the small PDS core protein has either an absent or a buried recognition site for glycosaminoglycan-chain synthesis. This assumption is supported by the observation that the glycosaminoglycan-free core protein is recognized less well than the chain-bearing species by antibodies directed against the N-terminal pentadecapeptide of the core protein. Work is under way to test this hypothesis by amino acid sequencing of purified core protein.

The pathogenesis of the clinical symptoms cannot be explained with certainty at the present stage of knowledge about the function of the small PDS. Ultrastructural studies have demonstrated the localization of the small PDS at the "d" band of collagen fibrils in unmineralized connective tissue (Scott and Orford 1981). Since the "d" band at the gap zone is not cationic, it has been suggested that type I collagen interacts with PDS core protein (Vogel et al. 1984; Scott and Haigh 1985). Competition between chain-free and chain-bearing core protein for collagen binding could therefore occur in the patient's tissues. Similarly, the small PDS may be associated with fibronectin. Codistribution of both macromolecules on the surface of cultured smooth-muscle cells has been demonstrated (Völker et al. 1985). Employing a sandwich enzyme-linked immunosorbent assay, we were able to demonstrate binding of PDS core protein and intact proteoglycan to heparin-binding fibronectin fragments (unpublished results). Core protein would also compete with PDS for binding to a cell-membrane receptor required for receptor-mediated endocytosis (Glössl et al. 1983). An altered turnover of PDS could therefore be taken

into account additionally, although the uptake properties of the mutant core protein have not yet been investigated. In summary, the multifunctional core protein may interact with several components of the extracellular matrix. At a reduced concentration of polyanionic glycosaminoglycan chains, potential functions such as binding of water or of calcium ions may be altered. An influence of an abnormal extracellular matrix on gene expression also seems feasible. Further work is needed to solve the many pathobiochemical problems presented by this patient.

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