

Brief Report

CLINICAL AND BIOCHEMICAL MANIFESTATIONS OF HYALURONIDASE DEFICIENCY

MARVIN R. NATOWICZ, M.D., PH.D.,
M. PRISCILLA SHORT, M.D., YU WANG,
G. RICHARD DICKERSIN, M.D., MARK C. GEBHARDT, M.D.,
DANIEL I. ROSENTHAL, M.D., KATHERINE B. SIMS, M.D.,
AND ANDREW E. ROSENBERG, M.D.

THE mucopolysaccharidoses are lysosomal storage disorders caused by a genetic deficiency of enzymes that catalyze the degradation of glycosaminoglycans (mucopolysaccharides). These disorders are clinically variable and commonly associated with mental retardation, short stature, coarse facial features, organomegaly, and an accumulation of glycosaminoglycans in tissues.

Hyaluronan (hyaluronic acid) is one of the major glycosaminoglycans and has a vital role in many physiologic processes.¹⁻⁴ Genetic deficiencies of most of the lysosomal enzymes that catalyze the degradation of glycosaminoglycans have been identified, with the exception of hyaluronidase, a lysosomal endoglycosidase that catalyzes the degradation of hyaluronan.⁵⁻⁷ We describe the clinical, pathological, and biochemical findings in a child with short stature and multiple periarticular soft-tissue masses who proved to have a storage disease of hyaluronan due to a genetic deficiency of hyaluronidase.

CASE REPORT

A 14-year-old girl had a normal early medical history except for frequent episodes of otitis media and a "ganglion" that was excised from her left wrist when she was 6 months old. At the age of 7½ years, she noted a soft-tissue mass over the lateral aspect of her left ankle. During the next year, additional periarticular masses developed involving the proximal right second finger, the left popliteal fossa, the left inferior patella, and the right and left lateral malleoli. Biopsies of the ankle and finger lesions and the skin were performed. At the age of 9½ years the patient had the first of several episodes of transient, painful swelling of the periarticular masses and generalized cutaneous swelling. These episodes accompanied or followed exertion or a febrile illness and were self-limited, resolving spontaneously within a period of 72 hours.

From the Division of Medical Genetics, Shriver Center for Mental Retardation, Waltham, Mass. (M.R.N., Y.W.); and the Departments of Neurology (M.R.N., M.P.S., K.B.S.), Pathology (M.R.N., M.P.S., G.R.D., A.E.R.), Radiology (D.I.R.), and Orthopedic Surgery (M.C.G.), Massachusetts General Hospital, Boston. Address reprint requests to Dr. Natowicz at the Division of Medical Genetics, Shriver Center for Mental Retardation, 200 Trapelo Rd., Waltham, MA 02254.

©1996, Massachusetts Medical Society.

Between the ages of 8½ and 14 years, her growth rate declined from about the 15th percentile to below the 5th percentile, some of the periarticular masses enlarged, and new masses and right-foot pain developed. Otherwise, her general health was good. At the age of 14 years, her head circumference was 53 cm (25th percentile), she weighed 43.8 kg (25th percentile), and she was 145.5 cm tall (<5th percentile). She had mildly dysmorphic craniofacial features with a flattened nasal bridge, bifid uvula, and a submucosal cleft palate. There was full range of motion of all joints and no scoliosis, kyphosis, adenopathy, or organomegaly. Her ophthalmologic and neurologic examinations were normal, and her academic performance was good. The family history and the results of routine blood chemical measurements were unremarkable.

Radiographic Findings

Radiographs of the ankles and knees at the age of nine years showed prominent bilateral nodular, periarticular soft-tissue masses. The masses were not calcified, there were no bony erosions, and the joint spaces were preserved. Magnetic resonance imaging of the left knee at the age of 10 years revealed a nodular synovium, a popliteal (Baker's) cyst, and a large joint effusion. Apart from the recent development of mild chondrocalcinosis of the right talonavicular joint, the patient's ankle and knee joints had not changed radiographically since the age of 10.

Radiographs of the pelvis at 12 years of age revealed multiple bilateral nodular, intraarticular soft-tissue masses and acetabular erosions (Fig. 1). Computed tomography and magnetic resonance imaging of the hip joints confirmed the presence of the masses and erosions and also revealed bilateral joint effusions and a possible cyst of the right trochanteric bursa. The results of other radiographic studies, including chest and spine x-ray films (at 10 and 14 years of age), radionuclide bone and liver-spleen scans (at 10 years of age), abdominal ultrasonography (at 10 years of age), and cranial magnetic resonance imaging (at 10 years of age), were normal.

METHODS

Histochemical and Electron-Microscopical Analyses

Fresh tissue specimens were processed routinely for light microscopy. Hematoxylin and eosin and special histochemical stains were applied according to standard procedures.^{8,9} Tissues for ultrastructural analyses were fixed and processed as described elsewhere.¹⁰

Biochemical Studies

Urinary mucopolysaccharides and oligosaccharides, lysosomal enzyme activities, and the results of a fibroblast sulfate-loading study were analyzed as described elsewhere.¹¹⁻¹⁸ Plasma and serum hyaluronidase activity was determined with human umbilical-cord hyaluronan (Sigma Chemical, St. Louis) (250 mg per liter) as the substrate. The results were similar for either type of specimen.¹⁸ Plasma hyaluronidase was also assayed with chondroitin (Seikagaku, Tokyo, Japan) (250 mg per liter) as the substrate. The results of these assays were normalized to standard curves of *N*-acetylglucosamine and *N*-acetylgalactosamine, respectively, and were expressed as milliunits per liter, a unit of activity representing the production of a micromole of reaction product per minute at 37°C. Plasma hyaluronan concentrations were determined by a ¹²⁵I-labeled hyaluronan-binding-protein assay (HA Test radiometric assay, Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions.

RESULTS

Histologic and Electron-Microscopical Findings

Histologic examination of the patient's ankle and finger masses showed similar pathologic changes. The ankle lesion consisted of synovium with marked



Figure 1. Radiograph of the Pelvis of a Patient with Hyaluronidase Deficiency, Showing Multiple Bilateral Bony Erosions Limited to the Acetabulum.

villonodular transformation (Fig. 2). The villi were bulbous and contained many large histiocytes that had abundant cytoplasm filled with clear vacuoles. The vacuoles stained with Alcian blue and colloidal iron, indicating the presence of mucopolysaccharide, and the degree of staining was reduced by pretreatment with hyaluronidase.

The finger lesion had the appearance of a ganglion cyst with vacuolated histiocytes lining the inner layer of the cyst wall. In both lesions other cell types, including synoviocytes, endothelial cells, and fibroblasts, were morphologically normal.

Ultrastructurally, the histiocytes from both the ankle and finger lesions were nearly filled with numerous large membrane-bound vacuoles whose appearance was consistent with that of lysosomes. The vacuoles contained a flocculent, medium-dense material, as well as a small number of dense secondary lysosomes (Fig. 2). Fibroblasts also contained the membrane-enclosed vacuoles with flocculent material but in lower quantities than the histiocytes. Endothelial cells and vascular smooth-muscle cells appeared normal. Fibroblasts in a separate skin-biopsy specimen also showed lysosomes filled with fibrillar material (data not shown).

Biochemical Findings

The results of analyses of urinary mucopolysaccharides and oligosaccharides and numerous lysosomal-enzyme activities were normal. The patient had no plasma hyaluronidase activity (Table 1); a mixing experiment that used various proportions of serum from the patient and pooled serum from control subjects provided no evidence of an inhibitor of enzyme activity (data not shown). The plasma hyaluronidase activity of the patient's father and mother

was 30 and 53 percent of normal, respectively, and two of her grandparents also had low or intermediate levels of enzyme activity (Table 1). The patient also had no detectable plasma hyaluronidase activity in assays in which chondroitin was used as the substrate, whereas the activity in plasma from her parents was 38 and 58 percent of normal (Table 1). The plasma concentrations of hyaluronan ranged from 918 to 2118 μg per liter in three specimens from the patient and were 10.4 and 8.4 μg per liter in plasma from her father and mother, respectively (mean [\pm SD] value in normal subjects, 23.7 ± 14.6 μg per liter). Plasma hyaluronidase activity was normal in 56 patients with 25 different lysosomal storage disorders, and there was no marked elevation of the plasma hyaluronan concentration in 16 patients with other storage diseases (data not shown).

DISCUSSION

Hyaluronan, one of the principal glycosaminoglycans of the extracellular matrix, is a high-molecular-weight polymer of repeating units of [*N*-acetylglucosamine (1- β -4) D-glucuronic acid (1- β -3)]_n that is synthesized in the plasma membrane of cells and translocated to the pericellular space. It is believed to have numerous important biologic functions, including the modulation of cell proliferation, migration, and differentiation and the regulation of extracellular water and protein homeostasis. It is also an integral structural component of cartilage and other tissues and acts as a lubricant in joints.¹⁻⁴ Hyaluronan is catabolized locally or after clearance by the lymphatic system and hepatic endothelial cells. Intracellular degradation occurs after endocytosis and is followed by digestion by hyaluronidase, a lysosomal endoglycosidase that cleaves the 1- β -4 *N*-acetylhexosaminide linkages.^{1,4}

We describe a patient with the mucopolysaccharidosis hyaluronidase deficiency. We based our conclusion on the following findings: pathologic storage of mucopolysaccharide in lysosomes of histiocytes and fibroblasts, the markedly elevated plasma hyaluronan concentration, and the complete deficiency of plasma hyaluronidase activity. The decreased plasma hyaluronidase activity in the patient's parents and in two of her grandparents, together with the other data, indicates that the enzyme deficiency is an autosomal recessive genetic disorder.

The principal abnormalities in our patient were periarticular soft-tissue masses composed of nodular aggregates of histiocytes, acquired short stature, and erosions of the acetabula. The first of these findings is unique for the mucopolysaccharidoses but not surprising. Hyaluronan concentrations are especially high in synovial fluid, and the highest concentrations of hyaluronan in solid tissues are in cartilage and skin.¹ In addition, much of the catabolism of hyaluronan in joints occurs locally.^{1,19} Consequently,

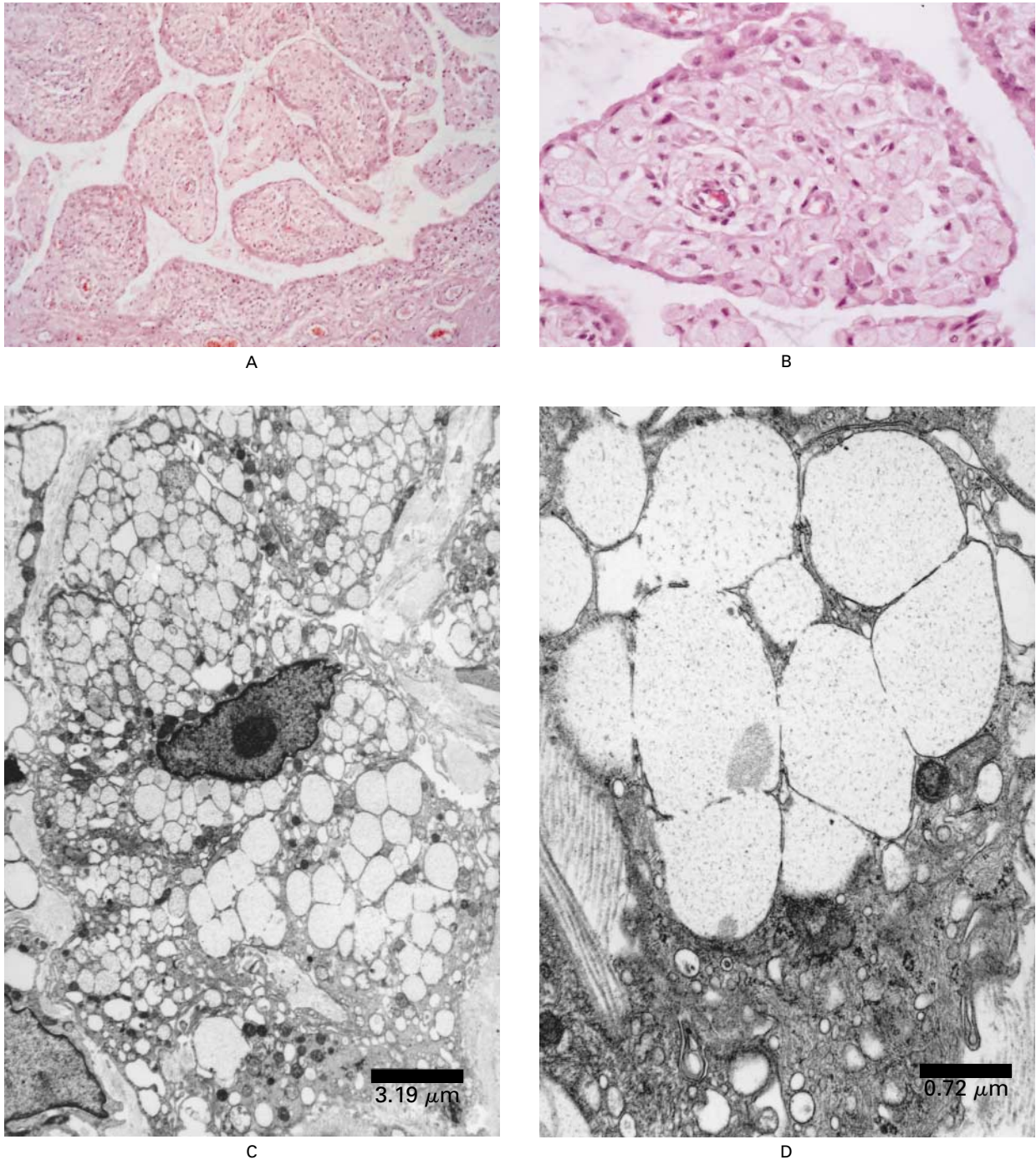


Figure 2. Histologic and Electron-Microscopical Findings in a Patient with Hyaluronidase Deficiency.

Panel A shows synovium from the ankle with prominent villonodular transformation (hematoxylin and eosin, $\times 125$). Panel B shows a cross section of a synovial frond that is expanded by numerous vacuolated histiocytes; the vacuoles are small, round, and apparently clear (hematoxylin and eosin, $\times 500$). In Panel C, an electron micrograph of a histiocyte from the finger cyst shows cytoplasm filled with membrane-bound granules that contain a medium-dense, flocculent material. Panel D shows a higher magnification of the view shown in Panel C. There are cytoplasmic granules with limiting membranes and medium-dense, flocculent contents.

TABLE 1. PLASMA HYALURONIDASE ACTIVITY IN THE PATIENT, HER FAMILY, AND CONTROL SUBJECTS.*

SUBJECT	HYALURONIDASE ACTIVITY	CHONDROITINASE ACTIVITY
	mU/liter (% of normal)	
Proband	0	0
Mother	2379 (53)	1662 (58)
Father	1349 (30)	1078 (38)
Maternal grandmother	2543 (57)	2505 (88)
Maternal grandfather	1441 (32)	1664 (58)
Paternal grandmother	791 (18)	1290 (45)
Paternal grandfather	3074 (69)	3454 (121)
Control subjects	4476±1144	2858±689

*Enzyme activities were determined as described in the Methods section. The values for the proband were determined by duplicate analyses of four separate specimens; the values for the parents were determined from duplicate analyses of two separate specimens for the hyaluronidase assay and from two duplicate analyses of a single specimen for the chondroitinase assay; the values for the grandparents were determined from two duplicate analyses of a single specimen; and a single duplicate analysis was performed for each control specimen. Blood samples from 100 normal subjects were used to establish the reference range for hyaluronidase activity, and blood samples from 20 normal subjects were used to establish the reference range for chondroitinase activity. Plus-minus values are means ±SD.

the loss of a major mechanism of hyaluronan catabolism, its degradation by hyaluronidase, could result in the development of periarticular soft-tissue masses. The histiocytic predominance at those sites might be the result of the hyaluronan-induced aggregation of macrophages.²⁰ The marked intracellular storage of substrate in macrophages was presumably due to receptor-mediated internalization of hyaluronan by these cells, in conjunction with the enzymatic deficiency.^{4,21,22} The lesser involvement of fibroblasts (and of the skin) and, possibly, other tissues may be due to less efficient internalization of hyaluronan, the delivery of hyaluronan to the lymphatic system, or other enzymatic or nonenzymatic degradative pathways.²³⁻²⁶

The patient's short stature might be the result of impaired function of growth-plate chondrocytes or abnormal properties of the extracellular matrix. Hyaluronan is internalized by chondrocytes by a receptor-mediated process²⁷ and might, if it accumulates, cause dysfunction of these cells; intracellular accumulation of macromolecular substrates and consequent chondrocytic abnormalities have been noted in other lysosomal storage disorders.^{28,29} Hyaluronan reduces the synthesis of constituents of the matrix of hyaline cartilage by chondrocytes and their precursors^{2,3} and interacts with other matrix components.^{1,4} Abnormal concentrations of hyaluronan could alter the normal biochemical and physical interactions between hyaluronan and other matrix components and thereby alter the assembly and sta-

bility of the skeletal matrix. Any of these pathophysiologic mechanisms could result in short stature.

It is curious that the periarticular aggregates of histiocytes caused bone erosions detected only in the acetabula. Activated macrophages secrete a variety of cytokines that can stimulate localized osteoclastic activity.³⁰ Their accumulation in an anatomically tight joint such as the hip joint, in which the cells are in close proximity to bone, may explain why these joints are the only sites where bone erosions were present.

The lack of hyaluronidase activity, coupled with continued biosynthesis of its substrate, suggests that the patient may be at risk for slow progression of her condition. The success of exogenous enzyme replacement in patients with type I Gaucher's disease,³¹ another lysosomal storage disorder that predominantly involves the reticuloendothelial system, suggests that the administration of hyaluronidase may be an appropriate form of treatment.

We are indebted to Drs. James England, Richard I. Kelley, Elizabeth M. Prence, Jerry N. Thompson, and Barbara Triggs-Raine for their critical evaluations of this work; to the members of the Division of Medical Genetics, Shriver Center for Mental Retardation, for performing the assays of lysosomal enzymes and metabolites; and to Dr. Joe Alroy, New England Medical Center, for performing the electron-microscopical analysis of the skin-biopsy specimens.

REFERENCES

1. Laurent TC, Fraser JRE. Hyaluronan. *FASEB J* 1992;6:2397-404.
2. Nathanson MA. Hyaluronates in developing skeletal tissues. *Clin Orthop* 1990;251:275-89.
3. Toole BP. Proteoglycans and hyaluronan in morphogenesis and differentiation. In: Hay ED, ed. *Cell biology of extracellular matrix*. 2nd ed. New York: Plenum Press, 1991:305-41.
4. Knudson CB, Knudson W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. *FASEB J* 1993;7:1233-41.
5. Meyer K. Hyaluronidases. In: Boyer PD, ed. *The enzymes*. Vol. 5. Hydrolysis (sulfate esters, carboxyl esters, glycosides), hydration. New York: Academic Press, 1971:307-20.
6. Barrett AJ, Heath MF. Lysosomal enzymes. In: Dingle JT, ed. *Lysosomes: a laboratory handbook*. 2nd ed. Amsterdam: Elsevier/North Holland Biomedical Press, 1977:19-146.
7. Kreil G. Hyaluronidases — a group of neglected enzymes. *Protein Sci* 1995;4:1666-9.
8. Histochemical methods for the demonstration of carbohydrates. In: Thompson SW, Hunt RD. *Selected histochemical and histopathological methods*. Springfield, Ill.: Charles C Thomas, 1966:370-575.
9. Cook HC. Carbohydrates. In: Bancroft JD, Stevens A, Turner DR, eds. *Theory and practice of histological techniques*. 3rd ed. Edinburgh, Scotland: Churchill Livingstone, 1990:177-213.
10. Nielson GP, Dickersin GR, Provenzal JM, Rosenberg AE. Lipomatous hemangiopericytoma: a histologic, ultrastructural and immunohistochemical study of a unique variant of hemangiopericytoma. *Am J Surg Pathol* 1995;19:748-56.
11. Humbel R, Marchal C, Fall M. Differential diagnosis of mucopolysaccharidosis by means of thin-layer chromatography of urinary acidic glycosaminoglycans. *Helv Paediatr Acta* 1969;24:648-50.
12. Humbel R, Collart M. Oligosaccharides in urine of patients with glycoprotein storage diseases. I. Rapid detection by thin-layer chromatography. *Clin Chim Acta* 1975;60:143-5.
13. Holmes EW, O'Brien JS. Separation of glycoprotein-derived oligosaccharides by thin-layer chromatography. *Anal Biochem* 1979;93:167-70.
14. Prence EM, Natowicz MR. Diagnosis of alpha-mannosidosis by measuring alpha-mannosidase in plasma. *Clin Chem* 1992;38:501-3.
15. Triggs-Raine B, Richard M, Wasel N, Prence EM, Natowicz MR. Mu-

tational analysis of Tay-Sachs disease: studies on Tay-Sachs carriers of French Canadian background living in New England. *Am J Hum Genet* 1995;56:870-9.

16. Natowicz MR, Stoler JM, Prence EM, Liscum L. Marked heterogeneity in Niemann-Pick disease, type C: clinical and ultrastructural findings. *Clin Pediatr* 1995;34:190-7.

17. Wenger DA, Williams C. Screening for lysosomal disorders. In: Hommes FA, ed. *Techniques in diagnostic human biochemical genetics: a laboratory manual*. New York: Wiley-Liss, 1991:587-617.

18. Natowicz MR, Wang Y. Human serum hyaluronidase: characterization of a clinical assay. *Clin Chim Acta* 1996;245:1-6.

19. Laurent UBG, Fraser JRE, Engstrom-Laurent A, Reed RK, Dahl LB, Laurent TC. Catabolism of hyaluronan in the knee joint of the rabbit. *Matrix* 1992;12:130-6.

20. Green SJ, Tarone G, Underhill CB. Aggregation of macrophages and fibroblasts is inhibited by a monoclonal antibody to the hyaluronate receptor. *Exp Cell Res* 1988;178:224-32.

21. Culty M, Nguyen HA, Underhill CB. The hyaluronan receptor (CD44) participates in the uptake and degradation of hyaluronan. *J Cell Biol* 1992;116:1055-62.

22. Bertolami CN, Berg S, Messadi DV. Binding and internalization of hyaluronate by human cutaneous fibroblasts. *Matrix* 1992;12:11-21.

23. Rodén L, Campbell P, Fraser JRE, Laurent TC, Pertoft H, Thompson JN. Enzymic pathways of hyaluronan catabolism. In: Evered E, Whelan J,

eds. *The biology of hyaluronan*. Chichester, England: John Wiley, 1989: 60-86.

24. Reed RK, Laurent UBG, Fraser JRE, Laurent TC. Removal of [³H]hyaluronan injected subcutaneously in rabbits. *Am J Physiol* 1990; 259:H532-H535.

25. Tammi R, Saamanen A-M, Maibach HI, Tammi M. Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture. *J Invest Dermatol* 1991;97:126-30.

26. Uchiyama H, Dobashi Y, Ohkouchi K, Nagasawa K. Chemical change involved in the oxidative reductive depolymerization of hyaluronic acid. *J Biol Chem* 1990;265:7753-9.

27. Hua Q, Knudson CB, Knudson W. Internalization of hyaluronan by chondrocytes occurs via receptor-mediated endocytosis. *J Cell Sci* 1993; 106:365-75.

28. Rimoin DL, Silberberg R, Hollister DW. Chondro-osseous pathology in the chondrodystrophies. *Clin Orthop* 1976;114:137-52.

29. Nogami H, Oohira A, Ozeki K, Oki T, Ogino T, Murachi S. Ultrastructure of cartilage in heritable disorders of connective tissue. *Clin Orthop* 1979;143:251-9.

30. Roodman GD. Role of cytokines in the regulation of bone resorption. *Calcif Tissue Int* 1993;53:Suppl 1:S94-S98.

31. Beutler E. Gaucher disease: new molecular approaches to diagnosis and treatment. *Science* 1992;256:794-9.

IMAGES IN CLINICAL MEDICINE

Images in Clinical Medicine, a weekly *Journal* feature, presents clinically important visual images, emphasizing those a doctor might encounter in an average day at the office, the emergency department, or the hospital. If you have an original unpublished, high-quality color or black-and-white photograph representing such a typical image that you would like considered for publication, send it with a descriptive legend to Karen Pedersen, *New England Journal of Medicine*, 10 Shattuck St., Boston, MA 02115. For details about the size and labeling of the photographs, the requirements for the legend, and authorship, please contact Karen Pedersen at 617-734-9800 (phone) or 617-739-9864 (fax), or the *New England Journal of Medicine* at images@edit.nejm.org (e-mail).
