# *N*-Acetylglucosamine-6-Sulfate Sulfatase in Man: Deficiency of the Enzyme in a New Mucopolysaccharidosis

LEONARD C. GINSBERG, PATRICIA V. DONNELLY, DANIELA T. DI FERRANTE, NICOLA DI FERRANTE,[53] AND C. THOMAS CASKEY

*Laboratories of Connective Tissue Research of the Department of Biochemistry (L.C.G., P.V.D., D.T.DiF., N.DiF.) and the Division of Medical Genetics of the Department of Medicine (C.T.C.), Baylor College of Medicine, Houston, Texas, USA*

## Summary

**The study of a 5-year old patient with a mucopolysaccharidosis different from those already known has given the opportunity to describe the clinical, biochemical and enzymic characteristics of *N*-acetylglucosamine-6-sulfate sulfatase deficiency.**

**The patient was delayed physically and mentally, was hyperactive, and had a short attention span. He had fair complexion and very blond, but coarse and excessive hair. He had mild hepatosplenomegaly and dysostosis multiplex, including hypoplastic odontoid process of the atlas, ovoid vertebral bodies, small femoral epiphyses, and modest enlargement of the ribs. He had a mild, bilateral conductive hearing loss. The urine gave a strongly positive spot test with Azur A paper. Quantitative measurement of urinary glycosaminoglycans demonstrated increased excretion and inadequate degradation of heparan sulfate and keratan sulfate. The microscopic examination of the stained peripheral leukocytes revealed metachromatic material which formed a ring on the inner aspect of the cellular membrane in 20% of the lymphocytes. The rate of degradation of $^{35}SO_4$-labeled intracellular glycosaminoglycans by cutaneous fibroblasts was delayed and inadequate.**

**The measurements of several lysosomal enzymes whose deficiencies are responsible for the known mucopolysaccharidoses gave results within normal ranges. Galactose-6-sulfate (Gal-6-S), *N*-acetylgalactosamine-6-sulfate (GalNAc-6-S), and *N*-acetylglucosamine-6-sulfate (GlcNAc-6-S) were prepared with chlorosulfonic acid, according to the method of Suzuki and Strominger (38). After chromatographic purification, aliquots of the three 6-sulfated monosaccharides were reduced with sodium borotritide. Measurements of Gal-6-S sulfatase, GalNAc-6-S sulfatase, and GlcNAc-6-S sulfatase were performed on extracts of normal cultured fibroblasts and of fibroblasts of the propositus, his parents, and several Morquio patients. Additionally, GalNAc-6-S sulfatase and GlcNAc-6-S sulfatase activities were also measured, using as substrate 500 nmol tetrasaccharide obtained from either chondroitin-6-sulfate or nonradioactive GlcNAc-6-S. The measurement demonstrated that the propositus has a defective GlcNAc-6-S sulfatase activity, but normal activities for Gal-6-S and GalNAc-6-S sulfatase.**

**These findings are in contrast with those found with fibroblast extracts of Morquio patients, since they have low or nondetectable activities of Gal-6-S and GalNAc-6-S sulfatase but normal GlcNAc-6-S sulfatase activity.**

**Extracts of leukocytes and fibroblasts of the propositus' parents, when tested with radioactive or nonradioactive substrates, had normal levels of GalNAc-6-S sulfatase activity but decreased levels of GlcNAc-6-S sulfatase activity (50–60% of normal).**

## Speculation

**The possible existence of two different sulfatases, specific for 6-sulfated hexoses with the glucose or galactose configuration, has been suggested (10).**

**The study of a patient with a novel mucopolysaccharidosis, characterized by defective degradation of keratan sulfate and heparan sulfate, has provided the opportunity of describing the clinical and biochemical features of GlcNAc-6-S sulfatase deficiency. These findings confirm that the defective degradation of keratan sulfate and chondroitin-6-sulfate typical of classic Morquio disease is caused by the deficiency of a sulfatase specific for Gal-6-S and GalNAc-6-S.**

## INTRODUCTION

The pathogenesis of several mucopolysaccharidoses has been clarified during the last ten years essentially by the experimental work performed in the laboratories of Dorfman and Neufeld. Using cultured skin fibroblasts, Neufeld and collaborators (12,13) demonstrated that those derived from affected patients had a defective degradation of intracellular glycosaminoglycans (GAG) which could be corrected by the addition to their cultures of concentrates of medium of normal fibroblasts or of fibroblasts derived from patients with a different mucopolysaccharidoses. These findings led Dorfman and collaborators (11) to the use of natural or synthetic substrates for the identification of enzymes involved in the degradation of the stored GAG. Thus, following these different and complementary approaches, the enzyme deficiencies responsible for the impaired degradation of dermatan sulfate and/or heparan sulfate (DS, HS) which occurs in Hurler/Scheie (3,27,40), Hunter (2,36), Sanfilippo A (20) and β-glucuronidase deficiency (17) diseases were identified. Subsequently, the enzyme deficiencies occurring in Sanfilippo B (29) and Maroteaux-Lamy (37) diseases have also been identified.

In contrast with the rather close sequence in which those discoveries were made, the identification of the enzyme deficiencies related to impaired degradation of keratan sulfate (KS) has been delayed and made more difficult by the impossibility of studying KS metabolism in cultured skin fibroblasts. These cells, in fact, do not synthesize KS.

Morquio disease became separated from the various osteochondrodysplasias when it was demonstrated that affected patients have excessive keratansulfaturia (30) and accumulate KS and chondroitin-6-sulfate (C-6-S) in cartilage (31). In 1974 Matalon et al. (26) performed enzyme measurements with biologically labeled, polymeric $^{35}SO_4$-chondroitin-4/6-sulfate and oligosaccharides derived from it and concluded that a hexosamine-6-sulfate sulfatase activity was absent or greatly decreased in homogenates of Morquio skin fibroblasts. As a result of these findings it was suggested that the impaired degradation of KS and C-6-S in Morquio disease could be ascribed to the deficiency of an enzyme normally active on N-acetylglucosamine-6-sulfate (GlcNAc-6-S) and galactose-6-sulfate (Gal-6-S) residues of KS and on N-acetylgalactosamine-6-sulfate (Gal NAc-6-S) residues of C-6-S (10). In 1976 Singh et al. (33) used as substrate tetrasaccharides prepared from C-6-S and suggested that the enzyme defective in Morquio disease was GalNAc-6-S sulfatase.

Because an impaired degradation of HS (which contains N-acetyl and N-sulfated glucosamine-6-sulfate residues) has not been found to be present in Morquio disease, Neufeld suggested that different 6-sulfatases might exist, specific either for the glucose or the galactose configuration of their substrates (quoted in (10)). If this hypothesis were correct, deficiency of a sulfatase normally active on Gal-6-S and on GalNAc-6-S should be associated with Morquio disease, while deficiency of GlcNAc-6-S sulfatase should be associated with a hitherto unknown syndrome of impaired degradation of KS and HS.

The study of a five year old patient with a mucopolysaccharidosis different from those already known has given us the opportunity to demonstrate the correctness of Neufeld's hypothesis.

In previous publications we have provided evidence that classical Morquio disease is associated with defective Gal-6-S and GalNAc-6-S sulfatase activity (8,33). Here we describe the clinical, biochemical and enzymic observations of the patient affected by GlcNAc-6-S sulfatase deficiency.

## Clinical description

G.G., a five year old Caucasian male, only live offspring of a nonconsanguineous couple, was the product of term pregnancy and normal delivery. At birth he weighed 3.5 Kg and had fronto-occipital circumference and body length of 31.5 and 49.5 cm, respectively. Thirteen hours after birth he developed cyanosis, thought to be caused by hyaline membrane disease, and for this episode he was kept one week in atmosphere of oxygen supplementation.

At eight months of age he could stand and use three words; at 11 months he could walk and at three years he could use short phrases, ride a tricycle and dress himself.

At age 14 months he had febrile seizures characterized by extension of the extremities. These episodes occurred again twice, and for the last time, at two and one-half years of age. Since then, the patient has been maintained on phenytoin sodium (Dilantin).

Because of his delayed physical and mental development, the patient has been evaluated repeatedly. When three years, three months of age he was found to have the mental language of a 21 month old child. At four years of age, he was found to be able to use several sentences, to be hyperactive and to have a short attention span. Six months later, a Peabody Picture Vocabulary test gave a score of 59 and a Slosson Intelligence test of 69. Six months later, these tests gave scores of 62 and 58, respectively, and an I.Q. of 50-60. He has been attending special education classes, up to the present time.

The family history does not reveal children similarly affected. The parents have had two additional pregnancies, one terminated spontaneously at the first trimester, the other terminated electively.

Physical examination reveals a hyperactive male, 100 cm high (less than third percentile), weighing 18.6 Kg, with fair complexion and capable of following instructions. The head and chest circumferences are 54 and 59.9 cm, respectively. The facies is not remarkable, but the hair, very blond, is coarse and excessive. There is no evidence of corneal clouding, gum hyperplasia, lymphoadenopathy or thyroid enlargement, cardiomegaly or cardiac murmurs; but there is mild hepatosplenomegaly, confirmed by radiological scanning.

The skeletal radiographic survey shows features of dysostosis multiplex, to include J-shaped sella turcica; hypoplastic and dysplastic odontoid process of the atlas; ovoid shape of the lower dorsal vertebral bodies, with bulging of the vertebral inplates; small femoral epiphyses with horizontal acetabular roofs; modest enlargement of the lateral portion of the ribs and of the medial ends of the clavicles; slight tapering of the proximal ends of the metacarpals and of the distal ends of the proximal phalanges.

Audiometric examination reveals mild, bilateral conductive hearing loss.

Routine laboratory examinations were normal and did not reveal any impairment of liver function or of the blood clotting system. Hematologic examination was normal, except for the presence of metachromatic granulations in peripheral leukocytes (see Methods and Results).

## METHODS

The urinary excretion of polymeric GAG and of oligosaccharides was measured on two sporadic urine specimens collected in January and April 1976 and on a 24 hour specimen collected in October 1976, using previously described techniques (7,9,23). Additional procedures, modified from those of Wolfe et al. (41) were used to investigate whether some urinary GAG did not precipitate with cetylpyridinium chloride (CPC) (42) and remained in the supernatant.

The presence of Reilly bodies in peripheral leukocytes was investigated as follows: 10 ml of venous blood were mixed in a vertical test tube with 4 ml of polyvinylpyrrolidone solution (PVP-40, Sigma Chemical Co. (43), M.W. 40,000; 3.5 g/100 ml saline) and 0.1 ml of aqueous 0.27 M ethylenediaminotetraacetate solution (2.05 g EDTA and 7.98 g of EDTA, tetrasodium salt/liter). Two hours later the supernatant was removed by aspiration and centrifuged at room temperature for 10 minutes at 700 g. The supernatant was discarded and aliquots of the sediment smeared on glass slides as for normal blood smears. The slides were air dried, fixed in absolute methanol for 10 minutes and stained for 2 minutes in 0.5% solution of toluidine blue (44) in 25% acetone in water. The slides were washed in three changes of absolute acetone for 10-15 seconds each, cleared for 1 minute in xylene and examined for intracellular metachromatic material with the microscope, under oil immersion.

Normal and mutant cultured skin fibroblasts (informed consent was obtained before performing skin biopsies), cultured as described previously (34) were removed by trypsinization from one or more 75 $cm^2$ plastic plates and collected by centrifugation (34). The pellets, suspended in 1 ml of saline and immersed in an ice bath, were sonicated for 20 seconds (Heat Systems (45) sonicator model W200R, 30 watts, microtip). The homogenates were dialyzed overnight at 4°C against saline and the retentates were cleared by centrifugation at 20,000 g for 10 minutes at 4°C. Aliquots of the clear supernatants were used for protein measurement (25) and enzyme assays. Additional plates of cultured skin fibroblasts from the propositus and a normal control were used as previously described (34) for the measurements of $^{35}SO_4$ uptake and degradation of $^{35}SO_4$-labeled intracellular GAG. The latter parameter was also evaluated after a concentrate of medium obtained from Sanfilippo A cultured fibroblasts was added to cultures of the propositus' fibroblasts.

Gal-6-S, GalNAc-6-S and GlcNAc-6-S were prepared with chlorosulfonic acid, according to the method of Suzuki and Strominger (38), using D-galactose (Fisher Scientific Co. (44)), N-acetyl-D-galactosamine (Sigma Chemical Co. (43), Lot 26C-0103-1) and N-acetyl-D-glucosamine (Chas. Pfizer & Co. (46), Lot 86146-05-EPD) as starting materials. The barium salts of the sulfated products, separated from the respective non-reacted material by precipitation with absolute ethanol, were first converted to the potassium salt by chromatography through Dowex 50 x 8 (48) in the $H^+$ form, followed by neutralization with a 1% KOH solution, and then desalted by gel filtration on Biogel P-2 (47) columns (2 x 180 cm, 50-100 mesh) packed and eluted with water. The products were purified by paper chromatography, using solvent B (butanol, glacial acetic acid, 1 N $NH_4OH$ 2:3:1) of Dietrich, Silva and Michelacci (6). The various bands, visualized on a pilot strip with the silver nitrate spray (39), were eluted with water, lyophilized and analyzed by infrared spectroscopy; for sulfate content (16), after hydrolysis with 25% formic acid for 14 hours at 110°C; for neutral sugars (32), reducing groups (21) and hexosamine content (14) in order to determine the sulfate/hexose molar ratios.

Subsequently, preparations of the three 6-sulfated monosaccharides were reduced with sodium borotritide according to Horton and Philips (19) and the specific activity of each [1-$^3$H]-labeled hexitol-6-sulfate was calculated as cpm/nmole sulfate (16).

## Enzyme studies

Measurements of α-L-iduronidase (18), L-idurono-2-sulfate sulfatase (15), α-N-acetylglucosaminidase (34), β-galactosidase (5), N-acetylgalactosamine-4-sulfate sulfatase (arylsulfatase B) (35) and β-glucuronidase (34) were performed using as a source of enzyme either the propositus' serum or leukocytes extracts.

Measurements of Gal-6-S sulfatase, GalNAc-6-S sulfatase and GlcNAc-6-S sulfatase were performed on extracts of normal cultured fibroblasts and of fibroblasts derived from the propositus, his parents and several Morquio patients with the following method. Fifty µl of fibroblasts extracts in saline and 10 µl of 0.6 M acetate buffer pH 4.8 (containing 5% bovine serum albumin, Miles (48)) were mixed in plastic centrifuge microtubes with 40 µl of water containing either 100 nmol of [1-$^3$H]-galactitol-6-sulfate (2700 cpm/nmole) or 150 nmol of [1-$^3$H]-N-acetylgalactosaminitol-6-sulfate (1200 cpm/nmole) or 180 nmol of [1-$^3$H]-N-acetylglucosaminitol-6-sulfate (1200 cpm/nmole). Control tubes, containing only the fibroblasts extract in buffer or each of the substrates in water, were also prepared. All these tubes were incubated at 37°C for 5 hours. Upon termination of the incubations the enzyme and substrates control tubes were mixed and all the tubes were immerged for 2 minutes in boiling water. After centrifugation for 1 minute at 10,000 g in a microfuge, each clear supernatant was aspirated, added to 0.8 ml of water and applied to a column of Dowex 1 x 8 (47) (5 x 20 mm, 200-400 mesh, $Cl^-$ form), packed in water. Because of their anionic charge, the sulfated substrates are retained on the column, while the desulfated products are not. Each water effluent, plus 0.3 ml of water wash, were collected directly into counting vials, containing 10 ml of Aquasol (New England Nuclear (49)), cooled and counted for 20 minutes in a Packard Tri-Carb spectrometer. Results were expressed as nmoles of product obtained in one hour/mg protein. These were calculated by subtracting the cpm of the control tubes (1% of the total cpm incubated in the case of galactosaminitol-6-sulfate and glucosaminitol-6-sulfate; 4% in the case of galactitol-6-sulfate) from the cpm of the corresponding tubes in which the substrate was incubated with fibroblasts extracts. Fibroblasts extracts with normal enzyme activities had a minimum of 3000 cpm above control values while those of mutant fibroblasts varied between 0 and 100 cpm above control values.

Additionally, GalNAc-6-S sulfatase activity of homogenates of normal fibroblasts, of Morquio fibroblasts and of the propositus' fibroblasts was measured with a micro-modification of the method described by Singh et al. (33), using as a substrate 500 nmol of tetrasaccharides obtained from C-6-S. GlcNAc-6-S sulfatase activity of the same homogenates was also measured as described above, using 500 nmol of non-radioactive, synthetic GlcNAc-6-S and an incubation period of 24 hours. In both cases, enzymic activity was expressed in terms of inorganic sulfate released in one hour per mg protein, the sulfate being measured with a modification of the rhodizonate method (16).

## RESULTS

The propositus' urine always gave a strongly positive "spot test" with Azur A paper (49). Quantitative measurements of the urinary GAG (Table I) demonstrated an increased excretion and inadequate degradation of hexuronate-con-

taining GAG as indicated by the high ratio polymeric GAG/oligosaccharides. HS measured as 2-deoxy-2-sulfoaminohexose (28) represented approximately 25% of the total. The latter GAG also appeared to be inadequately degraded.

Since it is known that KS (galactose-containing GAG) is not completely precipitated with CPC (1), it was measured not only in the CPC precipitate but also in the CPC supernatant. The latter was digested with pronase (50) and dialyzed; the non-dialyzable material was precipitated with absolute ethanol. The large amount of KS excreted by the patient was poorly degraded and almost equally distributed between CPC precipitate and supernatant.

The microscopic examination of the stained peripheral leukocytes revealed that in 20% of the lymphocytes, the intracellular metachromatic material formed a ring on the inner aspect of the cellular membrane, while in 1-2% of the granulocytes it was distributed in concentric rings or pretzel shaped structures.

Figure 1 shows that appreciable differences were not found when the rate of $^{35}SO_4$ uptake by the propositus' fibroblasts was compared to that of control fibroblasts. Vice versa, the rate of degradation of $^{35}SO_4$-labeled, intracellular GAG appeared to be delayed and inadequate throughout the period studied. This inadequate degradation was corrected when a concentrate of medium derived from Sanfilippo A cultured fibroblasts was added to the propositus' culture at the beginning of the experiment.

The infrared analyses of the purified 6-sulfated monosaccharides prepared for the measurement of the various 6-sulfatases showed a strong absorption band at 1235 $cm^{-1}$, typical of S-O stretching, and minor ones at 775, 825 and 995-1000 $cm^{-1}$, indicative of sulfate groups in the 6 position. No spectroscopic evidence was obtained for sulfate in the 4 position. Analyses of the monosaccharide and sulfate content of the three preparations gave the following sulfate/hexose molar ratios: 1.02, 0.8, 1.0, respectively for GlcNAc-6-S, Gal-6-S and GalNAc-6-S.

The measurement of several lysosomal enzymes whose deficiencies are responsible for the known mucopolysaccharidoses, when performed on the propositus' serum or on extracts of his leukocytes gave results well within the normal range (Table II). However, the measurement of the various 6-sulfatase activities performed with the various substrates prepared, either radioactive or non-radioactive, demonstrated (Table III) that the propositus has a defective GlcNAc-6-S sulfatase activity, but normal activities for Gal-6-S and GalNAc-6-S sulfatase.

These findings are in contrast with those found with fibroblasts extracts of Morquio patients, since they have low or non-detectable activities of Gal-6-S and GalNAc-6-S sulfatase but normal GlcNAc-6-S sulfatase activity.

Data not presented have demonstrated that normal enzymic activity was not inhibited when extracts of normal fibroblasts were mixed, in several proportions, with extracts of the propositus' fibroblasts.

Extracts of leukocytes and fibroblasts of the propositus' parents, when tested with radioactive or non-radioactive substrates (Table III) had normal GalNAc-6-S sulfatase activity and decreased levels of GlcNAc-6-S sulfatase activity.

## DISCUSSION

The conspicuous excretion of urinary GAG, with positive Azur A spot test, and the presence of metachromatic inclusions in the peripheral leukocytes indicate that the propositus is affected by a mucopolysaccharidosis.

Qualitative analyses of the urinary GAG have demonstrated that they consist essentially of polymeric KS and HS. Since keratansulfaturia does not give a positive Azur A test, this must be attributed to the increased excretion of HS and other hexuronate-containing GAG. The excretion of HS is certainly larger than that reported in Table I in terms of mg of 2-deoxy-2-sulfoaminohexose (22), because approximately 50% of the glucosamine present in HS is N-acetylated (4,24) and unreactive with the nitrous acid-indole reaction of Lagunoff and Warren (22,23).

Since this type of mucopolysaccharidurie and the particular distribution of intracellular metachromatic material have not been reported previously, it seemed possible that the patient could be affected by a new mucopolysaccharidosis, possibly of the type suggested by Neufeld to be caused by the deficiency of an enzyme normally participating in the degradation of KS and HS.

This possibility was supported by the clinical and radiological examinations of the patient (Table IV) which demonstrated a phenotype characterized by the association of findings typical of diseases in which HS or KS accumulate (28). In fact, the mental retardation, hyperactivity, excessive and coarse hair and history of seizures, typical of Sanfilippo diseases, were found to be associated with retarded growth, dysplasia of the odontoid process of the atlas and mild, diffuse skeletal alterations, typical of incipient Morquio disease.

On the other hand, it was clear that the patient did not have any of the known mucopolysaccharidoses. In fact, the measurements of the various lysosomal enzymes listed in Table II eliminated the diagnosis of Hurler/Scheie, Hunter, Sanfilippo B, Maroteaux-Lamy and β-glucuronidase deficiency. That of Morquio disease was very likely excluded because of the excessive heparansulfaturia and the delayed degradation of intracellular GAG by the patient's cultured fibroblasts. Finally, the correction of this parameter in presence of media concentrate from Sanfilippo A fibroblasts and the excessive keratansulfaturia excluded the diagnosis of Sanfilippo A.

To demonstrate that the patient was affected by a mucopolysaccharidosis with impaired degradation of KS and HS, it became necessary to prepare a substrate for the measurement of a sulfatase active on the sulfate present in position 6 of GlcNAc residues, since this is the only functional group common to the repeating unit of KS and HS (Fig. 2). And since the postulation of the existence of this sulfatase requires the assumption that at least another one must exist, active on the sulfate present in position 6 of Gal or GalNAc, we prepared several radioactive and non-radioactive substrates which would be suitable for the measurement of 6-sulfatases specific for the glucose or galactose configuration of their substrates.

We have already reported that extracts of cultured skin fibroblasts of Morquio patients, when incubated with tetrasaccharides prepared from C-6-S, fail to release measurable amounts of inorganic sulfate (8). We have also demonstrated that those extracts fail to desulfate either [1-$^3$H]-galactitol-6-sulfate or [1-$^3$H]-N-acetylgalactosaminitol-6-sulfate, while they desulfate normally either radioactive [1-$^3$H]-N-acetylglucosaminitol-6-sulfate or non-radioactive GlcNAc-6-S. As a result of these findings, it is clear that the impaired degradation and accumulation of KS and C-6-S in Morquio disease is caused by the deficiency of a sulfatase specific for the Gal-6-S residues of KS and the GalNAc-6-S residues of C-6-S (Fig. 2). Vice versa, extracts of cultured skin fibroblasts of our patient desulfate normally either tetrasaccharides from C-6-S or radioactive [1-$^3$H]-galactitol-6-sulfate or [1-$^3$H]-N-acetylgalactosaminitol-6-sulfate. However, the same extracts do not desulfate GlcNAc-6-S or have minimal activity when incubated with radioactive [1-$^3$H]-N-acetylglucosaminitol-6-sulfate.

The higher activities obtained with the non-radioactive substrates in the experiments summarized in Table III might be either due to the higher concentrations of substrates used (about twice the $K_m$ values found for the crude enzymes) or to higher enzyme affinities for substrates not reduced at position 1.

The enzymic activities measured when the various substrates were incubated with extracts of fibroblasts or leukocytes derived from the propositus' parents demonstrate their heterozygous state and the autosomal recessive character of the genetic defect.

In view of the feasibility of preparing adequate amounts of radioactive substrates of high specific activity, it is reasonable to presume that detailed knowledge of the various kinetic parameters of the enzyme GlcNAc-6-S sulfatase will allow the definition of a technique suitable for the prenatal diagnosis of the new disease.

## CONCLUSION

A five year old male had retarded growth (less than third percentile), mental retardation (I.Q. 50-60), hyperactivity, mild osteochondrodystrophy, hepatosplenomegaly, excessive and coarse hair, clear corneas and mild deafness. Excessive mucopolysaccharidurie, consisting of keratan and heparan sulfate, and unusual metachromasia of the peripheral lymphocytes were present. The release of $^{35}SO_4$ incorporated by his cultured skin fibroblasts was delayed. All the known mucopolysaccharidoses were excluded on the basis of specific enzyme measurements and of experiments in which the delayed release of $^{35}SO_4$-labeled glycosaminoglycans by his fibroblasts was corrected with extracts of fibroblasts derived from patients affected by known mucopolysaccharidoses. Since both keratan and heparan sulfate have N-acetylglucosamine-6-sulfate residues, this substrate was synthesized as described by Suzuki and Strominger (38). The product, which had proper analysis and infrared spectrum, was desulfated by extracts of normal fibroblasts and fibroblasts derived from Morquio patients but not by extracts of the propositus' fibroblasts. Similar results were obtained using as a substrate [1-$^3$H]-glucosaminitol-6-sulfate, obtained by reduction with sodium borotritide. Vice versa, extracts of the propositus' fibroblasts desulfated normally [1-$^3$H]-galactitol-6-sulfate, [1-$^3$H]-N-acetylgalactosaminitol-6-sulfate and 6-sulfated tetrasaccharides derived from chondroitin-6-sulfate, while those of Morquio patients did not.

It is concluded that Morquio disease and the mucopolysaccharidosis described are caused respectively by the deficiency of sulfatases specific for the galactose or glucose configuration of their 6-sulfated substrates.

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53. Requests for reprints should be addressed to: Dr. Nicola Di Ferrante, Department of Biochemistry, Baylor College of Medicine, Houston, TX. 77030.



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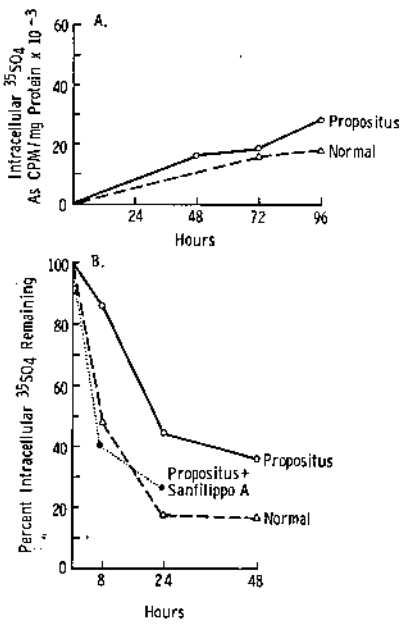


Fig. 1. Kinetics of $^{35}SO_4$ uptake and release by the propositus' cultured fibroblasts. The uptake of $^{35}SO_4$ is essentially normal (A), while the degradation of labeled, intracellular GAG (B) is delayed. The latter, however, is corrected when concentrate of Sanfilippo A medium is added to the plates at 0 time.

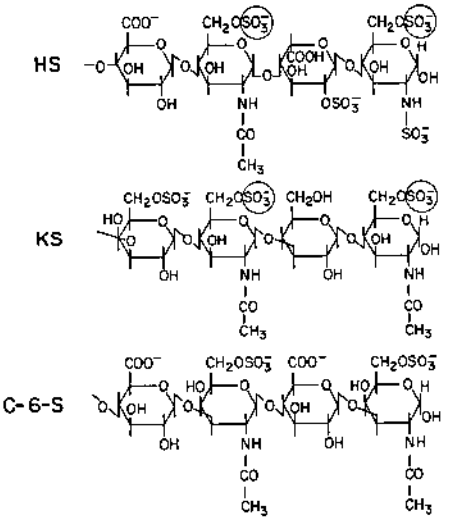


Fig. 2. The repeating units of heparan sulfate (HS), keratan sulfate (KS) and chondroitin-6-sulfate (C-6-S). The sulfate groups removed by N-acetylglucosamine-6-sulfate sulfatase are circled, while those removed by galactose-6-sulfate or N-acetylgalactosamine-6-sulfate sulfatase are shadowed. The former ones are not cleaved in the disease described here; the latter ones are not cleaved in Morquio disease.

TABLE I

Polymeric GAG and oligosaccharides excreted by the propositus, as mg/24 hours.

| | GAG-containing: | | |
|---|---|---|---|
| | Hexuronate | 2-deoxy-2-sulfoamino hexose | galactose |
| Polymeric (P) | 24.5 | 6.7 | 12.8[1] + 10.2[2] |
| Oligosaccharides (O) | 23.2 | 1.4 | 7.0 |
| Ratio P/O | 1.06 | 4.78 | 3.28 |

[1] CPC precipitable

[2] Non-precipitable with CPC.

TABLE II

Measurements of lysosomal enzymes on propositus' serum or leukocytes extracts.

Results are expressed as nmoles substrate changed/hour/mg protein.

| Enzyme | Propositus | Normal range |
|---|---|---|
| α-L-iduronidase[1] | 19.00 | 8 - 19.00 |
| L-idurono-2-sulfate sulfatase[2] | 1.00 | .80 - .90 |
| α-N-acetylglucosaminidase[1] | 1950 | 462 - 2200 |
| β-galactosidase[2] | 400 | 250 |
| N-acetylgalactosamine-4-sulfate sulfatase[1] (arylsulfatase B) | 100 | 70 - 160 |
| β-glucuronidase[2] | 230 | 35 - 300 |

Assays performed on leukocytes extracts[1] or serum[2].

TABLE IV

Salient clinical and radiological features of Sanfilippo, Morquio and N-acetylglucosamine-6-sulfate sulfatase deficiency diseases.

| Clinical features | Sanfilippo | Morquio | N-acetylglucosamine-6-sulfate sulfatase deficiency |
|---|---|---|---|
| Height | normal | < 3rd percentile | < 3rd percentile |
| Dysostosis multiplex | minimal | severe | mild |
| Odontoid process | normal | aplastic or hypoplastic | hypoplastic and dysplastic |
| Mental retardation | severe | absent | present (I.Q. 50-60) |
| Hyperactivity | present | absent | present |
| Hair | coarse, excessive | normal | coarse, excessive |
| Cornea | clear | cloudy | clear |
| Deafness | present | present | present |
| Facies | normal to coarse | coarse | normal |
| Hepatosplenomegaly | moderate | moderate | moderate |
| Seizures | present | absent | present |

TABLE III

Measurements of galactose-6-sulfate, N-acetylgalactosamine-6-sulfate and N-acetylglucosamine-6-sulfate sulfatase on extracts of normal fibroblasts, Morquio fibroblasts, the propositus' fibroblasts and on extracts of his parents' leukocytes and fibroblasts. Results are expressed as nmoles substrate desulfated/hour/mg protein.

| | 6-sulfated substrates | | | | |
|---|---|---|---|---|---|
| Fibroblasts | [1-$^3$H]-galactitol[1] | [1-$^3$H]-N-acetyl-galactosaminitol[1] | C-6-S tetra-saccharides | [1-$^3$H]-N-acetyl-glucosaminitol[1] | N-acetylglucosamine |
| Normal D.D. | 1.0 | 0.9 | | 1.8 | |
| L.J. | 1.0 | 0.8 | 20 | 1.9 | 24, 23[2] |
| K.B. | 1.6 | 0.8 | 24 | 1.8 | 28 |
| L.R. | 1.3 | 1.1 | | 1.6 | |
| K.P. | | | 19 | | 17 |
| H.J. | | | 14 | | 22 |
| Pooled normal fibroblasts extracts | 1.6 | 0.8 | | 1.5 | |
| MEAN ± SEM | 1.5 ± 0.09 | 0.9 ± 0.05 | 19 ± 2.2 | 1.8 ± 0.08 | 23 ± 1.9 |
| Range | 1 - 1.6 | 0.8 - 1.1 | 14 - 24 | 1.5 - 1.9 | 17 - 28 |

| | 6-sulfated substrates | | | | |
|---|---|---|---|---|---|
| Fibroblasts | [1-$^3$H]-galactitol[1] | [1-$^3$H]-N-acetyl-galactosaminitol[1] | C-6-S tetra-saccharides | [1-$^3$H]-N-acetyl-glucosaminitol[1] | N-acetylglucosamine |
| Morquio, line 593[3] | 0.3 | 0.15 | not detectable | 1.4 | 30 |
| 1361[3] | not detectable | not detectable | | 1.3 | |
| 1602[3] | 0.3 | 0.08 | | 1.7 | |
| R.R. | | | not detectable | | 21 |
| J.O. | | | not detectable | | 14 |
| MEAN ± SEM | 0.23 ± 0.11 | 0.08 ± 0.04 | | 1.5 ± 0.08 | 22 ± 4.8 |
| Range | 0 - 0.3 | 0 - 0.15 | | 1.3 - 1.7 | 14 - 30 |
| Propositus, G.G. | 1.3 | 1.3 | 15.3, 15.9[2] | 0.2, 0.16 | not detectable |
| father, T.G. | | 0.95 | | 1.1 | 23.6[4] |
| mother, S.G. | | 1.0 | | 0.9 | 21[4] |
| Normal leukocytes extracts | | | | | 45 |

Footnotes:

[1] Results were calculated by subtracting cpm of control tubes from cpm of tubes in which the substrates were incubated with fibroblasts or leukocytes extracts. Control tubes containing 180,000 cpm of [1-$^3$H]-N-acetyl-galactosaminitol-6-S, when processed as described under Methods, had 1000 cpm eluted with water from Dowex 1 x 8 columns; those containing 216,000 cpm of [1-$^3$H]-N-acetylglucosaminitol-6-S had 2200 cpm eluted with water; while those containing 270,000 cpm of [1-$^3$H]-galactitol-6-S had 6000 cpm eluted with water. Fibroblasts or leukocytes extracts with normal enzymic activity for a given substrate had a minimum of 3000 cpm above control values, while those of defective fibroblasts had between 0 and 100 cpm above control values.

[2] Performed on extracts derived from different cultures.

[3] Lines from the Human Genetic Mutant Cell Repository, Camden, N.J. 08103.

[4] Performed on leukocytes extracts.

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