Enzyme Replacement Therapy by Fibroblast Transplantation

LONG-TERM BIOCHEMICAL STUDY IN THREE CASES OF HUNTER'S SYNDROME

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ABSTRACT We have assessed the effectiveness of transplanted histocompatible fibroblasts as a long-lived source of lysosomal enzymes for replacement therapy in three patients with Hunter's syndrome, over periods ranging from 2.5 to 3.75 yr. The level of Hunter corrective factor excreted by all three patients increased after transplantation, as did the activity of α-L-idurono-2-sulfate sulfatase in serum, when measured directly with a radioactive disulfated disaccharide substrate. Sulfatase activity was also raised in leukocyte homogenates from the two patients that we were able to assess. These increases in enzyme activity were accompanied by corresponding increases in catabolism of heparan and dermatan sulfates, as shown by (*a*) a decrease in sulfate:uronic ratios of urinary oligosaccharides, (*b*) an increase in iduronic acid monosaccharide, and (*c*) a normalization of Bio-Gel P-2 gel filtration profiles. Both the increase in enzyme activity and increased catabolism were maintained during the period of study and were not affected by either a gradual decrease or total withdrawal of immunosuppressive therapy.

INTRODUCTION

The mucopolysaccharidoses are a group of recessively inherited disorders of connective tissue metabolism, each of which has a deficiency in one of the lysosomal exoglycosidase or sulfatase enzymes necessary for complete catabolism of glycosaminoglycans. In Hunter's syndrome, for example, there is an almost total absence of α-L-idurono-2-sulfate sulfatase (idurono-sulfate sulfatase), which is necessary for removal of sulfate groups from iduronic acid at the nonreducing terminus of heparan and dermatan sulfates. These deficiencies result in an increasing accumulation of incompletely degraded glycosaminoglycan, particularly in the visceral organs, connective tissues, and the central nervous system and in their excretion in large amounts in urine. Mental and physical deterioration are progressive, often resulting in early death.

Short-term increases in the catabolism of glycosaminoglycans have been produced in a number of patients with different forms of mucopolysaccharidosis (Hunter's, Hurler's, and the Sanfilippo syndromes) when given infusions of normal plasma (2–5) or leukocytes (6) as a means of enzyme replacement. In contrast, Dekaban et al. (7) and Erickson et al. (8) found that infusions of plasma or whole blood had little effect on patients with Hunter's, Hurler's, and Sanfilippo's syndromes, whereas Moser et al. (9) showed that infusions of HLA-compatible leukocytes in Sanfilippo B disease produced only a transient effect. The changes in urinary glycosaminoglycans noted in these patients were transient, presumably because most lysosomal enzymes have relatively short half-lives intracellularly (10–12). Studies on enzymes present in amniotic fluid (13), leukocyte homogenates (14), and serum (15), however, generally indicate a higher degree of stability. We attempted to increase the effectiveness of enzyme replacement therapy and to extend its duration, first, with a graft of allogeneic skin in a patient with Hunter's syndrome (16) and more re-

Preliminary results for patient N.G. have been published previously (1).

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cently by the transplantation of histocompatible fibroblasts from a clinically normal sibling, whose fibroblasts had been shown to correct the abnormal sulfate incorporation in the patient's own cells (1). In this report we describe the long-term biochemical results of transplanting allogeneic fibroblasts in three children with Hunter's syndrome. Samples of urinary glycosaminoglycans and their oligosaccharides taken before and after transplantation were compared quantitatively and qualitatively for evidence of increased catabolism after treatment. At the same time, urinary output of Hunter "corrective factor," which contains idurono-sulfate sulfatase, was monitored (17) and the levels of idurono-sulfate sulfatase measured directly in serum and leukocytes of patients and untreated cases of Hunter's syndrome, with radioactive disulfated disaccharide as substrate (15).

METHODS

Chemicals and reagents

Cell culture media were purchased from Flow Laboratories Ltd., Irvine, Ayrshire, Scotland and [^{35}S]sulfate from The Radiochemical Centre, Amersham, Buckinghamshire, England. Bio-Gel and Cellex-E were supplied by Bio-Rad Laboratories Ltd., Bromley, Kent, England, Dowex resin and pig mucosal heparin by Sigma Chemical Co., Kingston, Surrey, England, and 5-aminoacridine hydrochloride by Koch-Light Ltd., Colnbrook, Buckinghamshire, England. All other chemicals were obtained from British Drug Houses Ltd., Poole, Dorset, England and were of analytical reagent grade.

Clinical summaries

In all three patients the clinical diagnosis of Hunter's syndrome (18) was confirmed by the demonstration that their cultured fibroblasts could not correct known reference Hunter fibroblasts[1] and that they had a severe deficiency of Hunter corrective factor in their urine. Before grafting, the ability of the donor fibroblasts to correct abnormal $^{35}SO_4$ incorporation in the recipient's cells was confirmed by the method of Cantz et al. (17). All three patients were HLA identical with the sibling donor, against whom no mixed leukocyte reaction was detected. Approval for the transplants was obtained from Guy's Hospital and Medical School Committee on ethical practice. Individual details of the three patients were as follows:

N.G. This boy was 9 yr and 2 mo old at the time of his transplant in July 1974. Developmental retardation was detected at 18 mo. His IQ was estimated to be at an 18–24-mo level at the time of grafting. The patient and his sister, who was the donor, were both HLA 2,3;12,W5.

S.T. This boy was 4 yr and 8 mo old at the time of fibroblast grafting in March 1975. Developmental delay was noted at 1 yr. No firm estimate of his IQ could be made but he was thought to function at the level of an 18-mo-old child. The patient and his brother, who was the donor, were both HLA 3,9;12,5.

J.A. This boy was 5 yr and 3 mo old at the time of his transplant in December 1975. He was thought to be developing normally until about the age of 2 yr, when he was investigated for possible deafness and noted to have features suggestive of mucopolysaccharidosis. His IQ before grafting was estimated to be between 48 and 68. The patient and his sister, who was the donor, were both HLA 1,2;8,13.

All three patients studied were on an immunosuppressive regimen of 25 mg Imuran (Burroughs Wellcome & Co., Greenville, N. C.) and 30 mg prednisolone daily for at least 1 mo before fibroblast transplant. This program was continued for 12 mo in the first patient (N.G.) at which time he developed congestive cardiac failure and left lower lobe pneumonia, after which immunosuppressive therapy was stopped. Urine samples obtained after this time showed evidence of continued graft function and there was no evidence of clinical deterioration. The other two patients were given immunosuppressives for periods of 6 and 5 mo only after grafting, to minimize the possibility of complications.

Clinical observations

Patients were assessed before and 6 wk after transplant and at three monthly intervals thereafter. In addition to clinical examination, which included photographic recording of joint mobility, the following investigations were made; liver and spleen scans, radiological skeletal survey, and intellectual and developmental assessments.

Cell culture

Cultures of fibroblasts from donors and recipients were established by conventional methods with 0.2 × 0.3-cm skin biopsies taken from the volar surface of the arm. These were cultured in triplicate in glutamine-free RPMI 1640 medium (19) (Flow Laboratories Ltd.) supplemented with 4 mM L-glutamine that contained 50 mg of cloxacillin, 20 mg of gentamycin, and 200 ml of fetal calf serum per liter, in Falcon tissue culture flasks (No. 3024, Falcon Plastics; Division of BioQuest, Oxnard, Calif.) in a humidified incubator that contained an atmosphere of 5% CO_2 in air. When the primary cultures were confluent, the cells were removed by trypsinization with 0.25% (wt/vol) trypsin (Wellcome Laboratories, Beckenham, Kent, England) in serum-free Hanks' balanced salt solution and subcultured at a split ratio of 5:1. One further subculture at a split ratio of 6:1 was required to obtain sufficient cells for transplantation. Before transplantation, cells were again trypsinized for 20 min at 37°C in Hanks' balanced salt solution and washed three times in protein-free Hanks' balanced salt solution before resuspending them in 10 ml of the same solution for injection. Approximately 2×10^8 viable cells were injected as a cell suspension into four subcutaneous dorsal sites.

Isolation of urinary glycosaminoglycans and oligosaccharides

Urine collections (24 h) were stored at −20°C with 10 ml of toluene as a preservative. Glycosaminoglycans were precipitated from 100- to 200-ml aliquots of each urine specimen with 5-aminoacridine HCl (20) converted to their sodium salts by shaking with a suspension of Dowex 50 (Na^+ form) reprecipitated in 80% (vol/vol) ethanol, removed by centrifugation, and dried (21). Inorganic sulfate and phosphate were removed from 20-ml aliquots of the remaining supernatant solutions by drop-wise addition of 25% (wt/vol) aqueous $BaCl_2$ until precipitation ceased. Samples were clarified by cen-

[1] We are grateful to Dr. H. Kresse of the University of Münster for confirming these points. His present address is Medisinisch-Chemisches Institut und Pregl Laboratorium der Universitat Graz, Austria.

trifugation and the process repeated. Excess 5-aminoacridine and other cations were removed from the low molecular weight oligosaccharides by passing the supernatant solutions through a column (18 × 150 mm) of Dowex 50 H^+ (Dow Chemical Co., Midland, Mich.) and eluting with 300 ml of water. The pH of the eluates was adjusted to 6.5–7.0 with 2 M NaOH, the samples freeze-dried, redissolved in 10 ml of water, and stored at −20°C.

Analytical procedures

Columns (13 × 600 mm) of Bio-Gel P-2, 200–400 mesh, were packed in and eluted with 0.2 M sodium acetate, pH 6.8. Samples that contained ≅1 mg of uronic acid were applied to the columns, and the uronic acid contents determined in each 1.0-ml fraction by the carbazole method, with an automated procedure (22). Sulfate contents were determined by the modified rhodizonate method after hydrolysis of pooled fractions in 6 M formic acid for 24 h at 100°C (23, 24). Iduronic acid was measured by gas-liquid chromatography after methanolysis in 2 M anhydrous methanolic HCl, neutralization, and conversion into a trimethylsilyl derivative (25). Hexosamine was determined by the method of Tsiganos and Muir (26).

Assay of Hunter corrective factor

Hunter corrective factor was concentrated from urine and its activity determined by previously described methods (1, 15). Urines were frozen immediately after collection and assayed, usually within a few days. Urines stored for up to 6 mo showed no change in corrective factor activity, in agreement with the observations by Cantz et al. (27) on Hurler corrective factor. The response to corrective factor of patients' cells before and after treatment varied little over a period of 2 yr.

Measurement of α-L-idurono-2-sulfate sulfatase

After this investigation was begun it became possible to measure idurono-sulfate sulfatase directly (15) and a limited number of results are therefore presented here. Leukocytes, collected by centrifugation from heparinized blood after lysis of erythrocytes with hypotonic shock, were resuspended in 1 ml of 0.15 M NaCl and then subjected to six cycles of freeze-thawing, and then debris was removed by centrifugation. Sera and leukocyte homogenates were each dialyzed for 48 h against 8 liters of 0.15 M NaCl, stored frozen, and not thawed until assay. The substrate was ^{3}H-labeled *O*-(α-L-idopyranosyluronic acid 2-sulfate) (1 → 4)-2,5-anhydro-D-[1-^{3}H]mannitol 6-sulfate, prepared from heparin by deaminative cleavage, and subsequent reduction with sodium borotritide (28). Duplicate 30-μl samples of serum or leukocyte homogenate were incubated for 4 h at 37°C with 30 μl of substrate (4 mM in 0.25 M sodium acetate, pH 4.5, 57 mCi/mM sp act). Incubation mixtures were then diluted with 1.5 ml of water and added to a column (1.5 ml vol) of epichlorohydrin triethanolamine cellulose in the formate form. The tubes were rinsed with an additional 1.0 ml of water, which was added to the epichlorohydrin triethanolamine column and the radioactive reaction products separated from residual substrate, which remained bound to the column, by elution with 2 × 5-ml aliquots of 0.16 M sodium formate. Fractions were collected into scintillation vials that contained 10 ml of Lumagel (Lumac Systems Ac, Basel, Switzerland) and counted in a liquid scintillation spectrometer (Searle Ltd., High Wycombe, Buckinghamshire, England). Appropriate blanks of 30-μl substrate and 30-μl samples were incubated separately for 4 h, diluted with water, mixed, and then applied immediately to the column. Enzyme activity was expressed as nanomoles of substrate hydrolyzed per milligram protein per 24 h.

RESULTS

Changes in amount and composition of excreted glycosaminoglycans. There was an increase in total uronic acid output shown by all three patients within 48 h of transplantation. Excretion remained above pretransplantation levels for about 1 wk then fell to preimplantation levels. Output thereafter rose and fell in a cyclical manner throughout the period of observations, with elevated values recorded more than 20 mo after treatment in patient N.G.

Output of precipitated glycosaminoglycans was considerably greater than in normal controls before treatment so that the ratio (based on uronic acid) of oligosaccharides to precipitated glycosaminoglycans in Hunter urine varied between 3.0:1 and 6.5:1 in contrast to a normal mean of 27:1. After transplantation, mean uronic acid output increased by 72% in patient N.G. and by 19% in J.A., but in S.T. there was a decrease (Table I). There was an increase in output of both oligosaccharides and precipitated glycosaminoglycans so that the overall ratio of oligosaccharides to precipitated glycosaminoglycans remained relatively constant.

However, in one case (S.T.) the uronic acid content of later posttransplantation samples decreased to below pretreatment levels, this decrease being particularly marked in glycosaminoglycans precipitated with 5-aminoacridine.

Galactosamine accounted for 60–70% of the total hexosamine present in precipitated glycosaminoglycans (Table II), these values being slightly higher than those recorded for pooled samples of normal urine (29). The galactosamine content of this fraction decreased after transplantation in patients S.T. and J.A. Glucosamine was the principal amino sugar in the low molecu-

TABLE I
Mean 24-h Excretion of Urinary Uronic Acid before and after Transplantation

Patient	Uronic acid		Change
	Before	After	
	mg/24 h		%
N.G.	204±31 (11)	350±39 (44)	+72
S.T.	203±42 (6)	143±20 (15)	−30
J.A.	157±8 (8)	186±22 (20)	+19
Normal control	236±27 (4)		

Values represent the mean±SE from the numbers of analyses shown in parentheses.

TABLE II

The Ratio of Glucosamine to Galactosamine in Glycosaminoglycans and Oligosaccharides Isolated from each Patient before and after Transplantation of Fibroblasts

Patient	Precipitated glycosaminoglycans		Oligosaccharides	
	Before transplantation	After transplantation	Before transplantation	After transplantation
N.G.	1:2.20±0.18 (4)	1:2.48±0.14 (8)	2.21:1±0.18 (4)	1.89:1±0.13 (5)
S.T.	1:1.81±0.06 (4)	1:1.60±0.05 (4)	2.0:1±0.14 (5)	1.54:1±0.06 (7)
J.A.	1:1.98±0.77 (2)	1:1.74±0.06 (9)	1.85:1±0.07 (6)	2.03:1±0.02 (12)
Normal controls	1:1.70*	—	1.67:1±0.15 (2)	—

Number of samples analyzed is in parentheses.
* Data taken from Wessler (29).

lar weight oligosaccharide fraction of all three patients (Table II) and was present in greater proportions than in urine from age matched normal children. In patients N.G. and S.T. the glucosamine content of this fraction fell to a level close to, or below that of normal controls after treatment.

The oligosaccharides that were eluted from Dowex-50 were separated into four fractions of different hydrodynamic size (Fig. 1) by gel chromatography. Although broadly similar to the elution profiles seen when oligosaccharides previously eluted from Dowex-1 in 2 M HCl were similarly subjected to chromatography on Bio-Gel P-2 (1) there were, however, several differences. Fraction V was not present in any significant amount in the oligosaccharides eluted with water from Dowex-50, nor was fraction II. The four fractions resolved were numbered in order of decreasing hydrodynamic size and do not correspond to those previously separated from acid eluates. In normal urine, the largest component (fraction I) was of smaller size than a reference tetrasaccharide produced by the action of testicular hyaluronidase (EC 3.2.1.35) on chondroitin sulfate, whereas the smallest component (fraction IV) was eluted at a position that corresponded to that of

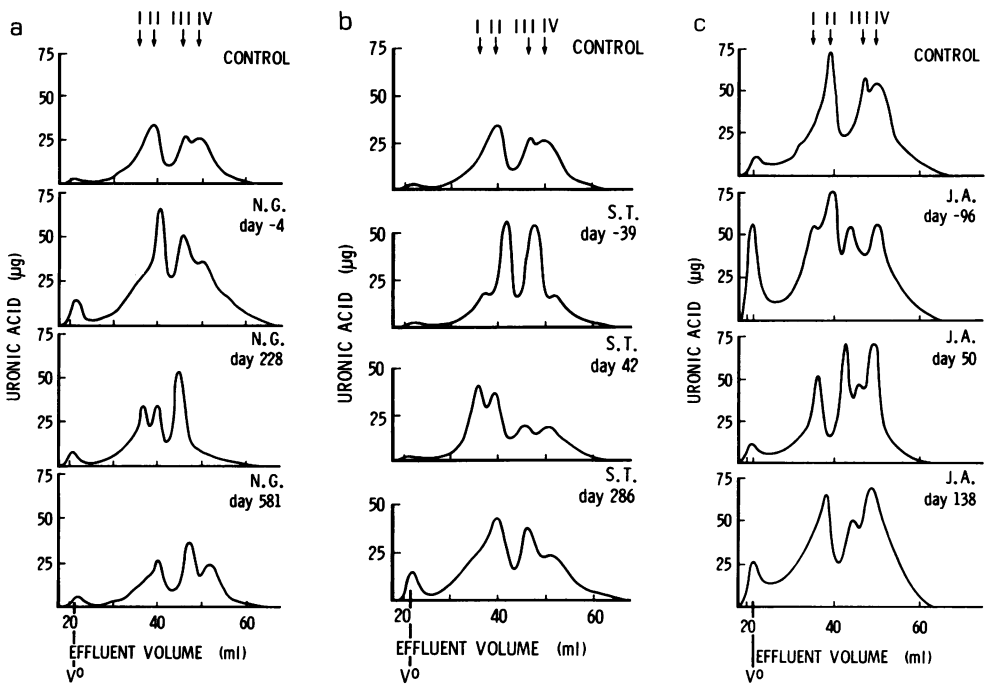

FIGURE 1 Elution profiles on Bio-Gel P-2 of oligosaccharides isolated from the urine of (*a*) patient N.G., (*b*) patient S.T., (*c*) patient J.A., and from normal urine. In each case a typical elution profile is shown from before transplantation and early and late posttransplantation together with the profile of oligosaccharides from urine of an age-matched control for comparison.

glucuronic acid. The intermediate fractions II and III were eluted at positions equivalent to the mono- and disulfated-unsaturated disaccharides produced by the action of chondroitin ABC lyase (EC 4.2.2.4) and chondro-4-sulphatase (EC 3.1.6.9) on chondroitin sulfate.

Urine from untreated patients contained a higher proportion of the larger oligosaccharides than normal urines and much less monosaccharide fraction. After transplantation the elution profiles gradually changed, so that the relative proportions of lower molecular weight oligosaccharides increased and larger molecular weight ones decreased, until about 1 yr after transplantation when elution profiles in the case of all three patients resembled those of age matched controls.

Pooled samples of fraction I (Fig. 1) from each patient and from normal controls were rechromatographed on Bio-Gel P-2, recollected, and their sulfate to uronic acid ratios determined. In normal urine this ratio was 1.02:1, in contrast to much higher ratios, ranging from 1.6 to 2.14:1, in samples taken from all three patients before transplantation (Table III). After treatment, the sulfate: uronic acid ratios decreased to approach normal values within 4 wk, this lower level being maintained throughout the period of observation.

Before treatment the iduronic acid content of the monosaccharide (fraction IV) was below that of control urines. After transplantation the proportion of iduronic acid in this fraction rose to normal values and there was a concomitant decrease in the iduronic acid content of the larger oligosaccharides (fractions I–III) (Table IV).

Changes in corrective factor and iduronate sulfatase levels. Protein concentrates from urine samples taken before and after transplantation. were tested for their ability to correct abnormal [^{35}S]sulfate incorporation by the patients' own fibroblasts, grown from biopsies taken before transplantation. Before transplantation the samples were virtually devoid of corrective-factor activity, which varied between 0 and 2 U/24 h (Fig. 2). After transplantation, fluctuating, but consistently higher levels of corrective factor activity were observed in the urines of all three patients. Mean output of corrective factor activity before and after fibroblast transplantation compared with normal age matched controls is shown in Table V. Mean activities increased from ≅1% of control levels to about 10% of control levels after treatment. Direct measurement of idurono-sulfate sulfatase activity in sera of all patients taken between 1.5 and 3 yr after transplantation gave a mean activity almost four times that obtained from untreated control Hunter patients (Table VI). Leukocyte homogenates from patients N.G. and J.A. also showed significant increases in activity compared with that of homogenates from untreated Hunter patients.

TABLE III

Sulfate:Uronic Acid Molar Ratios of Oligosaccharide Fraction I Separated on Bio-Gel P-2

Patient	Group A	Group B	Group C
N.G.	2.10 (3)	1.15 (5)	1.25 (7)
S.T.	2.14 (3)	0.54 (7)	0.87 (2)
J.A.	1.60 (6)	0.95 (7)	0.93 (4)
Control	1.02 (2)		

Group A samples were collected 0–3 mo before transplantation, group B samples from 1 wk to 6 mo after transplantation, and group C samples from 6 mo to 2 yr after transplantation. Each group was rechromatographed on Bio-Gel P-2 and sodium ions, which interfere with SO_4 determination, were removed on a column of Dowex-50 (H^+ form) before analysis. Numbers of samples pooled in each group are in parentheses.

TABLE IV

Changes in the Proportion of Iduronic Acid in Oligosaccharides and Monosaccharides Eluted from Bio-Gel P-2 in Patient N.G.

Day of treatment	Iduronate as a percentage of total uronic acid: Fractions I, II, and III combined	Iduronate as a percentage of total uronic acid: Fraction IV
	%	
−7	27.8	19.8
−1	21.7	17.6
58	10.4	18.1
65	8.0	27.5
Normal control	—	29.9

Samples taken before implantation are designated by negative numbers. The trimethylsilyl derivatives of iduronic acid and glucuronic acid were separated by gas-liquid chromatography. Iduronic acid values are expressed as a percentage of the combined value of both hexuronic acids.

Clinical changes. For ethical reasons the three patients selected for this experimental treatment were all grossly affected by the disease both physically and mentally. The three patients were observed for periods ranging from 2 yr and 6 mo to 3 yr and 9 mo as described in Methods. None of the patients showed consistent changes in joint mobility or liver and spleen size as revealed by serial scans. However, during this period there was no evidence of any further mental and physical deterioration. The patient followed for the longest period of time (N.G.) who was assessed as having a mental age of 1 yr 10 mo before transplant was reassessed as having a mental age in excess of 2 yr, 3.9 yr later, in contrast to the expected deterioration.

DISCUSSION

The effectiveness of transplanted, histocompatible fibroblasts as a source of normal enzyme for replace-

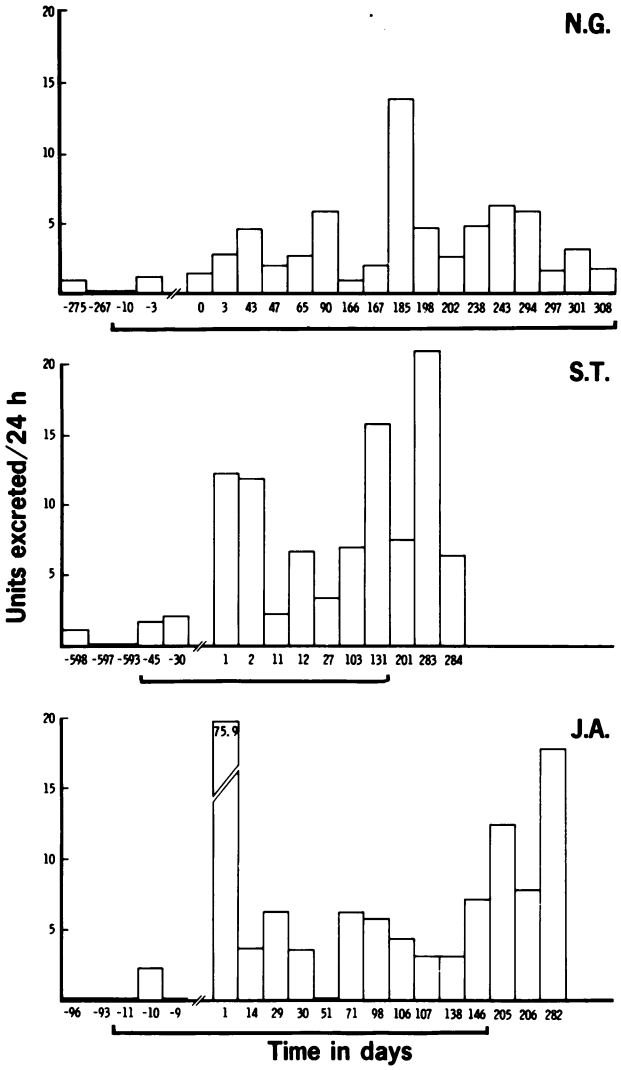

FIGURE 2 Total 24-h output of Hunter corrective factor isolated from the urine of each patient. The day of transplantation is designated day 0, the days before transplantation by negative numbers, and days after transplantation by positive numbers. The days on which immunosuppressive therapy was given are indicated by the horizontal bars beneath the ordinates.

ment therapy was assessed by increases in the activity of idurono-sulfate sulfatase, by the effect on the catabolism of accumulated heparan and dermatan sulfates and by the persistence of both effects over a long period of time.

Both enzyme activity itself and corrective factor activity were determined but were very low or absent in urine samples taken before treatment, or in sera and leukocyte homogenates from untreated patients. Immediately after transplantation, corrective factor activity appeared in the urine of each patient and persisted throughout the period of observation. The levels

TABLE V
Mean 24-h Excretion of Hunter Corrective Factor before and after Transplantation

Patient	Before	After	Control
N.G.	0.5±0.1 (9)	4.0±0.8 (15)	45.1 (4)
S.T.	0.5±0.2 (5)	5.0±1.6 (15)	49.0 (4)
J.A.	0.5±0.2 (5)	6.3±1.3 (13)	59.8 (3)

The values depicted represent the mean units of correction (as defined by Barton and Neufeld [30]) ±SE from all samples collected within the period of time shown for each patient in Fig. 2 with the exception of the first three post-transplantation days because these could include corrective factor derived from broken cells. Numbers of analyses are in parentheses.

of corrective factor activity were not constant but showed considerable fluctuations; nevertheless mean values of posttransplantation samples were always considerably higher than the mean values before trans-

TABLE VI
Activity of α-L-Idurono-2-Sulfate Sulfatase in Sera and Leukocytes from Patients who Received Fibroblasts Compared with Untreated Hunter Patients as Controls

		Leukocyte homogenates	
	Serum	I	II
Normal mean ±SEM	27.1±2.6 (7)	53.5±4.6 (6)	311±16.2 (4)
Treated Hunter patients			
N.G.	0.37	2.2[a]	75[b]
S.T.	0.44	—	—
J.A.	0.30	2.2[c]	135[d]
Mean±SEM	0.37±0.04		
Untreated Hunter controls			
D.F.	0.13	0.28	
R.N.	0.09	0.19	28
T.P.	0.08		
P.P.	0.07		
I.S.	0.08		
D.H.	0.20		5.3
Mean±SEM	0.11±0.01		

Final substrate concentration was 2.0 mM and values are expressed as nanomoles per milligram protein per 24 h. Leukocyte homogenates in column I were prepared as described in Methods, those in column II were separated by centrifugation in a dextrose gradient with the procedure described by Stanbury et al. (31). Sample *a* was collected 2 yr and 11 mo after transplant and sample *b* 51 wk later, sample *c* was collected 2 yr and 2 mo after transplant and sample *d* 16 wks later. Number of samples analyzed is in parentheses.

plantation (Fig. 2, Table V). The high level of corrective factor in the urine immediately after transplantation may have been caused by enzyme released from cells that were damaged during the transplantation procedure.

Direct measurement of idurono-sulfate sulfatase activity in the sera of all three patients and in leukocyte homogenates of two patients (N.G. and J.A.) several years after treatment suggested that the transplanted fibroblasts had survived and had continued to function and release enzyme.

Many of the changes in size and composition of the urinary glycosaminoglycans and oligosaccharides that were seen after transplantation were consistent with those that would be expected from increased catabolism of these compounds. The relative proportions of oligosaccharides separated by gel chromatography began to resemble those of normal urine, and the initial increase in output of uronic acid was mainly in the form of oligosaccharides of low molecular weight. The hexosamine composition of either glycosaminoglycans or oligosaccharides showed no consistent change, however. Glucosamine was the major constituent of oligosaccharides, which were presumably derived from heparan sulfate, whereas galactosamine was the major constituent of precipitated glycosaminoglycans, which were presumably derived principally from dermatan sulfate.

Among the more specific changes noted in all three patients was a marked fall in the molar ratio of sulfate: uronic acid of fraction I (Fig. 1), which was consistent with the removal of sulfate from the terminal nonreducing iduronic acid of partially degraded dermatan and heparan sulfates by idurono-sulfate sulfatase (32–34). The action of idurono-sulfate sulfatase would then enable free iduronic acid to be released. This was indeed found with patient N.G., where the iduronic acid content of the monosaccharide fraction increased, while the proportion of iduronic acid in the oligosaccharide fractions decreased.

It is notable that neither the increase in enzyme activity nor the changes in urinary glycosaminoglycans and oligosaccharides were affected by the gradual withdrawal or total cessation of immunosuppressive drugs.

It is known that normal skin fibroblasts can correct abnormal sulfate metabolism of fibroblasts from patients with mucopolysaccharidosis when they are grown together (35) and that this is because of the release of lysosomal enzymes and their active uptake by deficient cells (11, 36, 37). Furthermore, in in vitro studies with cells from patients with mucopolysaccharidosis IIIB (Sanfilippo B disease) it has been shown that 2–5% of normal intracellular levels of enzyme would induce 42–70% correction of their abnormal glycosaminoglycan metabolism (10). A relationship between amount of enzyme uptake and degree of correction has also been demonstrated in cells from patients with β-glucuronidase deficiency (38, 39) and Hunter's syndrome (40), whereas adsorptive endocytosis of a wide range of lysosomal hydrolases by fibroblasts has been shown in vitro (37, 41, 42). It is therefore possible that a relatively small number of transplanted normal fibroblasts might release idurono-sulfate sulfatase, which would be taken up by the patients' cells and bring about some catabolism of glycosaminoglycans in vivo. Although there is no direct evidence for this, it is compatible with the biochemical data. Exchange of lysosomal enzymes has been shown to occur in vivo with α-mannosidase and β-glucuronidase (43, 44). The possibility that some breakdown of glycosaminoglycans may occur in the urine cannot be entirely excluded, but this does not explain the increased excretion of polymeric glycosaminoglycans after transplant in patients N.G. and J.A. Moreover, the concentration of phosphate in urine (≅10 mM) was sufficient to inhibit idurono-sulfate sulfatase activity by more than 99% in our assay system.

Preliminary results showed that leukocytes of two patients possessed significantly more idurono-sulfate sulfatase than cells from untreated patients with Hunter's syndrome. The three patients were all severely mentally and physically affected before treatment. Lack of further deterioration after the transplant suggests that such treatment of patients before the disease has progressed this far may arrest or prevent further progress of the disease, particularly if a larger number of cells were transplanted. These results do not necessarily imply that other types of lysosomal storage diseases would respond to cell transplants, however.

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