

Induced Degradation of Glycosaminoglycans in Hurler's and Hunter's Syndromes by Plasma Infusion

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Communicated by Donald D. Van Slyke, October 28, 1970

ABSTRACT The effects of the administration of normal human plasma to patients affected by mucopolysaccharidoses I and II (Hurler's and Hunter's syndromes) have been evaluated. The infusion was followed by a decreased urinary excretion of relatively large molecular weight glycosaminoglycans and by an increased excretion of their products of degradation. Among the latter, products of the degradation of dermatan sulfate and heparan sulfate could be demonstrated.

The results indicate that normal human plasma may contain those "factors" that are involved in the normal degradation of dermatan sulfate and heparan sulfate, that are missing in the diseased states.

The study of the structure, function, and metabolism of glycosaminoglycans (GAG), once the domain of a handful of specialized biochemists, lately has increased in popularity mainly as a result of the discovery of a group of diseases in which the metabolism of these polymers appears to be deranged.

Brante's discovery (1952) of GAG in the liver of patients who died of gargoylism (1); Dorfman's and Lorincz's (1957) findings of dermatan sulfate (DS) and heparan sulfate (HS) in the urine of patients affected by the syndrome (2); Brown's (1957) isolation of HS from the liver of Hurler patients (3); McKusick's masterful description and classification (1965) of the various mucopolysaccharidoses (4); and Danes and Bearn's work (1965) on the histochemistry of cultured normal and abnormal fibroblasts (5, 6) represent the milestones that have led to the highly original studies of Neufeld and her collaborators (1968) (7, 8).

Using as a model for the respective diseases cultured fibroblasts obtained from patients with Hurler's and Hunter's syndromes (mucopolysaccharidoses I and II), Neufeld and her collaborators have demonstrated that the intracellular accumulation of GAG is due to a decreased rate of degradation rather than to an increased rate of synthesis (7). The same investigators have also provided preliminary information on the nature and properties of the "factors" that are missing in the disease states and are likely to be responsible for the defective degradation of GAG. Thus, supplementation of cultures of abnormal cells with normal cells or their media—or with abnormal cells of a different type or their media—prevents the intracellular accumulation of ³⁵S-labeled GAG and causes an increased passage of ³⁵S-labeled material from the cells to the

medium (9-11). Neufeld and coworkers have suggested that the latter material represents products of the degradation of GAG (7). Attempts to isolate, purify, and characterize the "corrective factors," which may be obtained from normal fibroblast media, have indicated that they are heat labile, have a high molecular weight (8, 12), and possess the physicochemical properties of proteins. It is conceivable that they may represent either the enzymes missing in the various mucopolysaccharidoses or protein "cofactors" that may be required to complement incomplete enzymes present in the disease states. The absence of complete chemical characterization of the products derived from the activity of these "corrective factors" makes it impossible at the present time to postulate their mechanism of action. Thus it has been suggested that they might be either proteases, cleaving the protein core of proteoglycans; carbohydrases, cleaving the carbohydrate chains at various sites; or even sulfatases, merely removing the sulfate-ester groups of intracellular GAG (8).

Intracellular accumulation of GAG in abnormal fibroblasts disappears when fetal-calf serum is replaced by human serum in the culture medium (13). This finding has led us to explore the possible effects that an infusion of normal human plasma might have on children affected by mucopolysaccharidoses. The results reported here clearly indicate that the plasma infusion has been followed in all cases by mobilization and degradation of GAG.

The patients used in this study were a 15-month-old caucasian boy (R. S.) affected by Hurler's syndrome (mucopolysaccharidosis I) and two caucasian brothers (S. R., 3-years-old and T. R., 5 years, 4 months old), affected by Hunter's syndrome (mucopolysaccharidosis II). The diagnoses were established on the basis of family pedigrees, clinical and radiological findings, and measurements of GAG on urine (14), plasma (15), and cultured skin fibroblasts and their media. The two brothers with Hunter's disease were hyperactive but their mental development was considered normal. This agreed with the character of the disease as it has occurred in other members of the family; all the affected males had been well educated and capable of leading useful, long lives. The mental adequacy of the Hurler patient could not be properly evaluated due to his young age.

MATERIALS AND METHODS

Individual units of fresh-frozen human plasma (originally collected with 10% acid citrate-dextrose solution and ranging in

Abbreviations: GAG, glycosaminoglycans; DS, dermatan sulfate; HS, heparan sulfate.

volume between 150–200 ml) were thawed, brought to room temperature, and cross-matched for compatibility with the patient's erythrocytes. Compatible plasma was infused intravenously at about 3 ml/min. 4-hr intervals were maintained between one infusion and the next. R. S. received a total of 1470 ml of plasma in a 36-hr period; S. R. 790 ml in 32 hr; and T. R. 1070 ml in 68 hr.

Urine was collected without preservative for 24-hr periods starting 1 day prior to the infusions and continuing for 2 or 3 days. Urinary GAG were precipitated with cetyl pyridinium chloride as described (14). The hexuronic acid-hexosamine-containing material present in the precipitate is defined as "large molecular weight urinary GAG," in contrast with that which is not precipitated by the detergent and is found in the supernatant. The latter material, referred to as "urinary GAG fragments" was collected on a column of Dowex 1-X2 resin (200–400 mesh, Cl-form) and eluted in various fractions with stepwise increasing concentrations of NaCl (16). The fractions thus obtained are considered to represent products of the further degradation of GAG. The ratio "large molecular weight GAG"/"GAG fragments" is 0.2–0.4 in normal children and 0.75–3.0, or higher, in patients affected by Hurler's, Hunter's, or Sanfilippo's syndromes (17). Both fractions were analyzed for (a) total hexuronic acid content with the "one step" borate-carbazole technique of Sajdera*; (b) DS content, with a quantitative modification (19) of the histochemical technique of Scott and Harbinson (20) based on the oxidation of L-iduronosyl residues of DS by periodate. The method, sensitive to less than 2 μ g of DS, is specific when glycogen and glycoproteins are removed; and (c) HS content, as sulfoaminohexose, with the nitrous acid reaction, as described by Lagunoff and Warren (21). Iduronic acid values were obtained by dividing by three the DS values; no attempts were made to calculate the percentage of acetyl-amino- or aminohexose present in HS.

RESULTS

Excretion of total urinary GAG (Fig. 1)

Before the infusion, in all three patients the excretion of "large molecular weight GAG" and "GAG fragments" was in accord with the values usually found in patients affected by mucopolysaccharidoses I and II (17). The values of the ratio "large molecular weight GAG"/"GAG fragments" were all above 1.00, clearly within the abnormal range.

1 day after the infusion began, the excretion of large molecular weight GAG increased moderately in both Hunter patients (Fig. 1 A, and B); it decreased sharply, however, on the third day and displayed a moderate rebound on the fourth day. The excretion of GAG fragments also increased on the second day and remained higher than the preinfusion values, despite some fluctuations. The values of the ratio "large molecular weight GAG"/"GAG fragments" decreased sharply after the infusion. In the Hurler patient (Fig. 1C), the excretion of large molecular weight GAG decreased sharply after the infusion, while that of the GAG fragments increased. The values of

the ratio, extremely high prior to the infusion, became almost normal.

Fractionation of GAG fragments

The cetyl pyridinium chloride supernatants, applied to columns of Dowex 1-X2, were eluted with increasing stepwise concentrations of NaCl (16). Fig. 2 shows the changes undergone by each fraction of the three patients during and after the plasma infusion.

Excretion of dermatan sulfate and heparan sulfate

The data in Fig. 3 indicate that the modifications in the excretion levels of the total "large molecular weight urinary GAG" of the three patients, before and during the infusion, reflect parallel changes in the excretion of "large molecular weight" DS and HS. The data in Fig. 4 indicate the distribution of DS and HS degradation products in the various fractions of the "urinary GAG fragments" of the three patients. It is evident that the degradation products of these two GAG account for a modest part of the total "GAG fragments" in the two Hunter patients but represent a considerable part of the total "GAG fragments" in the Hurler patient. A conspicuous amount of the "GAG fragments" excreted by the three patients during the infusion is represented by degradation products of hexuronic acid-containing GAG other than DS and HS. Similar analyses of urine of control children demonstrate that approximately 0.5 mg of DS (as iduronic acid) is excreted daily as high molecular weight urinary GAG and 1.0 mg as urinary GAG fragments; HS was below the minimum amount measurable in either fraction (17).

DISCUSSION

The various techniques used for the measurement of urinary GAG measure only those having a relatively high molecular weight, but are inadequate for the measurement of their products of degradation. Various experiments (22) have demonstrated that depolymerization of GAG decreases their susceptibility to precipitation with quaternary ammonium salts or to retention on anion-exchange resins.

Since a defective process of degradation is responsible for the accumulation of GAG in the course of mucopolysaccharidoses, their high urinary excretion in these diseases very likely represents material that normally would have been degraded and not measured with the usual techniques employed. The method used in this study allows the measurement of the traditional, relatively high molecular weight, urinary GAG and also of some of their degradation products; the results confirm that a defect of degradation is responsible for the accumulation of GAG in the course of mucopolysaccharidoses and support the statement (8) that "the amount of mucopolysaccharide excreted in the urine of Hurler children is roughly comparable to that catabolized by normal children."

Very few attempts have been made to modify the course of the mucopolysaccharidoses. Wolfson *et al.* (23) studied and reviewed the merits of long-term corticosteroid therapy and concluded against its recommendation. DeJong *et al.* (24) investigated the effects of ascorbic acid depletion on the clinical and biochemical course of Hurler's syndrome but could not suggest adoption of such therapy. Histochemical findings, demonstrating a reduction of GAG content of cultured fibroblasts in the presence of retinol (water-soluble vitamin A) (25), induced Danes and Bearn (26) to administer the vitamin

* Sajdera, S. W., personal communication. 5 ml of borate-sulfuric acid reagent (9.5 g sodium tetraborate + 190 ml of H₂O were carefully added to a 9-lb bottle of concentrated sulfuric acid) was added to 0.5 ml of sample and 0.2 ml of 0.1% carbazole in 95% ethanol. After mixing, the tubes were heated at 100°C for 15 min. Absorbancies at 522 nm were identical to those obtained with Bitter and Muir's method (18).

to normal children and to children affected by Hurler's and Hunter's syndrome. Measurements of the urinary GAG indicated a 2-fold or higher increase, both in the control and affect-

ed children, but no changes in the clinical symptomatology were reported. Madsen and Linker (27) administered retinol to patients affected by Hurler's, Hunter's, Sanfilippo's, and

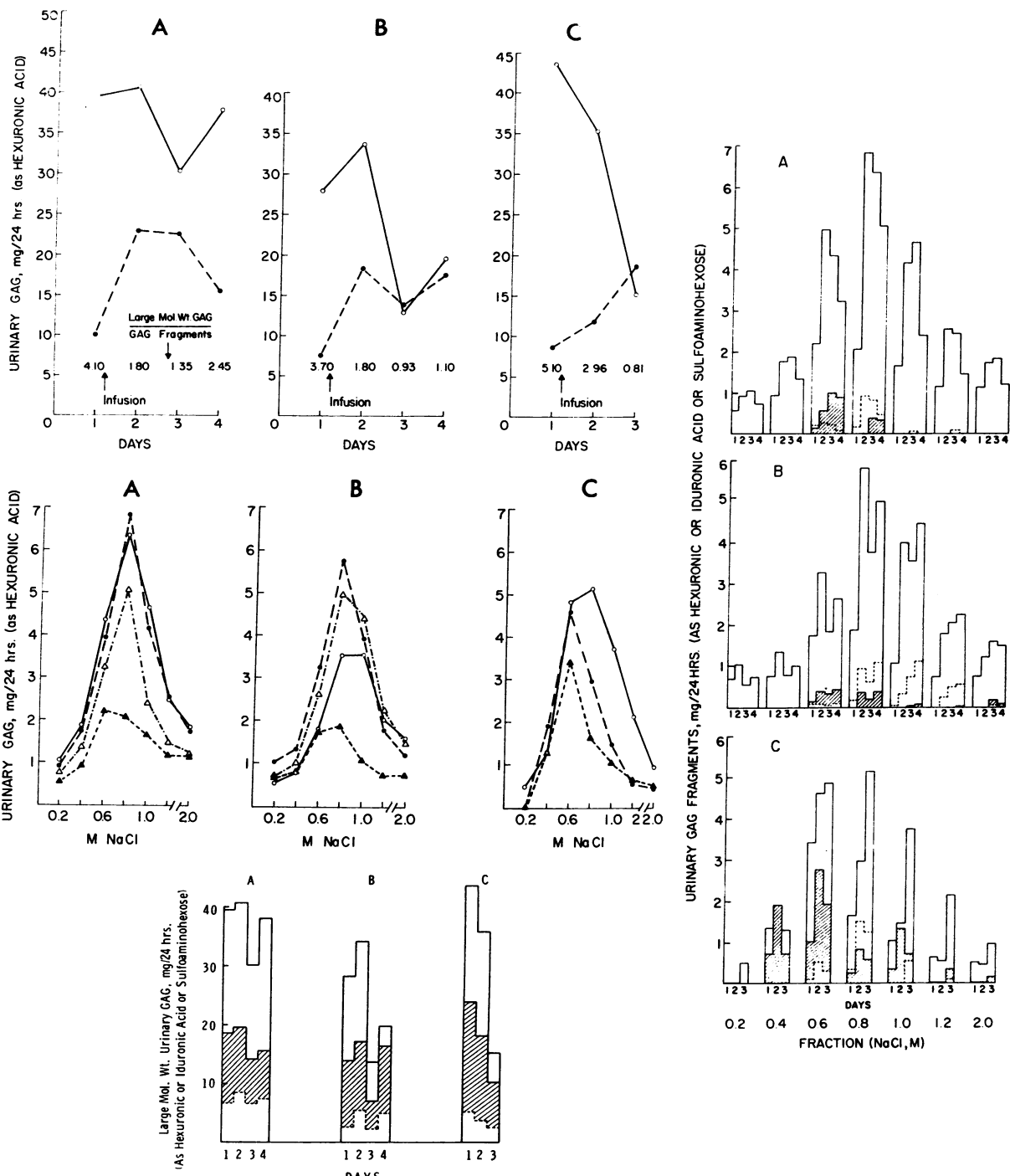


FIG. 1 (Top left). Excretion of "large molecular weight urinary GAG" (—) and of "urinary GAG fragments" (---) in three patients before and during plasma infusion. (A) T.R.; (B) S.R.; (C) R.S.

FIG. 2 (Center left). Quantitative changes in the amount of "urinary GAG fragments" excreted by the three patients before and during plasma infusion. Fractionation was on Dowex 1-X2 columns. A, B, and C as in Fig. 1. \blacktriangle — \blacktriangle first day of experiment; \triangle — \triangle second day; \square — \square third day; Δ — Δ fourth day.

FIG. 3 (Bottom left). Changes in the amounts of "large molecular weight" total urinary GAG (as total hexuronic acid, \square), dermatan sulfate (as iduronic acid, \blacksquare), and heparan sulfate (as sulfoaminohexose, \boxplus) excreted during plasma infusion. A, B, and C as in preceding Figs.

FIG. 4 (Right). Changes in the amounts of total urinary GAG, dermatan sulfate, and heparan sulfate excreted as "GAG fragments" during plasma infusion. GAG fragments were fractionated on Dowex 1-X2 columns. A, B, C, and symbols as in Fig. 3.

Morquio-Brailsford's syndromes; extensive clinical and biochemical observations failed to support Danes' and Bearn's enthusiasm for the treatment. Onisawa and Lee (28) described an increased urinary excretion of DS and other GAG in two Hunter and one Scheie patients treated with prednisolone, and concluded that the treatment reduced the fixation of GAG in tissues. They (29) did not find any modification of the urinary GAG after administration to the same patients of vitamin A or vitamin C.

Human plasma infusions in patients with mucopolysaccharidoses seemed to fit the recommendations of Crocker (30) for any theoretically useful therapeutic trial to be attempted for inborn errors of metabolism. Thus far, two patients with Hurler's syndrome and five with Hunter's syndrome have been treated. All the patients were hyperactive and difficult to manage prior to the infusion but became quiet and almost "sedated" during and after the infusion. Striking changes occurred in the skin; thick and cold before infusion, it became soft and warm and somewhat elastic during and after the infusion. The "claw hands" of a Hunter patient could be extended almost completely; the cold, rigid, and poorly vascularized extremities of a Hurler patient improved dramatically. The improved behavior of several patients lasted from a few weeks to about 1 month, to return abruptly to that observed prior to the infusion. It was frequently reported that during the "remission" period, the children would entertain themselves to some extent and show more attention to their environment.

The measurement of the urinary excretion of GAG in the three patients described demonstrates that after the beginning of the plasma infusion (1) some mobilization of GAG occurred, followed by their degradation (Fig. 3A, B, C); (2) the very limited ability the patients had to degrade DS and HS (see first columns of Fig. 4 A, B, C) increased during the infusion. This increase was more evident in the youngest patient (R. S.); (3) about 20–25% of the "large molecular weight urinary GAG" excreted by Hurler's and Hunter's patients represents compounds other than DS and HS. This aliquot decreased during plasma infusion and correspondingly increased in the "GAG fragments." These data agree with results obtained recently (31, 32) and indicate that the metabolism of other GAG, besides DS and HS, is compromised in the course of long-standing Hurler's and Hunter's syndromes.

Recently, Mapes *et al.* (33), after infusing plasma in two patients with Fabry's disease, demonstrated a striking but transitory increase in the plasma content of the enzyme ceramide trihexosidase and a corresponding decrease in the plasma concentration of its substrate, galactosyl-(β 1-4)-galactosyl-(β 1-4)-glucosyl-(β 1-1')-ceramide. Thus, they have proposed that enzyme replacement by plasma infusion be used as a means of therapy for the disease.

Since the nature of the "factors" missing in the course of various mucopolysaccharidoses is not yet known, we could only measure changes in the degradation of the substrates excreted in the urine. Our results indicate that infusion of normal human plasma is followed by partial reversibility of the GAG accumulation typical of Hurler's and Hunter's syndromes.

One may speculate on the possible mechanisms involved in GAG mobilization following plasma infusion. Because of the solubility of GAG in aqueous systems, the possible role of the fluid load was explored. A Hunter patient received a continuous infusion of 5% dextrose in 0.2% NaCl for 14 days, at a rate of 650 ml/day. 50 ml of fresh human plasma administered

every day for 5 days caused variations of the urinary excretion of "large molecular weight GAG" and "GAG fragments" compatible with mobilization and degradation of stored GAG. For the next 2 days, the plasma was withheld, while the infusion of fluid continued; the ratio "large molecular weight GAG"/"GAG fragments" promptly reverted to pathological values but decreased again to normal values when the daily plasma infusions were resumed. Thus, we conclude that the mobilization of stored GAG was not the result of a "solvent load."

In view of the hyaluronidase activity present in human plasma (34), the possible role of this enzyme must be considered. Plasma hyaluronidase has a pH optimum of 3.5–4.0; accordingly, it is considered to be of lysosomal origin. Aronson and Davidson (35) have stated that the lysosomal enzyme may be active only within intact lysosomes, where such a favorable low pH could be maintained. Thus, a generalized action of the enzyme may be excluded. Assuming that part of the enzyme could ultimately gain access to the lysosomes, it is likely that the high concentration of DS and HS existing therein (36) would inhibit its action (35). However, according to the data of Patel *et al.* (37), the deficiency of hyaluronidase in Hurler, Hunter, and Sanfilippo's syndromes is not secondary to the presence of endogenous inhibitors but rather represents a basic enzymatic defect responsible for the accumulation of GAG other than DS and HS in cultured Hurler fibroblasts and in patients with long-standing mucopolysaccharidoses. If this were the case, administration of lysosomal hyaluronidase could cause the depolymerization of hyaluronidase-sensitive GAG but not extensive depolymerization of DS and HS. Thus, the possibility must be considered that human plasma may also contain "factors" more specifically involved in the degradation of DS and HS. Our results indicate that after plasma infusion, these two GAG undergo a process of degradation which, although greater than that taking place in control children of the same age, nevertheless does not reflect the magnitude nor the duration of the clinical changes noticed in the treated children. It is conceivable that the temporary replacement of the missing factors may cause degradation of DS and HS to residues too small to be measured with our methods.

In conclusion, the striking, albeit transitory, clinical and biochemical changes observed in patients affected by mucopolysaccharidoses I and II after infusion of normal human plasma lend hopes to the possibility of influencing the course of these diseases once purified preparations of "corrective factors" become available.

This work is dedicated to the memory of Theodore T. Tsaltas. Supported by the U.S. Public Health Service (AM 10811.05, HE 05435-10, and RR-00188) and aided by a grant from the National Foundation—March of Dimes.

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