Enzyme therapy in Fabry disease: Differential *in vivo* plasma clearance and metabolic effectiveness of plasma and splenic α-galactosidase A isozymes*

(lysosomes/glycoproteins/trihexosylceramide/vascular endothelium/lipidosis)

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ABSTRACT   A pilot trial of enzyme replacement with splenic and plasma α-galactosidase A (α-D-galactosidase; α-D-galactoside galactohydrolase, EC 3.2.1.22) isozymes was undertaken in two brothers with Fabry disease, an X-linked glycosphingolipid storage disease. Six unentrapped doses (2000 units/kg) of each isozyme were administered intravenously to the respective recipient during a 117-day period. The circulating half-life of the splenic isozyme was about 10 min, whereas that for the plasma isozyme was approximately 70 min. No immune response was detected by skin and immunodiffusion tests or by alterations in the maximal activity or clearance kinetics for either isozyme after successive administrations. After each dose of the splenic isozyme, the concentration of the accumulated circulating substrate, trihexosylceramide (globotriaosylceramide), decreased maximally ($\approx$50% of initial values) in 15 min and returned to preinfusion levels by 2–3 hr. In marked contrast, injection of the plasma isozyme decreased the circulating substrate levels 50–70% by 2–6 hr; the concentrations gradually returned to preinfusion values by 36–72 hr.

Fabry disease, an inborn error of glycosphingolipid metabolism, results from the defective activity of the lysosomal hydrolase, α-galactosidase A (α-GAL A; α-D-galactosidase; α-D-galactoside galactohydrolase, EC 3.2.1.22) (1–4). Trihexosylceramide [globotriaosylceramide; Gb₃Cer; $Gal(\alpha 1 \rightarrow 4)Gal(\beta 1 \rightarrow 4)$-$Glc(\beta 1 \rightarrow 1')Cer$], the enzyme's major glycosphingolipid substrate, accumulates in the plasma (5) and particularly in the vascular endothelial lysosomes (6–8) of hemizygous males afflicted with this X-linked disease. The preferential and progressive deposition of Gb₃Cer in the vascular endothelium is responsible for the major manifestations of the disease and may be enhanced by the receptor-mediated uptake of low-density lipoproteins (LDL), which carry the accumulated circulating substrate (7). Thus, efforts to treat Fabry disease must be directed to the depletion of Gb₃Cer in the circulation and vascular endothelium.

The rationale for enzyme replacement in this lysosomal storage disease was based on *in vitro* correction of the metabolic defect by addition of exogenous α-GAL A to the media of cultured skin fibroblasts obtained from hemizygotes with Fabry disease (9). In 1970, we reported the first clinical trial (10) based on the finding that normal plasma contained active α-GAL A (2). Single infusions of fresh normal plasma, containing about 6000 units of enzymatic activity, were administered to three patients with Fabry disease; the infused activity disappeared from the circulation with a half-life of about 95 min and approximately 50% of the circulating substrate was cleared for a period of several days (10, 11). Subsequently, Brady and coworkers (12) partially purified a tissue form of α-GAL A from

human placenta and intravenously administered single doses to two patients (6000 and 11,000 units, respectively). The exogenous activity was rapidly cleared from the recipient's circulation with a half-life of about 11 min. The plasma substrate level was decreased about 50% at 45 min with a return to the preinfusion level by 48 hr. In addition, the administered activity was detected in percutaneously biopsied liver at 1 hr. These trials demonstrated the feasibility of this approach and indicated that the kinetics of the disappearance of the tissue and plasma forms of the enzyme were different. Recent studies in our laboratories demonstrated that highly purified splenic and plasma α-GAL A isozymes differed in their isoelectric points, electrophoretic migration on polyacrylamide gels, and sensitivity to neuraminidase treatment (13). However, double immunodiffusion studies against splenic anti-α-GAL A showed a line of identity for both isozymes (unpublished results). These findings were consistent with the plasma form being a glycoprotein that was significantly more sialylated than the splenic isozyme. Because the disappearance from plasma of sialylated and asialoglycoproteins in animals has been the subject of recent investigations (14–20), it was of interest to determine if these observations could be extended to a homologous lysosomal hydrolase administered to humans. We report here the results of a clinical trial of multiple intravenous injections of unentrapped α-GAL A isozymes for treatment of Fabry disease.

MATERIALS AND METHODS

Patients. Cases I and II were brothers, aged 25 and 26 years, respectively. Hemizygosity for Fabry disease was biochemically confirmed (4, 5, 21). Both patients were admitted to the Clinical Research Center prior to each infusion for baseline clinical and laboratory studies. Plasma volumes were determined by standard ⁵¹Cr-labeled erythrocyte dilution studies (case I = 3125 ml; case II = 2833 ml).

Enzyme Assays. α-GAL A activity was determined in heparinized plasma, isolated leukocytes, and cultured skin fibroblasts by using the synthetic substrate, 4-methylumbelliferyl-α-D-galactopyranoside (4-MU-α-D-Gal; Research Products International Corp., Elk Grove Village, IL) (4, 22). Purified preparations of α-Gal A were assayed by using Gb₃Cer (23). One unit of enzymatic activity hydrolyzed 1 nmol of 4-MU-α-D-Gal (or, when specifically noted, 1 nmol of Gb₃Cer) per hr at 37°C.

Purification of α-GAL A Isozymes. Splenic and plasma α-GAL A isozymes for clinical administration were partially purified without the use of detergents and under aseptic con-

Abbreviations: α-GAL A, α-galactosidase A; Gb₃Cer, globotriaosylceramide (trihexosylceramide); LDL, low-density lipoprotein; 4-MU-α-D-Gal; 4-methylumbelliferyl-α-D-galactopyranoside.
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ditions. Plasma α-GAL A was purified from human Cohn fraction IV-1 by the method of Bishop *et al.* (23). α-GAL A from human spleen was purified by sequential ammonium sulfate precipitation, affinity chromatography on concanavalin A-Sepharose, and chromatography on DEAE-cellulose and Sephadex G-200 according to unpublished methods. The partially purified isozymes were stable at 4°C or −20°C for up to 10 months. Prior to use, these preparations were tested for bacterial endotoxin concentration, residual thrombin activity, hepatitis B surface antigen, toxicity in mice and guinea pigs, and sterility; approval was obtained from the Food and Drug Administration for their use.

Quantitation of Gb₃Cer. Gb₃Cer was isolated from heparinized plasma samples by the method of Vance and Sweeley (24) and quantitated by gas/liquid chromatography of the alditol acetate derivatives, as described (25).

In Vitro **Studies.** Neuraminidase treatment, column isoelectric focusing, and polyacrylamide gel electrophoresis of the plasma and splenic isozymes were carried out as described (13). The stabilities of the administered enzymes were determined *in vitro.* Mixtures of each isozyme (48 units) and heparinized Fabry whole blood (1.0 ml) were buffered with 25 mM Hepes (0.25 M stock solution at pH 6.9) to pH 7.4 in screw-capped tubes. Individual mixtures were incubated at 37°C from 0 to 180 min and were centrifuged (2000 × *g* for 15 min), and the supernatants were assayed for α-GAL A activity.

The possible binding of either isozyme to a component in Fabry or normal plasma or to purified LDL was assessed. Splenic or plasma α-GAL A was mixed with Fabry or normal plasma containing 25 mM Hepes and the mixture was adjusted to pH 7.2. In addition, each isozyme was mixed with LDL (final concentration, 6.6 mg/ml). Each mixture contained approximately 500 units of enzyme per ml and was incubated at 37°C for 10 min before electrophoresis. As a control for the binding studies, each isozyme (13 units) was incubated with either 2.6 or 26 μg of rabbit anti-human splenic α-GAL A antibody (IgG fraction) at pH 7.0 in 10 μl for 5 min at 37°C. From each mixture, 1 μl was applied to cellulose acetate gels (250 μm; Kalex Scientific, Manhasset, NY) and was electrophoresed for 45 min at 200 V (constant voltage) (*ca.* 5 mA) in a Beckman Microzone unit in the pH 7 buffer system (XIV) of Van Someren *et al.* (26). The enzymatic activity was visualized by incubation of the gel for 20 min at 37°C with an overlay of 5 mM 4-MU-α-D-Gal in Whatman 3MM filter paper followed by intensification for 1 min in ammonia vapor. The same strip was then stained for protein with Coomassie brilliant blue (27).

Analogously, the possible binding of Gb₃Cer to a component in the splenic or plasma enzyme preparations was investigated. [³H]Gb₃Cer (21,000 cpm/nmol), labeled in the terminal galactosyl moiety (28), was incorporated with 5% yield into purified LDL (1 × 10⁵ cpm/mg of LDL) according to the method of Clarke and Stoltz (29). The LDL-adsorbed Gb₃Cer (700 cpm/μl) was then mixed 1:1 with 2.5 units of splenic or plasma α-GAL A per μl or heparinized normal plasma (pH 7.2, 25 mM Hepes) and the mixture was incubated at 37°C for 5 min. From each mixture, 1 μl was applied to the cellulose acetate gel and the gel was electrophoresed and stained as described above. Then, the individual lanes were cut into sections, solubilized, and assayed for radioactivity.

In Vivo **Studies.** Informed consent was obtained separately for each procedure. The preparations of splenic and plasma α-GAL A activities (2000 units/kg) were administered intravenously into the right anticubital vein over 15 sec on days 1, 3, 10, 52, 91, and 117. Case I received only the splenic isozyme and case II received only the plasma isozyme. Plasma and leukocytes were isolated from heparinized blood obtained from the left antecubital vein at selected intervals before and after

each infusion. The levels of α-GAL A activity in plasma and leukocytes and the concentrations of Gb₃Cer in plasma were determined. The plasma disappearance curves were analyzed by curve peeling to give multiexponential equations of the form $a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$. The metabolic clearance rates were calculated as $1/[\int_0^\infty (a_1 e^{-k_1 t} + a_2 e^{-k_2 t}) dt]$.

Potential immune response to each administered enzyme preparation was determined by Ouchterlony double immunodiffusion of the patient's undiluted serum against serial dilutions (1:1 to 1:1024) of the respective isozymes and by intradermal and scratch tests with 0.1 ml of the respective isozyme preparation before and after each injection. The potential immune clearance of the administered isozymes was assessed by comparing the maximal recovered activity and the half-life of the disappearance of enzymatic activity from plasma after each administration.

RESULTS

Characterization of the Administered Isozymes. The partially purified α-GAL A isozymes from human spleen and plasma Cohn fraction IV-1 had essentially identical physical and kinetic properties (Table 1). Both isozymes bound to concanavalin A-Sepharose, indicating that the native isozymes were glycoproteins. However, the splenic isozyme had a single isoelectric point at pH 4.3 [using the column micromethod of Behnke *et al.* (30)], whereas the plasma isozyme had a pI of 3.7 and migrated more electronegatively in 6% polyacrylamide disc gels at pH 7.0. After neuraminidase treatment, the pI of the plasma enzyme, its substrate affinity, and its migration on polyacrylamide gels were essentially the same as those of the treated or untreated splenic isozyme (13). The administered preparations had low endotoxin levels, were negative for thrombin activity and hepatitis B antigen, and were sterile.

Plasma Disappearance Kinetics of Infused Isozymes. Infusion of the isozymes did not result in any change in vital signs or other complications. Coagulation studies were normal before and after each infusion. Fig. 1 compares the typical disappearance kinetics of equivalent doses of plasma and splenic isozymes from the circulation. Maximal recovery from plasma for both the plasma and splenic activities was approximately 50% of the injected dose at 1 min, based on the plasma volumes of the respective recipients. The splenic isozyme was rapidly cleared with a $t_{1/2}$ of approximately 10 min. In marked con-

Table 1. Comparison of the administered α-GAL A preparations

| Property	Isozyme	
	Splenic	Plasma
Enzymatic activity		
(4-MU-α-D-Gal)	18,000 U/ml	5,630 U/ml
Specific activity		
(4-MU-α-D-Gal)	20,500 U/mg	1,450 U/mg
(Gb₃Cer)	3,280 U/mg	234 U/mg
pH optimum	4.5	4.6
Isoelectric point, pI	4.3	3.7
After neuraminidase		
treatment	4.3	4.2
K_m (4-MU-α-D-Gal)	2.5 mM	1.9 mM
After neuraminidase		
treatment	2.5 mM	2.6 mM
Thermostability, $t_{1/2}$ at 55°C	7 min	7 min
Dose/injection (2000 U/kg)	120,000 U	90,000 U
Buffer composition		
Sodium chloride	120 mM	120 mM
Sodium phosphate	10 mM	25 mM
pH	6.5	6.0
Endotoxin	1.3 ng/ml	1.0 ng/ml

U, units.
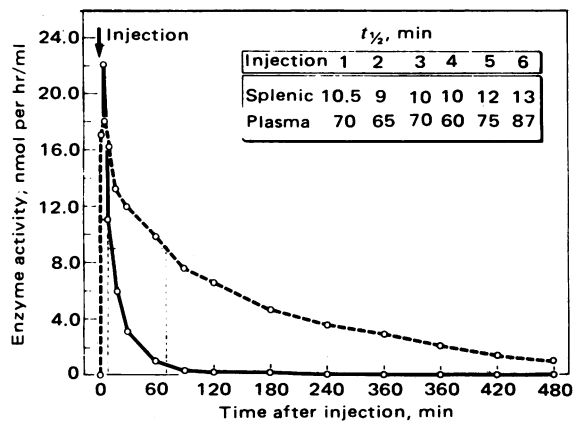
FIG. 1. Disappearance from plasma of α-GAL A activity after the third intravenous injection of the splenic (——) and plasma (- - -) isozymes. The $t_{1/2}$ values for each injection are shown in the inset.

trast, the plasma isozyme disappeared more slowly with a $t_{1/2}$ of about 70 min. The metabolic clearance rate of the splenic enzyme was 253 ml/min, which was about 8 times faster than that of the plasma enzyme. These clearance curves were compatible with distribution of the respective isozyme in at least two compartments, the plasma enzyme being retained longer in the injected compartment. No exogenous activity was recovered in peripheral leukocytes from either recipient at 1 and 4 hr after injection 1.

Fig. 2 contrasts the *in vivo* disappearance kinetics of the splenic and plasma isozymes with their *in vitro* stabilities when incubated in buffered whole blood maintained at pH 7.4 and 37°C. Under the *in vitro* conditions, both isozymes had half-lives greater than 200 min, indicating their relative stabilities under these conditions. As shown in Fig. 3, electrophoresis of mixtures of each isozyme with normal or Fabry plasma or with LDL showed no alteration in the electrophoretic mobility of either isozyme. In all mixtures, the plasma isozyme migrated to the front of the human serum albumin band whereas the splenic isozyme migrated to a less electronegative position. Neither isozyme comigrated with LDL. In contrast, when either enzyme preparation was mixed with rabbit anti-splenic α-GAL

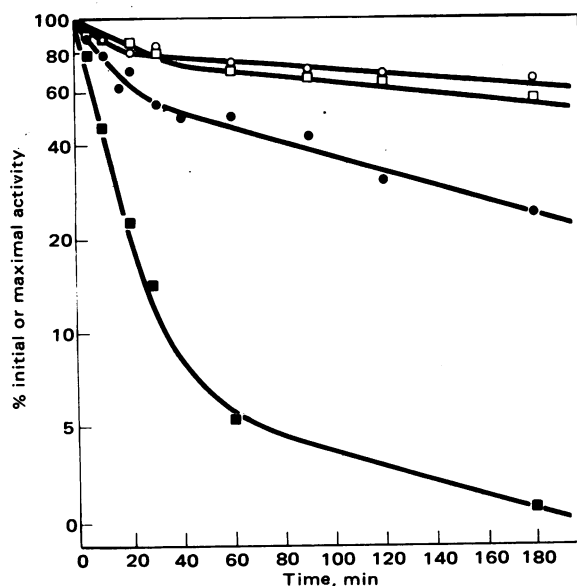
FIG. 2. *In vitro* stabilities and *in vivo* disappearance from plasma of α-GAL A isozymes. The values for the *in vivo* disappearance of the isozymes represent the means of 3–5 separate injections. *In vitro*: ○, plasma; □, splenic. *In vivo*: ●, plasma; ■, splenic.
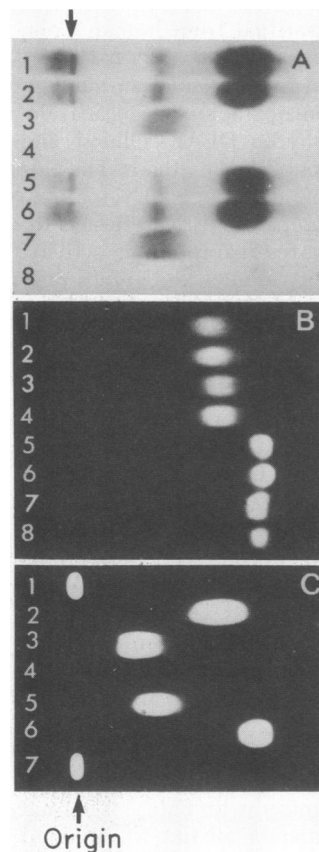

FIG. 3. Cellulose acetate electrophoresis of α-GAL A isozymes in the presence of plasma (*A* and *B*), LDL (*A* and *B*), or antibody (*C*). (*A* and *B*) Lanes 1–3 contain mixtures of the splenic isozyme with Fabry plasma (lane 1), normal plasma (lane 2), and LDL (lane 3). Lane 4 contains splenic isozyme only. Lanes 5–7 contain mixtures of the plasma isozyme with Fabry plasma (lane 5), normal plasma (lane 6), and LDL (lane 7). Lane 8 contains plasma isozyme only. The same chromatogram was stained for protein (*A*) and 4-MU-α-D-Gal activity (*B*). (*C*) Rabbit anti-human splenic α-GAL A (IgG fraction) was mixed with splenic isozyme (lanes 1 and 3) and plasma isozyme (lanes 5 and 7) at two antibody concentrations, 2.6 mg/ml (lanes 1 and 7) and 0.26 mg/ml (lanes 3 and 5). Lanes 2 and 6 contain only spleen or plasma isozyme, respectively; lane 4, antibody alone (2.6 mg/ml).

A antibody, the electrophoretic mobility was markedly decreased, demonstrating that this system could detect noncovalent binding of the isozymes to other proteins.

There were no detectable alterations in the maximal activity or clearance kinetics for either isozyme with successive injections (Fig. 1 *Inset*), indicating the absence of neutralizing or precipitating antibodies to either enzyme. In addition, no antibodies were detected by double immunodiffusion studies or by intradermal and scratch tests before and after each administration.

Effect of Infusions on Circulating Gb₃Cer Levels. A marked difference in the clearance of the circulating substrate was observed after the administration of these isozymes. As shown in Fig. 4 *left*, the splenic isozyme effected a rapid decrease in the plasma concentration of Gb₃Cer. The level of the circulating substrate decreased to approximately 50% of the preinfusion values at 15 min after injection followed by a rapid return to preinfusion levels by 2–3 hr. In contrast, the plasma isozyme resulted in a prolonged depletion of the circulating substrate (Fig. 4 *right*). At 2 hr after injection, the levels of Gb₃Cer were decreased to 30–50% of the preinfusion values. Significantly, low levels were retained up to 12—24 hr and slowly returned to preinfusion levels after 36–72 hr. When the total amount of substrate cleared with time was calculated by integrating the mean concentrations of Gb₃Cer, the plasma isozyme appeared to have cleared about 25 times more substrate over time than the splenic isozyme. For each administration, the total amount of circulating lipid cleared, based on the respective recipient's plasma volume, was less than the potential quantity of natural substrate that the administered enzymatic activity could hydrolyze *in vitro*.

In order to determine whether a component other than the enzyme in either preparation could bind Gb₃Cer and perhaps mediate its clearance, radiolabeled Gb₃Cer adsorbed to LDL was incubated with an aliquot of the splenic or plasma isozyme preparation or of normal plasma and the mixtures were elec-
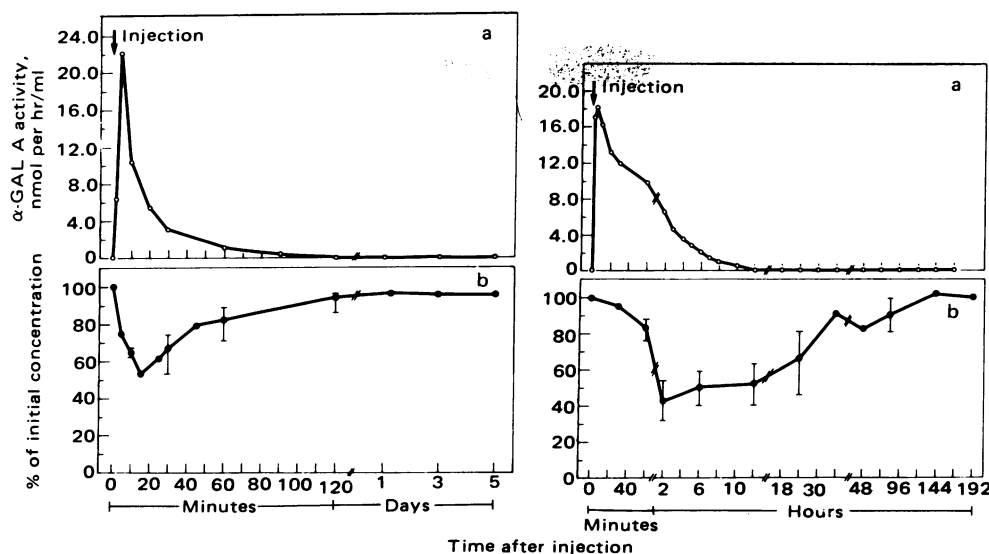
FIG. 4. Effect of intravenously administered splenic (*Left*) and plasma (*Right*) α-GAL A activities on concentrations of Gb₃Cer in plasma from case I (*Left*) and case II (*Right*). (*a*) Plasma disappearance of α-GAL A activity (injection 3); (*b*) means and ranges of Gb₃Cer concentrations in plasma after injections 1, 3, 4, and 6 (plasma) and injections 1, 3, 4, 5, and 6 (splenic).

trophoresed. In each mixture, two peaks of radioactivity were detected, a major peak at the point of application and a second peak which corresponded to the position of LDL. Essentially no alteration in the distribution of radioactivity was observed when the LDL–substrate complex was mixed with either enzyme preparation or normal plasma.

DISCUSSION

The results of these studies demonstrated significant *in vitro* and *in vivo* differences between the splenic and plasma α-GAL A isozymes that may be crucial to the effectiveness of enzyme therapy for Fabry disease. In contrast to the α-GAL A isozyme purified from human spleen, the plasma isozyme (*i*) was more anodic on polyacrylamide and cellulose acetate electrophoresis and was sensitive to neuraminidase treatment, (*ii*) was retained in the recipient's circulation with a 7-fold longer half-life, and (*iii*) was about 25 times more effective in the clearance of circulating Gb₃Cer. In addition, these clinical trials demonstrated that multiple doses of either partially purified isozyme, administered unentrapped over a 117-day period, did not elicit an immune response in the recipients.

The *in vitro* demonstration that the α-GAL A isozyme partially purified from human plasma was more acidic than the splenic form and was affected by neuraminidase treatment was consistent with previous studies (31) demonstrating that plasma forms of various lysosomal hydrolases are more electronegative than their respective tissue counterparts. These physical and kinetic findings were corroborated in our laboratory by using highly purified plasma α-GAL A [specific activity = 44,600 units/mg (13)] and the splenic isozyme purified to apparent homogeneity (1,450,000 units/mg (unpublished results)]. In addition, immunodiffusion studies of these highly purified preparations revealed a line of identity for both isozymes against rabbit anti-human splenic α-GAL A antibody (unpublished results). These results suggest differences in the posttranslational modification of the two isozymes, the plasma form being more electronegative, consistent with it's being more sialylated than the tissue isozyme.

Recently, attention has been focused on the role of oligosaccharide moieties in the molecular recognition processes that determine the clearance and tissue uptake of sialylated and desialylated glycoproproteins (14–17), including lysosomal hydrolases (18–20). Ashwell and Morell (14) demonstrated that the terminal sialic acid residues on many circulating mammalian glycoproteins rendered these molecules more electronegative and were essential for their prolonged retention in the

circulation. Desialylation, exposing the penultimate galactosyl moieties, signaled their rapid clearance from the circulation via selective recognition of these asialoglycoproteins by hepatocytes and uptake into their lysosomal apparatus (16). Similar plasma clearance kinetics have been demonstrated for native and desialylated plasma or tissue forms of various purified lysosomal hydrolases injected intravenously into rodents (17–20). The different *in vivo* plasma disappearance kinetics of the α-GAL A isozymes described here were similar to those observed in the rodent studies (17–20) and suggest the occurrence of this molecular recognition process for homologous hydrolases administered to humans. We postulate that the differential clearance kinetics were due to the presence of sialic acid residues on the plasma form of α-GAL A, but this remains to be demonstrated conclusively.

Although the ultimate fate of the injected isozymes could not be determined, it is presumed that the splenic isozyme was primarily taken up by the liver (i.e., hepatocytes) or reticuloendothelial cells in the spleen, consistent with the findings for injected tissue forms of lysosomal hydrolases in animals (17–19) and previous human trials (32–34). The retention of the plasma isozyme in the circulation may permit its uptake in part by circulating blood elements, vascular endothelium, or other visceral tissues; alternatively, the plasma isozyme may be slowly processed by other circulating enzymes (e.g., neuraminidase) for tissue uptake or it may be slowly inactivated. It is unlikely that the isozymes were degraded in the circulation, because they were relatively stable under physiologic conditions *in vitro* (Fig. 2). In addition, binding of either isozyme to a component in the recipient's plasma that could have mediated its disappearance was not demonstrated (Fig. 3).

Whatever the ultimate fate of these isozymes, their differential metabolic effectiveness was underscored by their remarkably different substrate clearance and reaccumulation kinetics. The effect of the splenic isozyme on the circulating substrate was rapid and transient and paralleled the rapid disappearance of the isozyme from the plasma (Fig. 4 *left*). The splenic isozyme presumably was taken up primarily by the lysosomal apparatus of the liver (12, 15, 32, 33), where it may have hydrolyzed accumulated substrate. In contrast, the prolonged retention of the plasma isozyme in the circulation was associated with significantly more substrate clearance (Fig. 4 *right*).

As yet, the mechanism by which the administered α-GAL A activity reduced the levels of Gb₃Cer in the plasma pool is obscure. The rapidity with which the phenomenon occurred,

after injection of either the plasma or splenic isozymes, suggested direct hydrolysis of the glycosphingolipid in one or more of the plasma compartments (e.g., various lipoprotein pools). Arguments against this possibility are the unfavorable pH and the relative inactivity of the plasma or tissue α-GAL A isozyme when incubated at pH 7.4 with the natural substrate (ref. 13; unpublished results) or with whole blood from patients with Fabry disease (12). A second possibility is the clearance of the enzyme from the circulation into a tissue, presumably the liver, in which the lysosomal catabolism of the glycosphingolipid substrate occurs, followed by a decrease of the plasma pool of accumulated substrate to maintain an equilibrium between plasma and tissue pools. This mechanism has no physiological analogy and seems to be unlikely due to the rapidity of the decrease and subsequent reaccumulation of substrate to preinfusion levels, especially after injection of the splenic enzyme. However, evidence for the rapid exchange of neutral glycosphingolipids between plasma and extraplasma pools has been reported (35). A third possibility is that the enzyme becomes adsorbed onto the surface of certain blood cells or vascular endothelial cells where the pH is favorable for substrate catabolism. Alternatively, the circulating substrate may have become bound to a component in the administered preparations, other than the enzyme, which mediated its removal from the circulation. However, no evidence for such a component in the administered preparations was detected by *in vitro* studies with radiolabeled Gb₃Cer. Although the site for substrate hydrolysis or depletion of the circulating substrate is not known, similar decreases in the levels of circulating substrates have been reported after enzyme replacement in patients with Fabry (10–12) and other (32, 33) lysosomal storage diseases.

The potential immunologic complications associated with the administration of exogenous enzymes have been the subject of concern (36). These pilot studies demonstrated the absence of any side effects or immune response to multiple doses of these unentrapped and partially purified isozymes over a period of almost 4 months. Further studies evaluating the biochemical, immunologic, and clinical effects of long-term trials in selected patients are required before the efficacy and safety of enzyme therapy for Fabry disease can be established.

1. Brady, R. O., Gal, A. E., Bradley, R. M., Martensson, E., Warshaw, A. L. & Laster, L. (1967) *N. Engl. J. Med.* **276** (21), 1163–1167.
2. Mapes, C. A., Anderson, R. L. & Sweeley, C. C. (1970) *FEBS Lett.* **7**, 180–182.
3. Kint, J. A. (1970) *Science* **167**, 1268–1269.
4. Desnick, R. J., Allen, K. Y., Desnick, S. J., Raman, M. K., Bernlohr, R. W. & Krivit, W. (1973) *J. Lab. Clin. Med.* **81**, 157–171.
5. Vance, D. E., Krivit, W. & Sweeley, C. C. (1969) *J. Lipid Res.* **10**, 188–192.
6. Pompen, A. W. M., Ruiter, M. & Wyers, H. J. G. (1947) *Acta Med. Scand.* **128**, 234–255.
7. Johnson, D. L. & Desnick, R. J. (1978) *Biochim. Biophys. Acta* **538**, 195–204.
8. Desnick, R. J., Klionsky, B. & Sweeley, C. C. (1977) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Frederickson, D. S. (McGraw-Hill, New York), 4th Ed., pp. 810–840.
9. Dawson, G., Matalon, R. & Li, Y. T. (1973) *Pediat. Res.* **7**, 694.
10. Mapes, C. A., Anderson, R. L., Sweeley, C. C., Desnick, R. J. & Krivit, W. (1970) *Science* **169**, 987–989.
11. Sweeley, C. C., Mapes, C. A., Anderson, R. A., Desnick, R. J. & Krivit, W. (1971) in *Lipid Storage Diseases, Enzymatic Defects and Clinical Implications*, eds. Bernsonn, J. & Grossman, H. J. (Academic, New York), p. 165.
12. Brady, R. O., Tallman, J. F., Johnson, W. G., Gal, A. E., Leahy, W. R., Quirk, J. M. & Dekaban, A. S. (1973) *N. Engl. J. Med.* **289**, 9–14.
13. Bishop, D. F. & Sweeley, C. C. (1978) *Biochim. Biophys. Acta* **525**, 399–409.
14. Ashwell, G. & Morell, A. (1974) *Adv. Enzymol.* **41**, 99–218.
15. Fiddler, M. B., Wold, F. & Desnick, R. J. (1975) *Intl. J. Biochem.* **6**, 793–799.
16. Hudgin, R. L., Pricer, W. E., Ashwell, G., Stockert, R. J. & Morell, A. G. (1974) *J. Biol. Chem.* **249**, 5536–5543.
17. Fiddler, M. B. & Desnick, R. J. (1977) *Arch. Biochem. Biophys.* **179**, 398–408.
18. Stahl, P., Rodman, J. S. & Schlesinger, P. (1976) *Arch. Biochem. Biophys.* **117**, 594–605.
19. Furbish, F. S., Steer, C. J., Barranger, J. A., Jones, E. A. & Brady, R. O. (1978) *Biochem. Biophys. Res. Commun.* **81**, 1047–1053.
20. Bearpark, T. & Stirling, J. L. (1977) *Biochem. J.* **168**, 435–439.
21. Desnick, R. J., Sweeley, C. C. & Krivit, W. (1970) *J. Lipid Res.* **11**, 31–38.
22. Johnson, D. L., Del Monte, M. A., Cotlier, E. & Desnick, R. J. (1975) *Clin. Chim. Acta* **63**, 81–90.
23. Bishop, D. F., Wampler, D. E., Sgouris, J. T., Bonefeld, R. J., Anderson, D. K., Hawley, M. C. & Sweeley, C. C. (1978) *Biochim. Biophys. Acta* **524**, 109–120.
24. Vance, D. E. & Sweeley, C. C. (1967) *J. Lipid Res.* **8**, 621–628.
25. Yang, H. J. & Hakomori, S. I. (1971) *J. Biol. Chem.* **246**, 1192–1200.
26. Van Someren, H., Van Henegouwen, H. B., Los, W., Wurzer-Figurelli, Doppert, B., Vervloet, M. & Khan, P. M. (1974) *Humangenetik* **25**, 189–201.
27. Weber, K., & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406–4412.
28. Suzuki, Y. & Suzuki, K. (1972) *J. Lipid Res.* **13**, 687–689.
29. Clarke, J. T. R. & Stoltz, J. M. (1976) *Biochim. Biophys. Acta* **441**, 165–169.
30. Behnke, J. N., Dagher, S. M., Massey, T. H. & Deal, W. C., Jr. (1975) *Anal. Biochem.* **69**, 1–9.
31. Swallow, D. M., Stokes, D. C., Corney, G. & Harris, H. (1974) *Ann. Hum. Genet.* **37**, 287–302.
32. Brady, R. O., Penchev, P. G., Gal, A. E., Hibbert, S. R. & Dekaban, A. S. (1974) *N. Engl. J. Med.* **291**, 989–993.
33. Johnson, W. G., Desnick, R. J., Long, D. M., Sharp, H. L., Krivit, W., Brady, B. & Brady, R. O. (1973) in *Enzyme Therapy in Genetic Diseases*, eds. Desnick, R. J., Bernlohr, R. W. & Krivit, W., Birth Defects: Original Article Series (Williams & Wilkins, Baltimore), pp. 120–124.
34. deBarsy, Th., Jacquemin, P., van Hoof, F. & Hers, H. G. (1973) in *Enzyme Therapy in Genetic Diseases*, eds. Desnick, R. J., Bernlohr, R. W. & Krivit, W., Birth Defects: Original Article Series (Williams & Wilkins, Baltimore), pp. 184–190.
35. Barkai, A. & di Cesare, J. L. (1975) *Biochim. Biophys. Acta* **398**, 287–293.
36. Desnick, R. J., Fiddler, M. B., Douglas, S. D. & Hudson, L. D. S. (1978) in *Enzymes of Lipid Metabolism*, eds. Mandel, P., Freysz, L. & Gatt, S. (Plenum, New York), pp. 753–764.