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Enzyme uptake   lysosomes
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 β -glucuronidase

Human β -Glucuronidase. I. Recognition and Uptake by Animal Fibroblasts Suggests Animal Models for Enzyme Replacement Studies

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Summary

As part of an effort to develop an animal model for studies of uptake of human β -glucuronidase, fibroblasts were established from primary explants of connective tissue from nine different animal species, and examined for their ability to take up human platelet β -glucuronidase. Endogenous fibroblast β -glucuronidase was inactivated by heating extracts to 65° for 30 min. Human β -glucuronidase was stable to this treatment. Uptake of human β -glucuronidase by animal fibroblasts was measured as heat-stable β -glucuronidase present in fibroblasts after exposure to partially purified human platelet β -glucuronidase for 48 hr. Although all animal fibroblasts examined exhibited some uptake capacity for human β -glucuronidase, the uptake capacity of different animal fibroblasts varied over a 10-fold range. The uptake capacity of bovine fibroblasts was at least 80% that of human fibroblasts. Rat and hamster fibroblasts showed about half the uptake capacity of human fibroblasts. The rat fibroblasts resembled the human fibroblasts in the kinetics of uptake of high uptake (platelet) enzyme, poor uptake of human placental enzyme, and lack of appreciable turnover of enzyme taken up over 4 days. Heating extracts of rat organs containing added human β -glucuronidase at 65° selectively inactivated rat enzyme.

Speculation

These results indicate that uptake of human platelet β -glucuronidase by fibroblasts is a reflection of a general biologic process shared by many animal species. As such, the rat, and probably several other animal species, might serve as animal models for study of that process in the whole animal. Studies in the whole rat should provide information regarding the uptake and metabolic fate of the different forms of human β -glucuronidase that have large differences in uptake activity and corrective potency for human fibroblasts. This information may prove important in choosing the organ source for purified enzyme, or desirable modifications of purified human enzyme for enzyme replacement therapy.

Most inherited lysosomal storage diseases are caused by a deficiency of a single lysosomal enzyme with resulting accumulation in lysosomes of one or more undegraded metabolites (see review, Reference 13). The remarkable progress in defining the molecular mechanisms of this group of diseases, and the observations that cultured fibroblasts in many of these disorders are "corrected" by adding the missing enzyme to deficient cells have

generated considerable interest in the possibility of enzyme replacement therapy for this group of disorders (2). Animal model systems are desirable for testing this approach since many questions about the uptake, tissue distribution, and fate of native or modified enzymes cannot be answered by human experimentation. A number of animal model systems exist for storage diseases with pathology similar to that found in the corresponding enzyme deficiency disease in man. These include dogs with galactocerebroside β -galactosidase deficiency (16), cattle with mannosidosis (11), and cats with GM₁ gangliosidosis (3).

Although there is no animal model for β -glucuronidase deficiency mucopolysaccharidosis, previous studies with human β -glucuronidase deficiency mucopolysaccharidosis suggested this disorder as a good model to test the feasibility of enzyme replacement therapy in man (9, 14, 15). As with other mucopolysaccharide storage diseases, the deficient fibroblasts show exaggerated accumulation of [³⁵S]mucopolysaccharides in culture, and their biochemical defect is corrected by addition of the missing enzyme. Studies using this *in vitro* model of enzyme replacement have shown that β -glucuronidase of bovine and human origin is taken up and is corrective (9, 14), that human β -glucuronidase varies markedly in corrective potency depending on the organ source from which it is purified (1), and that the corrective activity resides in a discrete subpopulation of the β -glucuronidase molecules with a characteristic isoelectric range (7). It has been suggested that the "high uptake" form of the enzyme bears a component which accounts for its recognition and uptake by human fibroblasts. This may be the component which is abnormal in the lysosomal enzymes in I-cell disease, where enzyme is produced which is catalytically active but non-corrective for fibroblasts (6, 10). Whether the recognition component on lysosomal enzymes that is responsible for high uptake and high corrective potency in cultured fibroblasts will also influence the uptake, tissue distribution, and fate of infused enzyme in the deficient patient is not known. The answer, which could be important to the prospects of lysosomal enzyme replacement for lysosomal enzyme deficiency diseases, might be obtained in animal studies. Human fibroblasts have been reported to take up and be corrected by bovine β -glucuronidase (9). This led us to suspect that some animal fibroblasts would recognize human β -glucuronidase. If so, the study of animal fibroblasts for the ability to recognize and take up human β -glucuronidase might indicate which animals might be suitable for study of the uptake, distribution, and fate of infused enzyme in whole animal studies. The greater thermostability of human β -glucuronidase than β -glucuronidase from animal sources (8) might allow one to identify human β -glucuronidase in animal tissues and thereby examine the uptake and fate of infused human β -glucuronidase in experimental animals.

This paper (1) compares the thermostability of human and animal fibroblast β -glucuronidase, (2) compares fibroblasts established from a variety of animal species for the ability to recognize and take up human β -glucuronidase (recognized by its heat stability), (3) shows many similarities in the uptake properties of rat and human fibroblasts for human β -glucuronidase, and (4) demonstrates the relative stability of human β -glucuronidase in heated extracts of rat organs. A preliminary report of this work was presented previously (5).

MATERIALS AND METHODS

ESTABLISHMENT AND GROWTH OF ANIMAL FIBROBLAST LINES

Biopsies were obtained from connective tissue of 9 different animal species (cow, lamb, pig, chicken, golden hamster, Holtzman rat, rabbit, AKR mouse, and dog). Explants were grown in 60 mm (Falcon) plastic dishes under glass cover slips and fed three times per week with 5.0 ml minimum essential medium (MEM) (Gibco) containing 20% heat inactivated fetal calf serum; penicillin, 100 units/ml; and streptomycin, 100 μ g/ml. When the 60-mm dishes became confluent, they were subcul-

tured to two 100-mm dishes and frozen in Ham's F-12 medium (Gibco) containing 10% fetal calf serum and 10% glycerol. Cells were thawed for growth and maintained in Eagle's MEM containing 10% fetal calf serum. When plates were confluent, they were routinely subcultured at one-sixth the density (1:6 split) except for the rapidly growing fibroblasts (bovine, rat, rabbit) which did well with 1:8 to 1:16 splits.

ISOLATION AND PURIFICATION OF HUMAN β -GLUCURONIDASE ACTIVITY FROM PLATELETS AND PLACENTA

Platelet and placenta enzyme isolations followed the immunoadsorbent procedure described earlier, followed by CM-Sephadex adsorption and elution with 0.2 M sodium phosphate, pH 5.8 (7). Fractions with highest β -glucuronidase activity were pooled, brought to 20% glycerol concentration, and frozen at -80° . Activity was stable for over 1 year under these conditions.

UPTAKE OF β -GLUCURONIDASE ACTIVITY BY FIBROBLASTS

Enzyme uptake by duplicate subconfluent dishes (100 mm) of fibroblasts was measured during a 48-hr incubation at 37° . β -Glucuronidase was added to 0.15 M saline containing 10% heat-inactivated (60° for 1 hr) fetal calf serum and filtered through a 0.45 μ millipore filter. Dishes received aliquots (0.1–1.0 ml) of enzyme (250–2,500 units) in 10 ml final volume of MEM containing 10% heated fetal calf serum. After 48 hr, dishes were washed three times with Puck's Saline G. Cells were lysed by adding 1.0 ml of 1% sodium deoxycholate (DOC) in 0.05 M Tris, pH 8.0. After 6 min, the cells and DOC were scraped from the plates into tubes and mixed vigorously. The lysates were added to 4.0 ml 10% heated fetal calf serum in saline, diluting the cell lysates to 0.2% DOC. Cell lysates were stored frozen until assayed for β -glucuronidase.

ASSAYS

β -Glucuronidase was assayed fluorometrically (8). To 100 μ l substrate (10 mM 4-methylumbelliferyl-glucuronide) in 0.2 M sodium acetate buffer, pH 4.8, 25 μ l enzyme were added and the incubation carried out for 60 min at 37° . One unit of enzyme activity is defined as the activity which releases 1 nmol 4-methylumbelliferone/hr. Protein was determined by the method of Lowry *et al.* (12).

RESULTS

ESTABLISHMENT OF ANIMAL CELL LINES

Fibroblasts were successfully established from all of the animals attempted except the DBA mouse. However, several animal cell lines were not successfully cultured for long periods. Chicken fibroblasts did not survive subculturing. Hamster fibroblasts grew poorly after removal from the freezer and rarely survived the third passage. The AKR mouse fibroblasts survived serial propagation, but grew very slowly. Canine cells were difficult to recover from freezing, and sheep fibroblasts tended to detach from the plates while still subconfluent after several passages. Some of these cell lines might be adapted to culture if one were to optimize the growth conditions for each cell line. However, attention was directed to cell lines which grew well under the conditions used for human fibroblasts. Under these conditions, rabbit, rat, and bovine fibroblasts grew very well. Although human and mouse fibroblasts were easily detached for subculturing by trypsinization, most of the other cell lines required the cells to be scraped free with a rubber policeman.

THERMAL INACTIVATION OF ANIMAL FIBROBLAST β -GLUCURONIDASE

In previous studies (8), we found that bovine β -glucuronidase in fetal calf serum was inactivated by heating to 60° . Added human β -glucuronidase was resistant to heating. Deoxycholate

0.2% (at pH 8.0) increases the heat sensitivity of bovine β -glucuronidase. We therefore incorporated deoxycholate into the cell lysis procedure to prepare lysates from different animal fibroblasts and sought heat inactivation conditions under which the endogenous animal β -glucuronidase would be inactivated, but the human enzyme would be stable. Figure 1 presents experiments which demonstrate that heating the cell lysates at 60° for varying times inactivated all of the animal fibroblast β -glucuronidases. The human enzyme was stable for over 90 min under these conditions.

UPTAKE OF HUMAN PLATELET ENZYME BY ANIMAL FIBROBLASTS

The fact that endogenous animal fibroblast β -glucuronidase can be totally inactivated by heating conditions under which the human enzyme is stable means that any of these fibroblasts can be exposed to human enzyme and the amount of human enzyme taken up can be measured as the heat-stable enzyme present in the cells at the end of the uptake period. Table 1 presents the comparison of the uptake of the same platelet enzyme preparation by different animal fibroblast cultures. The results in Table 1 indicate considerable variability in uptake of the human "high uptake" enzyme. Although the differences seen are certain to be influenced by certain uncontrolled variables in these comparisons such as variation in cell passage number, in generation time of the fibroblast strains, and cell population density from experiment to experiment, at least limited general conclusions can be drawn from these comparisons. The bovine fibroblasts take up human enzyme nearly as well as human fibroblasts. Rat and hamster fibroblasts also have appreciable uptake capacity. None of the animal fibroblast lysates inhibited or inactivated added human platelet enzyme during heating. The meaning of very low uptake of "high uptake" enzyme by some of the fibroblast strains is not clear. None of the animal fibroblasts appeared to destroy the added human enzyme during the 48-hr exposure. With all of the animal fibroblasts studied, over 95% of the added human β -glucuronidase could be accounted for after the 48-hr

Table 1. Uptake of human platelet β -glucuronidase by animal fibroblasts¹

Animal	No. of experiments	% of added β -glucuronidase activity taken up	
		Mean	Range
Deficient human (CR)	6	24	19-30
Cow	8	19	15-24
Rat	10	15	14-16
Hamster	6	12	12-13
Pig	9	9.3	7.4-11
Sheep	6	6.7	5.4-8.0
Rabbit	9	5.1	3.0-9.4
Mouse	9	2.5	2.2-2.9

¹ Subconfluent fibroblasts were exposed to human platelet enzyme (250-2,500 units/dish) for 48 hr. Then the cells were washed, lysed in deoxycholate (DOC) as described in "Materials and Methods," and stored frozen until assayed. β -Glucuronidase measurements on the cell lysates after heating at 60° for 30 min indicated the amount of added human enzyme which had been taken up. Control plates (no added enzyme) were done for each experiment to be sure that the heat step completely inactivated the animal fibroblast enzyme. Enzyme assays of mixtures of control plate lysate plus added human platelet enzyme gave, in all cases, over 90% of the expected residual (human) activity after heating.

exposure to cells, either in the cell lysates, or in the medium removed at 48 hr. Similarly there was no evidence for extracellular conversion of added high uptake enzyme to low uptake enzyme by the animal fibroblasts. We previously reported that high uptake enzyme is converted to low uptake enzyme after it is taken up by human fibroblasts (7). The possibility was considered that the animal fibroblasts exhibiting low uptake might secrete an enzyme which destroys the uptake activity of human platelet β -glucuronidase. This, in turn, could explain their apparent low uptake. This possibility was examined by first exposing human platelet β -glucuronidase to human, rat, and mouse fibroblasts for 48 hr, removing the medium, and determining the residual uptake activity of enzyme still in the medium by adding it to human fibroblasts during a second uptake period. In these experiments depletion of the original uptake activity was greatest by human fibroblasts (65-85%), next by rat fibroblasts (50%), and least by mouse fibroblasts (0%). Thus depletion of uptake activity from the medium correlated with high uptake rather than low uptake. These observations suggest that the differences in uptake observed in fibroblasts of different animal origin reflect differences in specific recognition and uptake of the human enzyme among the different animal fibroblasts used. Inactivation of the uptake activity of added enzyme did not explain the low uptake by murine fibroblasts.

Although bovine fibroblasts and whole animal experiments in cattle might be suitable for certain other lysosomal enzyme studies, *e.g.*, in α -mannosidase deficiency (11), the rat, because of its size and availability, appeared to us most suitable for studies of human β -glucuronidase in the whole animal. For this reason, the rat fibroblast system was studied further.

COMPARISON OF UPTAKE CHARACTERISTICS OF RAT AND DEFICIENT HUMAN FIBROBLASTS

Figure 2 shows the time dependence of enzyme uptake in growing (freshly split) fibroblasts of human and rat origin over 48 hr. Figure 3 shows the proportionality between enzyme added and enzyme taken up by subconfluent rat fibroblasts in the 48-hr. assay. Human and rat fibroblasts appear to have similar kinetics of uptake, and uptake by rat fibroblasts is proportional to the amount of enzyme added.

We previously reported the half-disappearance time of human platelet enzyme taken up by deficient human fibroblasts to exceed 2 weeks (14). Figure 4 compares the stability of enzyme

Fig. 1. Heat inactivation of β -glucuronidase in lysates of fibroblasts from various animal species. The heat stability of partially purified human platelet β -glucuronidase added to the deoxycholate lysate mixture is also shown.

Fig. 2. Comparison of the kinetics of uptake of human β -glucuronidase activity by rapidly growing rat fibroblasts and deficient human fibroblasts. Human (●—●) and rat (▲—▲) fibroblasts were split 1:8 into 100-mm dishes and fed at 0 hr with medium (10 ml) containing 1,500 units human platelet β -glucuronidase. Plates were removed at intervals for measurement of heat-stable (60° for 30 min) β -glucuronidase in deoxycholate lysates from these plates.

taken up by human and rat fibroblasts over 96 hr. In both cases no significant decrease in intracellular enzyme is apparent. Thus the intracellular stability of human β -glucuronidase in human and rat fibroblasts appears comparable, at least for 4 days.

Brot *et al.* (1) previously reported that deficient human fibroblasts could distinguish between high uptake enzyme and low uptake enzyme. In those studies, between 10 and 30% of platelet β -glucuronidase (high uptake) was taken up by human cells, whereas placental β -glucuronidase was only poorly recognized and taken up by human cells in culture (0.5–1.5%). When rat and human fibroblasts were exposed to the same placental β -glucuronidase preparation under the conditions used for Table 1, 0.7% was taken up by human fibroblasts and 0.6% was taken up by rat fibroblasts. Thus rat fibroblasts also appear to discriminate between high uptake and low uptake forms of β -glucuronidase.

EXTENSION OF HEAT INACTIVATION STUDIES TO RAT ORGANS

The thermal inactivation conditions used for endogenous β -glucuronidase in fibroblasts require only slight modification to inactivate rat β -glucuronidase in extracts of rat organs. Rat liver, spleen, brain, and kidney β -glucuronidase can be inactivated by heating at 65° with 90% or greater recovery of added human β -glucuronidase as shown in Table 2.

DISCUSSION

The recognition component which accounts for the specific uptake of lysosomal enzyme by fibroblasts is most likely a common factor shared by a large family of lysosomal enzymes. This recognition component is apparently important to normal intracellular enzyme localization, at least in some human cell types,

since it is defective in mucopolipidosis II and mucopolipidosis III, diseases characterized by intracellular deficiency of this family of hydrolases in cultured fibroblasts (6, 10). The studies presented here indicate that fibroblasts from several other mammalian species also recognize the factor responsible for platelet enzyme uptake by human fibroblasts. Clearly, there are differences between bovine fibroblasts, which take up human β -glucuronidase nearly as well as human fibroblasts, and murine fibroblasts, which take up the enzyme far less efficiently, if at all. Whether these differences result from a different number of binding sites for the enzyme on the cell surface, different affinity of these sites for the enzyme, or differences in the rates of endocytosis is not known. What does appear clear is that fibroblasts from a number of mammalian species have apparently similar mechanisms for recognition and uptake of lysosomal enzymes. These species may provide animal models for study of the uptake, tissue distribution, and fate of infused human enzyme.

Others have pointed out the value of animal models for enzyme therapy to work out methods for the delivery of sufficient quantities of the specific active enzyme to the target tissue and to the appropriate subcellular sites for efficient substrate metabolism (2). Thorpe, Fiddler, and Desnick (17) recently described a murine model for determining the *in vivo* fate of administered bovine β -glucuronidase in β -glucuronidase-deficient mice. This mouse strain is not β -glucuronidase-deficient in the clinical sense, as there are no storage abnormalities associated with the partial deficiency for β -glucuronidase. However, the reduced levels of murine β -glucuronidase provide a lower background of endogenous β -glucuronidase in the mouse organs. In some respects, the mouse model is similar to the one reported here in that it relies on differences in thermostability of donor and recipient β -glucuronidase to demonstrate the administered en-

Fig. 3. Dose-dependence of uptake of human platelet β -glucuronidase by rat fibroblasts in 48 hr. Subconfluent rat fibroblasts were exposed to increasing amounts of added β -glucuronidase and uptake measured at 48 hr.

Fig. 4. Comparison of the stability of human platelet β -glucuronidase activity in rat fibroblasts and deficient human fibroblasts. Human fibroblasts and rat fibroblasts were exposed to 10 ml medium containing 1,500 units human platelet β -glucuronidase for 48 hr. At zero time, the medium was removed and replaced with MEM with 10% heated fetal calf serum containing no β -glucuronidase activity. Plates were removed at the times shown to determine the residual human (heat-stable) β -glucuronidase in lysates of the human (●—●) and rat (▲—▲) fibroblasts.

Table 2. Heat inactivation of rat organ β -glucuronidase¹

Organs	Units/g	% Rat enzyme activity remaining after heating	% Added human enzyme activity remaining after heating
Liver	72,125	<0.02	97
Spleen	75,240	0.02	114
Kidney	3,120	1.15	98
Brain	1,626	<0.7	90

¹ One gram of each tissue was homogenized in a Waring Blendor in 3 ml buffer containing Tris-HCl, 0.05 M, pH 8.0; deoxycholate (DOC), 1%; and frozen-thawed three times in an alcohol-Dry Ice bath. The extract was centrifuged twice at 15,000 rpm for 30 min and 20 min. Heat inactivations were done for 30 min at 65° on 0.1-ml aliquots of the supernatants diluted to 1 ml with buffer (DOC final concentration 0.2%) with or without 200 units added human platelet β -glucuronidase. Human platelet β -glucuronidase was stable for over 90 min under these inactivating conditions in the presence of 10% heated fetal calf serum or 1 mg/ml bovine serum albumin in the Tris-DOC buffer.

zyme in various localizations. However, in that model, the administered enzyme is the heat-labile enzyme and the administered enzyme is detected as that which disappears on heating. That model, as described, is not useful to follow the fate of infused human enzyme, which is heat stable. One might be able to modify the thermal heat inactivation conditions used in the mouse studies to allow one to recognize infused human enzyme in mouse tissues. However, the studies reported here indicate that murine fibroblasts were the least efficient of all examined in the recognition and uptake of human "high uptake" enzyme. Thus the mouse may represent a poor choice for analyzing for differences in metabolic fate of the different forms of human

enzyme, even if one could identify human enzyme in murine tissues. Nevertheless, the mouse model was shown to be quite effective in defining the uptake, tissue distribution, and metabolic half-life of administered bovine enzyme (17). Since bovine enzyme is commercially available, that model can be used relatively easily to test methods to modify either the bovine enzyme or the host to influence the uptake, tissue distribution, or half-life of infused bovine enzyme (4, 18).

For a variety of reasons, including immunologic compatibility, the most likely source of lysosomal enzyme for replacement in human lysosomal disorders is human enzyme. For this reason, an animal model which allows one to study the fate of infused human enzyme is particularly desirable. The primary purpose of these fibroblast studies was to provide a basis for selection of an animal model to study the uptake, tissue distribution, and fate of infused human enzyme. Human β -glucuronidase is long lived in human fibroblasts, where the half-disappearance time exceeds 2 weeks. Furthermore, human β -glucuronidase from different organ sources varies tremendously in uptake activity and corrective potency in human fibroblasts. The studies on rat fibroblasts suggest that these rat cells are similar to human fibroblasts in their behavior toward high uptake (platelet) and low uptake (placental) human β -glucuronidase. The human enzyme can be identified in extracts of rat organs by its stability to thermal inactivating conditions which inactivate endogenous β -glucuronidase in rat organs. Thus, it appears feasible to utilize the rat animal model to characterize the uptake, tissue distribution, intracellular localization, and half-life of infused human enzyme which has been purified from various human organ sources. Although the rat appears the most attractive animal for initial experiments, the limited studies with fibroblasts from other species suggest that the hamster, calf, and, possibly, the rabbit could also be used to test the generality of interesting observations made with the rat model. The animal models suggested by the fibroblast experiments presented here can also be used to study ways to modify the human enzyme to influence its tissue distribution or its metabolic half-life after infusion.

CONCLUSION

As part of an effort to develop an animal model for studies of uptake of human β -glucuronidase, fibroblasts were established from primary explants of connective tissue from a variety of mammalian species. In order to measure the human enzyme in extracts containing non-human enzyme, thermal inactivation conditions that preferentially destroy animal β -glucuronidase activities were determined. All fibroblast lines examined exhibited some uptake capacity for human platelet β -glucuronidase. The uptake capacity of bovine fibroblasts resembled the high uptake capacity of human fibroblasts. Rat and hamster fibroblasts incorporated the human enzyme at about half the rate as human fibroblasts. Cultured rat fibroblasts resembled human fibroblasts in terms of their kinetics of uptake of high uptake (platelet) enzyme, their low turnover of incorporated enzyme, and their poor uptake of human placental enzyme. Selective heat inactivation of rat enzyme in extracts of rat organs containing human β -glucuronidase was also demonstrated.

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enzyme uptake   storage disease
 β -glucuronidase

Human β -Glucuronidase. II. Fate of Infused Human Placental β -Glucuronidase in the Rat

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Summary

Human placental β -glucuronidase could be identified in rat organs by heating organ extracts to 65° which selectively inactivated endogenous rat enzyme. Infused enzyme was rapidly cleared from rat plasma ($t_{0.5}$ of 3.5 min). From 50-60% of the infused dose was accounted for in rat liver and spleen 24 hr after infusion. Removal of abdominal viscera, including the spleen, and interruption of the portal circulation before infusion slowed the plasma disappearance ($t_{0.5}$ of 60 min) and allowed significant uptake by bone and other organs. Subcellular fractionation of liver 18 hr postinfusion localized the human enzyme in the mitochondrial-lysosomal fraction. The half-disappearance times of infused human enzyme were 2.6 days in rat liver and 5.8 days in rat spleen. Periodate treatment of human placental β -glucuronidase destroyed 90% of its binding to concanavalin A-Sepharose, reduced its heat stability, and abolished its rapid clearance from rat plasma after infusion.

Speculation

Experiments with the rat model for evaluation of the uptake, distribution, intracellular localization, and metabolic fate of infused human β -glucuronidase can provide a number of answers about enzyme replacement therapy that could not be obtained

easily by human experimentation. The experiments presented suggest that placental β -glucuronidase is cleared predominantly by organs with significant reticuloendothelial components. The clearance mechanism appears to recognize the carbohydrate structure of the enzyme, but may recognize carbohydrate components different from those responsible for enzyme uptake by fibroblasts. Delineation of the cell-specific receptors responsible for organ-specific localization of native and modified human enzymes such as β -glucuronidase may be important to developing a rational basis for enzyme replacement therapy in lysosomal storage disease.

Enzyme replacement therapy for lysosomal storage disorders due to single enzyme deficiency diseases was initially suggested by Hers (13). Interest in enzyme replacement (see reviews, References 2 and 8) was greatly stimulated by the discoveries of Neufeld and coworkers (21) of biochemical correction of cultured fibroblasts *in vitro* by lysosomal enzymes. For a number of reasons, outlined elsewhere (23), β -glucuronidase deficiency mucopolysaccharidosis seemed attractive as an experimental model system to test the feasibility of enzyme replacement in man. However, an animal model system seemed desirable to answer questions about the uptake, fate, and distribution of infused human enzyme that could not be answered in man.