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# ENZYMIC EFFECTS OF β-GLUCOSIDASE DESTRUCTION IN MICE

# CHANGES IN GLUCURONIDASE LEVELS

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## Summary

1. The injection into mice of a single dose of conduritol B epoxide, a covalent inhibitor of glucosidases, quickly produced changes in tissue levels of β-D-glucuronidase (EC 3.2.1.31). The specific activity of the enzyme decreased in liver, spleen and kidney while brain showed little change. The inhibitor did not act on glucuronidase in vitro, so the effect of the inhibitor is complex, possibly a result of the loss of glucosidase activity. Since glucuronidase contains glucose, we suggest that the transport of the enzyme between subcellular regions and tissues involves loss of part of the glucose moieties.

2. Levels of glucocerebrosidase (D-glucosyl-N-acylsphingosine glucohydrolase, EC 3.2.1.45) dropped very rapidly after epoxide injection, reaching a minimum at 1 h in liver. There was a noticeable restoration of activity within the next 1—2 h. Aryl β-glucosidase (EC 3.2.1.21) decrease somewhat less than cerebrosidase, reaching a minimum within 2 h. It too showed some recovery of activity within 3 h.

3. Acid phosphatase rose slightly in liver but not in brain. α-L-Fucosidase and angiotensin-converting enzyme were not affected by the epoxide injection. The latter two enzymes are known to contain glucose.

4. Injection of a hemolyzing agent, phenylhydrazine, produced an increased level of glucuronidase in liver and spleen within 6 days, but not in kidney. This enhancement was a little less in mice previously injected with the glucosidase inhibitor.

5. Mice injected with the epoxide once a day eight times showed a distinct rise in brain glucuronidase level, as well as a rise in brain weight. However, the other organs showed only the same decrease in glucuronidase specific activity noted with the single injection protocol. It is suggested that the difference is

due to the blood-brain barrier, which could slow the loss of brain glucuronidase from the extracellular fluid.

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## Introduction

While Gaucher's disease appears to be primarily a genetic disease in which $\beta$-glucosidases are defective, other striking protein abnormalities can be seen in Gaucher patients. These include high serum levels of acid phosphatase [1], angiotensin-converting enzyme [2], $\beta$-acetylglucosaminidase [3], $\beta$-D-glucuronidase [3], $\beta$-galactosidase [3], and $\alpha$-fucosidase [3]. Intracellular enzyme abnormalities have also been noted: high acid phosphatase in spleen [4], low glutathione reductase in erythrocytes [5], and high angiotensin-cleaving enzyme in spleen [2]. In addition, high levels of some proteins have been seen: immunoglobulins in serum [6,7], ferritin in Gaucher cells [8], and transcobalamin II in serum [9]. Amyloid deposits have also been seen in a Gaucher patient [10].

This study is an attempt to produce some of the above changes by destroying $\beta$-glucosidase activity in mice with an irreversible inhibitor, conduritol B epoxide. Some of the findings reported here are interpreted to indicate that impaired glucoside metabolism can affect the metabolism of glucose-containing glycoproteins.

## Materials and Methods

$\beta$-D-Glucuronidase was assayed with the reagents and procedure from Sigma Chemical Co. (St. Louis, MO, Bulletin 105) with the addition of Triton X-100. The incubation mixture contained 0.15 ml of 0.2 M acetate pH 4.5, 0.25 mg Triton, 0.05 ml of 30 mM sodium phenolphthalein monoglucuronate, and 0.05 ml of tissue homogenate. After 60 min at 56°C, the incubation was stopped with 1.25 ml of 0.1 M 2-amino-2-methyl-1-propanol (pH 11) containing 0.2% sodium lauryl sulfate.

Activity of the angiotensin-converting enzyme was determined with hippuryl histidylleucine [2,11]. The incubation tubes contained 2 mg of kidney or 10 mg of the other tissues.

$\alpha$-L-Fucosidase was assayed by modifying a published method [12] to solve the problem of the low solubility of the substrate. 4-Methylumbelliferyl fucoside (Research Products International, Elk Grove Village, IL, 0.2 $\mu$mol) was added to the incubation tube in chloroform/methanol solution and deposited by evaporation. To this was added 0.1 ml of pH 5.5 buffer, made from 0.4 M phosphate and 0.2 M citric acid. The tissue homogenate in 0.1 ml (0.5 or 1 mg brain, 0.63 or 1.25 mg liver) was incubated at 37°C for 1 h, and the free methylumbelliferone determined as above.

Acid phosphatase was assayed with 0.1 ml of homogenate (0.5 or 1 mg brain, 0.31 or 0.63 mg liver), 0.25 ml of 16 mM $p$-nitrophenyl phosphate, and 0.25 ml of pH 4.7 buffer, made from 0.2 M phosphate and 0.1 M citric acid. After incubation as above, the reaction was stopped with 0.5 ml of 0.25 M NaOH.

Protein was determined by the method of Hess and Lewin [13] after precipitation and washing with trichloroacetic acid.

TABLE I

GLUCURONIDASE ACTIVITY IN MICE INJECTED WITH CONDURITOL B EPOXIDE

Mice were injected at a dose level of 300 mg/kg and killed 5 h later. Activities are expressed in nmol/h per mg tissue.

| | Brain (mg) | | Kidney (mg) | | Liver (mg) | | Spleen (mg) | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 | 5 | 1 | 2 | 0.5 | 1 | 0.5 | 1 |
| Control mice | 1.78 | 1.78 | 15.6 | 15.0 | 25.3 | 23.4 | 31.8 | 27.8 |
| Conduritol B epoxide-treated mice | 1.69 | 1.78 | 9.5 | 11.0 | 17.7 | 19.9 | 25.1 | 22.5 |
| Percent decrease due to conduritol B epoxide | 3 | | 33 | | 22 | | 20 | |

The procedures for other analyses and reagents are given in the accompanying paper [14]. The mice used were type CF-1, 16 days old, injected in groups of eight intraperitoneally with saline or conduritol B epoxide in saline.

## Results

Mice injected with a single dose of conduritol B epoxide (300 mg/kg body weight) were found to have lost almost all of their tissue glucocerebrosidase and an appreciable part of their aryl glucosidase within 5 h [14]. Glucuronidase showed less loss of activity (Table I). Only brain, of the four organs assayed, showed no effect. A decrease in liver and spleen glucuronidase specific activity had been found before in mice treated with daily injections of the epoxide for several weeks [15] and in cultures of neuronal tumor cells treated with a different inhibitor of $\beta$-glucosidases, $N$-hexyl glucosyl sphingosine [16]. A small decrease in activity had also been seen in white cells of Gaucher patients [17].

Since the substrates for glucuronidase and glucosidase differ in structure only at the C-6 carbon atom of the sugar, we thought that the epoxide might simply act directly on both enzymes. However, incubation of relatively high concentrations of the inhibitor with normal mouse homogenates showed no distinct effect on glucuronidase (Table II) while aryl glucosidase was largely destroyed [14]. The effect therefore seemed to be indirect, presumably resulting from the destruction of glucosidase.

*Changes with time*

The time course of enzyme decrease in liver and brain was examined after a

TABLE II

EFFECT OF CONDURITOL B EPOXIDE ON MOUSE TISSUES IN VITRO

Activities are for D-glucuronidase, in nmol phenolphthalein released/h per mg tissue. The inhibitor was incubated for 1 h at 37°C with 0.2 ml of tissue homogenate in pH 4.5 acetate (5 mg brain, 1 mg spleen and liver, 2 mg kidney). Then 0.05 ml of substrate was added and incubation was continued for 1 h at 56°C. 2 and 10 denote the amount of conduritol B epoxide/tube ($\mu$g).

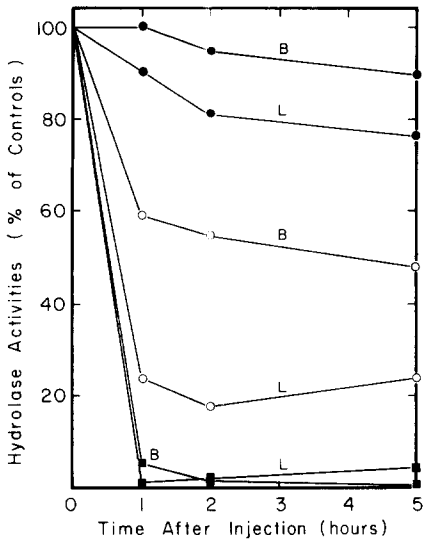| | Brain | | Kidney | | Liver | | Spleen | |
|---|---|---|---|---|---|---|---|---|
| | 2 | 10 | 2 | 10 | 2 | 10 | 2 | 10 |
| Control activities | 1.2 | | 12.7 | | 27.6 | | 23.1 | |
| Experimental activities (% of control) | 108 | 108 | 98 | 96 | 94 | 101 | 101 | 100 |

Fig. 1. Changes in glycosidase activities in liver (L) and brain (B) as the result of injecting 16-day old mice intraperitoneally with conduritol B epoxide, 300 mg/kg body weight. Top curves: glucuronidase (●); middle curves: aryl glucosidase (○); lowest curves: glucocerebrosidase (■).

single injection of conduritol B epoxide at the 300 mg/kg level (Fig. 1). Gluco-cerebrosidase was found to disappear almost completely within 1 h, more so in liver than in brain. The increase in brain loss by the second hour is probably sig-nificant, indicating a slower entrance and release of the inhibitor from brain, possibly because of the blood-brain barrier. Aryl glucosidase also decreased very rapidly. With this enzyme too, brain showed additional decrease in activ-ity over a further period of time. With both enzymes liver seemed to show a slight amount of glucosidase resynthesis after the second hour. Glucuronidase loss followed a similar but delayed pattern: brain activity did not drop until the second hour and continued to drop, while liver showed its major decrease during the first 2 h and possibly a little additional decrease during the next 3 h. While some of the slopes in Fig. 1 are rather small, they probably cannot be attributed to variation in groups of animals as the different enzymes showed different patterns even though measured in the same homogenates. However, the small decrease in brain glucuronidase is inconsistent with the lack of effect in our previous in vivo experiment (Table I).

The primary conclusion from the time study is that (1) brain behaves like liver but more slowly, presumably due to slower absorption and release of con-duritol B epoxide and (2) the changes in glucuronidase could be the result of the changes in glucosidase levels since they followed them in time.

*Subcellular distribution*

Glucoronidase comprises a mixture of enzymes: there is a distinct lysosomal form and a mixture of microsomal forms [18,19]. It seemed possible that the epoxide-induced loss was primarily in one form of the enzyme so that a large loss of only one form would appear as a relatively small loss when the entire mixture is assayed. An initial attempt to localize the effect was made by assay-

ing the enzyme in the soluble and membrane-bound portions of the tissue homogenate. The soluble enzyme is presumably derived mainly from lysosomes, while the membrane-bound enzyme is presumed to be mainly microsomal [20]. However, evidence for this presumption seems to be lacking. The livers and brains of eight mice were pooled 5 h after injection of saline or conduritol B epoxide (300 mg/kg). In this experiment, the decrease in liver glucuronidase (Table III) was greater than before, and the effect was similar in both soluble and membranous fractions. Glucuronidase specific activity was enhanced in the brains of conduritol B epoxide-treated mice, the effect being localized in the soluble portion (26% increase vs. 10% decrease in membranes). Aryl glucosidase was much more diminished in liver, as noted before, and the effect seemed to be greater in the membrane-bound enzyme, especially with brain.

The findings suggest that there is some difference between easily solubilized and tightly bound glucuronidases with respect to changes resulting from glucosidase destruction in liver. The situation in brain seems to be different with respect to the solubilizable enzyme.

In order to obtain a more detailed estimate of the subcellular distribution of glucuronidase activities in liver, we injected additional mice with conduritol B epoxide or saline as above but homogenized the liver in 0.25 M sucrose instead of water. The fractions were separated according to the protocol of Schlesinger et al. [21] and assayed for glucuronidase and protein. The findings (Table IV) revealed again decreasing specific activities in the total homogenates as the result of conduritol B epoxide treatment, the effect increasing between 2 and 5 h. The soluble (cytosolic) activity, which accounted for about 15% of the total, decreased 25% within the first 2 h but returned to normal within 5 h. The microsomal activity, 31% of the total, decreased only 5% in 2 h but 35% in 5 h. The lysosomal fraction, which contained 30% of the tissue enzyme, decreased

TABLE III

SUBCELLULAR DISTRIBUTION OF ENZYMES IN MICE TREATED WITH EPOXIDE

Activities are in nmol/h per mg tissue. Glucosidase assays were run with 1 mg of tissue; glucuronidase assays were run with 5 mg brain and 1 mg liver. Numbers in parentheses are the percent decrease found in the conduritol B epoxide-treated mice (a + sign signifies an increase).

|  | Aryl glucosidase | | Glucuronidase | |
| --- | --- | --- | --- | --- |
|  | Brain | Liver | Brain | Liver |
| Total homogenate |  |  |  |  |
| Control | 3.95 | 3.19 | 1.69 | 22.9 |
| Conduritol B epoxide | 1.57 | 0.26 | 1.87 | 13.1 |
|  | (60%) | (92%) | (+11%) | (43%) |
| Membranes |  |  |  |  |
| Control | 2.54 | 2.40 | 0.56 | 6.8 |
| Conduritol B epoxide | 0.83 | 0.22 | 0.50 | 4.8 |
|  | (67%) | (92%) | (10%) | (30%) |
| Cytosol |  |  |  |  |
| Control | 0.60 | 0.21 | 0.81 | 14.7 |
| Conduritol B epoxide | 0.48 | 0.05 | 1.03 | 8.5 |
|  | (20%) | (76%) | (+26%) | (43%) |

TABLE IV

GLUCURONIDASE ACTIVITIES IN LIVER SUBCELLULAR FRACTIONS

Groups of 8 mice each were injected with conduritol B epoxide (300 mg/kg) or saline and killed 2 or 5 h later (number in parentheses). Subcellular fractions were obtained by sucrose centrifugation and the pellets were suspended in sucrose and assayed. Activities are in nmol/h per mg protein; protein contents are in mg/g tissue. CBE, conduritol B epoxide.

| | Glucoronidase | | | | Protein | | | |
|---|---|---|---|---|---|---|---|---|
| Time after injection: | 2 h | | 5 h | | 2 h | | 5 h | |
| | Con-trol | CBE | Con-trol | CBE | Con-trol | CBE | Con-trol | CBE |
| Total homogenate | 92 | 86 | 94 | 73 | 211 | 215 | 218 | 214 |
| Nuclei + debris | 50 | 42 | — | — | 70 | 72 | 59 | 55 |
| Lysosomes + mitochondria | 462 | 360 | 401 | 245 | 13 | 12 | 12 | 16 |
| Microsomes | 111 | 106 | 128 | 83 | 49 | 46 | 54 | 55 |
| Cytosol | 37 | 28 | 36 | 37 | 64 | 64 | 72 | 69 |

22% in 2 h and 39% in 5 h. It is interesting to note that the lysosomal enzyme responded to the inhibitor somewhat faster than the microsomal enzyme.

Glucocerebrosidase, by contrast, was found to occur at by far the highest specific activity in the lysosomal fraction, but an appreciable portion of the total tissue activity resided in the microsomes (about 60% of that seen in the lysosomes).

*Multiple injections of conduritol B epoxide*

It seemed possible that some changes resulting from glucosidase destruction were slow, due to slow metabolism of some glucose-containing proteins. We therefore administered eight daily injections of conduritol B epoxide (100 mg/kg) into 16-days old mice. Two groups of six each were used as controls (saline) and two as experimentals. All were killed 5 h after the last injection and assays were carried out as before.

The conduritol B epoxide treatment seemed to produce a significant increase in the size of brain and liver, which were weighed individually (Table V). The increases were 13 and 9%, respectively.

The changes in glucuronidase of liver, spleen, and kidney were similar in magnitude to those obtained with a single (larger) conduritol B epoxide injection. However, brain showed a distinct increase, as noted in Table III and in a previous study involving four weeks of conduritol B epoxide injection into very young mice [15]. The latter study reported a glucuronidase level that was three times the specific activity in the controls. There was also a marked increase in t-mannosidase and $\beta$-hexosaminidase, but a slight decrease in $\alpha$-glucosidase and $\alpha$-galactosidase. Thus one cannot conclude that destruction of $\beta$-glucosidase produces a rise in all brain hydrolases.

Our data for aryl glucosidase (Table V) confirmed our previously noted partial loss of activity after conduritol B epoxide treatment. The point of interest here is that eight injections of conduritol B epoxide produced no greater inhibition than one (larger) injection.

TABLE V

EFFECT OF CHRONIC ADMINISTRATION OF CONDURITOL B EPOXIDE

Groups of 16-days old mice were injected daily with 100 mg conduritol B epoxide/kg eight times, then killed 5 h later. Enzyme activities are in nmol/h per mg tissue.

|  | Controls | Epoxide treated | |
|---|---|---|---|
| Weight | | | |
| Body (g) | 15.3 | 15.9 | |
| Brain (mg) | 361 | 407 * | |
| Kidney (mg) | 230 | 245 | |
| Liver (mg) | 907 | 993 ** | |
| Spleen (mg) | 92 | 101 | |
| Glucuronidase | | | |
| Brain | 1.58 | 1.96 | (+24%) |
| Kidney | 9.99 | 6.91 | (−31%) |
| Liver | 18.9 | 14.3 | (−24%) |
| Spleen | 20.3 | 14.9 | (−27%) |
| Aryl glucosidase | | | |
| Brain | 3.27 | 1.93 | (−41%) |
| Kidney | 1.16 | 0.40 | (−66%) |
| Liver | 3.46 | 1.16 | (−66%) |
| Spleen | 2.26 | 0.72 | (−68%) |

* $P < 0.01$.
** $P < 0.05$.

## Effect of phenylhydrazine

A further examination was made of the relationship between glucosidase and glucuronidase by injecting control mice and conduritol B epoxide-treated mice with saline or phenylhydrazine [14]. Phenylhydrazine is a hemolytic agent that stresses spleen and liver with erythrocyte components which must be hydrolyzed. Kampine et al. [22] found that phenylhydrazine injections into rats produced appreciable increases in the specific activities of several splenic hydrolases (the increases were greater in total activities). We found that mice react the same way with respect to cerebrosidase and aryl glucosidase, and that the liver responds like spleen [14]. Evidently glucuronidase levels are also increased (Table VI). Within 2 days after the start of phenylhydrazine injection into normal mice, no effect or a decrease was observed (lines 1 and 3) but by 6 days a distinct increase was visible: 48% in liver and 39% in spleen. Kidney was not affected, as expected, since it is not involved in erythrocyte processing.

The conduritol B epoxide treatment, as noted before, produced a decrease in glucuronidase within 5 h (the first time point shown). The reduction persisted for at least 2 days (lines 1 and 2, column 2) in animals subsequently injected only with saline. There may have been a rebound phenomenon, since by 6 days the conduritol B epoxide-treated animals had 11% more activity in liver and 26% more activity in spleen.

Injection of phenylhydrazine into mice deficient in glucosidases (lines 2 and 4) produced a slight increase in the glucuronidase level in liver after 2 days and a distinct increase in both liver and spleen by 6 days. The effects were thus quite similar to those observed in normal mice but a little smaller: 40% increase in liver and 19% in spleen.

TABLE VI

GLUCURONIDASE ACTIVITY IN MICE INJECTED WITH SALINE, EPOXIDE, AND/OR PHENYL-HYDRAZINE

Groups of 16-days old mice were injected with saline or conduritol B epoxide and 5 h later they were injected with saline or phenylhydrazine. The latter injection was repeated twice more, then groups of mice were killed at ages 18 and 22 days. Enzyme activities are in μg phenolphthalein liberated/h per mg tissue, based on assays at two levels of tissue. CBE, conduritol B epoxide.

| | Age at time of killing (days) | | |
| --- | --- | --- | --- |
| | 16 | 18 | 22 |
| Liver | | | |
| Saline/saline | 9.2 | 8.9 | 7.2 |
| CBE/saline | 6.6 | 6.8 | 8.0 |
| Saline/phenylhydrazine | — | 9.1 | 10.6 |
| CBE/phenylhydrazine | — | 7.5 | 11.2 |
| Spleen | | | |
| Saline/saline | 11.6 | 10.6 | 5.5 |
| CBE/saline | 7.5 | 6.5 | 6.9 |
| Saline/phenylhydrazine | — | 7.4 | 7.6 |
| CBE/phenylhydrazine | — | 6.5 | 8.2 |
| Kidney | | | |
| Saline/saline | 4.5 | 4.4 | 3.8 |
| CBE/saline | 3.5 | 3.4 | 3.6 |
| Saline/phenylhydrazine | — | 4.1 | 3.9 |
| CBE/phenylhydrazine | — | 3.2 | 3.5 |

A side observation is that the specific activities of glucuronidase changed with age appreciably in the control animals (injected four times with saline). Spleen showed an especially large decrease during the 6 day period: 53%. While the stressing of the animals during the first 2 days may have influenced the glucuronidase levels, it is possible that the changes are typical for mice approaching weaning.

*Other enzymes*

Angiotensin-converting enzyme in Gaucher spleen was reported to be about nine times the normal concentration [2] but we found no distinct change in mice injected with conduritol B epoxide and killed 5 h later. The activity in normal kidneys was far higher than in the other organs: 5.1 nmol/min per mg tissue vs. 0.13 units in brain, 0.04 units in liver, and 0.08 units in spleen. Our multiple injection experiment also showed no changes.

Acid phosphatase was unchanged in the experimental mouse brains but 9% above normal in liver by 5 h. Most of this increase took place between the 2nd and 5th hours. However, no change was seen in the animals injected over a period of a week.

$\alpha$-L-Flucosidase showed no changes in brain or liver during the 5 h period after conduritol B epoxide injection. In the experiment involving eight conduritol B epoxide injections, we found a small rise in specific activity in brain only (13%). This increase is probably significant, since the activities in the two control groups (6 mice each) were 1.30 and 1.39 units, and, in the experi-

mental groups, 1.54 and 1.51 units. The increase is thus like the one we noted with brain glucuronidase.

## Discussion

The primary finding of this study is that glucuronidase levels decrease in the organs other than brain of animals whose glucosidase levels have been drastically lowered. The effect can be explained by making three hypotheses: (1) glucuronidase contains glucose bound in glycosidic linkage, (2) this glucose is released by the action of aryl glucosidase during the normal handling of glucuronidase, and (3) the glucuronidase molecules, during the course of transport or uptake by subcellular membranes, successively lose sugar moieties.

The first hypothesis is based on the finding that glucuronidase from rats and mice contains an appreciable amount of glucose [19,23,24]. Different forms have been isolated with differing glucose contents. For example, form C from rat preputial gland contains 1.5% glucose while forms A and B contain 1/9 as much [23]. The mode of linkage is not yet known.

The second hypothesis is based on the difference in properties of cerebrosidase and aryl glucosidase. Many phenolic and aliphatic $\beta$-glucosides occur in plants and, to a certain extent, in mammalian diet. There is therefore a use for a glucosidase of low specificity. There seems to be such an enzyme in the intestine, able to hydrolyze glucocerebroside, galactocerebroside, phlorizin, and possibly other naturally occurring glycosides [25]. Liver also contains a glycosidase of wide specificity, active at a slightly alkaline pH [26]. Our mouse liver homogenates, under the assay conditions we used, did not show the presence of this enzyme [14]. Our data showed that two kinds of aryl $\beta$-glucosidase exist in brain [14], one of them being more susceptible to conduritol B epoxide. Very recent reports [27—29] have described the existence of a microsomal glucosidase (probably a $\beta$-glucosidase) that removes glucose residues from freshly synthesized glycoprotein molecules. The removal steps are believed to control the disposition of the proteins within the cell regions. It is possible that this enzyme was inhibited in our conduritol B epoxide experiments, and that abnormal handling of glucuronidase by the cells resulted.

The literature on Gaucher's disease is not yet clear as to the number of glucosidases that are defective. While some work seems to show that only one glucosidase is lacking, and that this enzyme, in normal individuals, can hydrolyze both cerebroside and aryl glucosides, other studies have indicated the existence of two different defective enzymes. An example of the latter group is a chromatographic study of Gaucher liver in which two cerebrosidase peaks could be separated, both being abnormally low [30]. We suggest that the natural substrate for one of these enzymes is the glucose that is in glucose-containing glycoproteins. Several such proteins are now known, besides glucuronidase: $\alpha$-L-fucosidase [31] and angiotensin-converting enzyme. The latter had previously been thought to contain no glucose, but a very recent oral report (Soffer, R.L., personal communication) established the existence of a low level of glucose in the glycoprotein. In addition, several glucose-containing peptides have been isolated from tissues [32,33].

Our third hypothesis is derived from the many recent reports indicating the

importance of the structure of oligosaccharide side chains in lysosomal hydrolases. Much evidence supports the hypothesis that lysosomal enzymes, shortly after synthesis and attachment of carbohydrate side chains, are secreted from cells into the extracellular fluid and then reabsorbed for packaging within the lysosomes. This hypothesis seems to apply to rat liver glucuronidase, which was studied with labeled amino acid [34]. The microsomal enzyme fraction reached a maximal specific activity within 30 min, and the cytosolic enzyme reached a plateau within 30 min, but the lysosomal enzyme did not become radioactive for at least 240 min. Apparently some of the secreted enzyme molecules are not reabsorbed by the cells and instead enter the blood stream, to be taken up by other organs or possibly excreted into urine. The reuptake process is apparently determined by the presence of terminal sugars on the glycoproteins, such as glucose, galactose, and mannose [35]. It is to be expected that enzymes having abnormal terminal residues, due to lack of adequate glycosidase activity in the cell region that modifies the oligosaccharide structure, will show changes in body distribution and levels.

Our data on time-related changes in glucuronidase distribution within liver cells may indicate that the lysosomal form cannot be retained by the lysosomes if some glucose residues cannot be released from the enzyme. This concept explains why the first change we observed (Table IV) was a loss of enzyme activity from the lysosomal fraction. Perhaps the glucose-rich form is indeed reabsorbed by the cells from the extracellular fluid by pinocytosis (which might be relatively non-specific) but the enzyme leaks rapidly from the newly formed lysosomes, back to the extracellular space.

Gaucher patients exhibit a slightly low glucuronidase level in leukocytes [17] and a high level in plasma [3], a difference consistent with our data and hypothesis. However, platelets have a distinctly high concentration of glucuronidase [36]. Perhaps they derive part of this enzyme from the plasma. We don't know if the enzyme is deficient in Gaucher organs, but there is the possibility that, over a long period of time, the Gaucher body may adapt to the problem of handling glucuronidase molecules with terminally linked glucose.

Our data showed that brain and liver reacted differently, possibly because the blood-brain barrier slows loss of glucuronidase from brain extracellular fluid into the blood stream. Thus the enzyme containing terminal glucose residues would be expected to accumulate in the extracellular compartment. It is possible that this phenomenon explains why the infantile form of Gaucher's disease (unlike the other forms) involves so much damage to the brain. The extracellular accumulation of glucose-containing proteins is probably much more rapid in the young child than in the adolescent or adult. A high concentration of certain proteins, such as glucuronidase, might cause damage to the outer surfaces of the brain plasma membranes.

Our experiment with phenylhydrazine poisoning showed that the spleen and liver responded to an overload of erythrocytes by increasing their levels of glucuronidase, presumably to handle the problem of hydrolyzing glucuronides in erythrocytes. The conduritol B epoxide pretreatment failed to influence this response strongly. This result is consistent with our interpretation of the conduritol B epoxide effect: loss of aryl glucosidase must be severe in order to influence glucuronidase metabolism. Since there is appreciable resynthesis of

the glucosidase within a few days after conduritol B epoxide injection, there is a rapid recovery in the level of glucuronidase molecules having the normal glucose distribution.

## Acknowledgement

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