changes in emotional behavior became most apparent within a few minutes after injection of the stronger doses of CPZ, and they gradually disappeared during the next few hours. Chlorpromazine had no apparent effect upon water intake. Norepinephrine also failed to alter water intake to a measurable extent, although 45 percent of the rats injected with NE (and none of those injected with CPZ) drank for a few seconds before starting to eat.

Additional investigations with CPZ, in which we examined the interaction between pretreatment with CPZ and subsequent hypothalamic injection of NE, further demonstrate the failure of CPZ to interfere in any way with the eating induced by centrally-injected NE (*7*). These experiments tested the effects upon NE-induced eating of CPZ in a single hypothalamic injection, a single intraperitoneal injection, and a series of daily intraperitoneal injections over a period of at least 1 month. In none of these experiments was CPZ found to have any blocking effect upon the amount of food eaten after hypothalamic injection of NE. In these experiments, NE was injected into the rats pretreated with CPZ, always at a time when they were not eating to CPZ, but when the emotional effects of CPZ, such as increased aggression to central injection and sedation and ptosis to intraperitoneal injection, were clearly evident. The presence of these emotional effects was interpreted as an indication that CPZ was pharmacologically active in the brain when NE was injected. These results therefore suggest that, in the "adrenergic feeding" system of the brain, CPZ does not block the postsynaptic receptors, contrary to what would be expected on the basis of its peripheral actions.

Instead, all of our findings suggest a quite different hypothesis: that the central effects of hypothalamically injected CPZ are mediated by endogenous NE, and that a possible mode of action is a CPZ-induced increase in turnover of endogenous NE (*2*). This hypothesis would explain our findings that CPZ, like NE, elicits eating when injected directly into the adrenergic feeding center of the hypothalamus and that the magnitude of this CPZ effect is positively correlated with the magnitude of the eating response elicited by NE. This hypothesis would also predict that hypothalamic pretreatment with NE-depleting drugs should reduce eating induced by CPZ, but not that induced by NE. We have recently performed this experiment and have confirmed this prediction (*7*).

Since Bradley *et al.* (*8*) found that CPZ has an effect similar to that of NE only on neurones that were inhibited by NE (it blocked neurones excited by NE), our results may be interpreted as suggesting that, instead of eliciting eating directly by excitation, CPZ (and NE) elicit eating by inhibiting the hypothalamic system (possibly involving the ventromedial nucleus) which normally suppresses eating.

The classical view has been that CPZ is pharmacologically an adrenergic blocking agent and clinically a tranquilizer. These effects are supposed to be opposite to those of imipramine, a potentiator of adrenergic effects and an antidepressant. However, these two drugs are chemically similar, and CPZ and other phenothiazines have been found to be effective, like imipramine, in treating certain patients with depressions (*9*). It is suggested here that the unique clinical effectiveness of CPZ can be attributed to the complexity of its action, part of which is the central adrenergic effect demonstrated in the present study (*10*).

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10. This hypothesis does not rule out the possibility that CPZ may derive some of its clinical effects from an adrenergic blocking effect on peripheral, or even on certain central, neurones. A few investigators have suggested that CPZ, at least in the peripheral nervous system, inhibits the uptake of NE into the nerve endings, in a fashion similar to that of imipramine-like drugs [(*1*) and J. Häggendal and B. Hamberger, *Acta Physiol. Scand.* **70**, 277 (1967)]. However, since hypothalamic injection of the potent uptake inhibitor, desmethylimipramine, fails to elicit the consistent eating elicited by CPZ (our unpublished data), we feel that an effect on uptake cannot be the primary explanation of the eating effect of CPZ. Instead, we feel that this adrenergic action of CPZ in the hypothalamus probably results from its effect of accelerating the synthesis and release of catecholamines (*2*).
11. Supported by PHS grant MH 13189 and Nutrition Foundation grant 390. We thank Arnold Kriegstein and Lillian Rochlitz for technical assistance, Elizabeth Sherwood for implanting the cannulas, and Smith Kline and French laboratories for donating the chlorpromazine hydrochloride (Thorazine).

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Hurler's Syndrome: Deficiency of a Specific Beta Galactosidase Isoenzyme

Abstract. *A marked deficiency of a specific thermolabile β-galactosidase isoenzyme (*p*H optimum 3 to 5) was found in liver and kidney tissues of five patients with the Hurler's syndrome (types 1 to 3).*

The Hurler's syndrome is a genetically transmitted disorder of mucopolysaccharide metabolism characterized by excessive visceral storage of chondroitin sulfate B and heparitin sulfate (*1*) and excessive cerebral storage of gangliosides GM_1, GM_2, and GM_3 (*2*). At least six phenotypically distinct types of the Hurler's syndrome have been delineated (*1*). The fundamental enzymic defect in each type is unknown. One possibility is a deficiency of a hydrolytic enzyme which participates in the cleavage both of mucopolysaccharides and glycolipids. This possibility is enhanced by evidence from (i) morphological studies indicating that the storage substances are in lysosomes (*3*), (ii) kinetic studies which demonstrate an impairment in the deg-

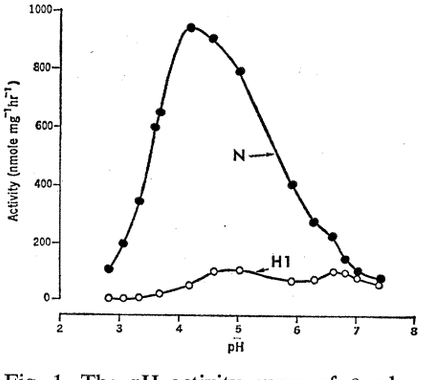

Fig. 1. The *p*H activity curve of β-galactosidase from normal (*N*) and Hurler's type 1 (*H1*) liver. Activity was determined with *p*-nitrophenyl galactoside as substrate ($10^{-3}M$) in citrate-phosphate buffer ($0.1M$) and is expressed as nmoles of *p*-nitrophenyl galactose cleaved per milligram of protein per hour at 37°C.

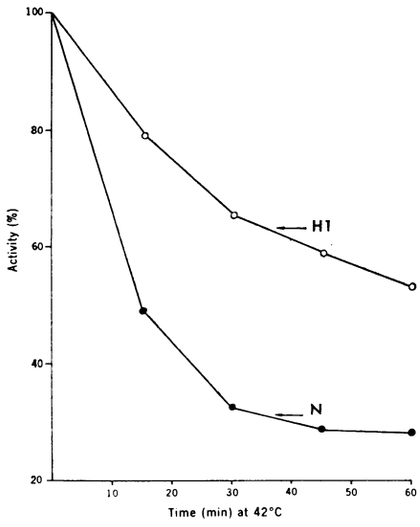

Fig. 2. Thermostability of β-galactosidase from normal (*N*) and Hurler's type 1 (*H1*) liver. Tissue homogenates were incubated at 42°C for varying intervals, and enzyme activity for *p*-nitrophenyl galactoside was assayed. Activity is expressed as a percentage of the initial activity of each enzyme before heating.

radation of mucopolysaccharides in Hurler's (types 1 to 3) fibroblasts (*4*), and (iii) enzymic studies which demonstrate a specific deficiency of lysosomal β-galactosidase (*p*H optimum 3 to 5) in many tissues of patients with the Hurler's syndrome (types 1 to 3) (*5*, *6*).

The relation of the β-galactosidase deficiency to the fundamental enzymic defect in the Hurler's syndrome is obscure. The deficiency could account for the accumulation of galactose-containing sphingolipids in this disease, since impaired cleavage of galactose from ganglioside GM_1 has been demonstrated in Hurler's tissues (*6*). Whether β-galactosidase also participates in the degradation of the stored mucopolysaccharides is an open question.

It seemed worthwhile to explore the properties of β-galactosidase in Hurler's tissues to determine whether abnormalities of this enzyme might be present. This report documents a marked deficiency of a specific thermolabile β-galactosidase isoenzyme in tissues from patients with the Hurler's syndrome (types 1 to 3).

The patients studied included five who died from the Hurler's syndrome: three with type 1 (Hurler form), one with type 2 (Hunter form), and one with type 3 (Sanfilippo form) (*7*). Organs were obtained at autopsy and were stored frozen at −20°C prior to analysis. Controls included 14 patients, similar in age to the Hurler's patients, who died of disorders not involving the liver, one patient with Niemann-Pick disease, and two with generalized gangliosidosis. Control tissues were stored for periods similar to those of the Hurler's tissues. β-Galactosidase activity was assayed with either *p*-nitrophenyl or 4-methylumbelliferyl derivatives of galactose as substrates (*8*). Assays were performed on tissue homogenates (in 0.25*M* sucrose) and on supernatants obtained by high-speed centrifugation (100,000*g* for 45 minutes) of the homogenates; supernatants contained 90 percent of the β-galactosidase activity of the homogenates. Vertical starch-gel electrophoresis was carried out by the method of Smithies (*9*), with commercially available apparatus and starch (Otto Hiller, Madison, Wisconsin). The electrode buffer was varied between *p*H 6.0 to 7.6 with the use of citrate-phosphate or sodium phosphate buffers (5 m*M* gel buffer, 0.04*M* electrode buffer). The 4-methylumbelliferyl derivatives of glucose and galactose were incubated at *p*H 4.9 in 0.1*M* acetate buffer with the developed gels, and enzyme activities were located by viewing the fluorescence of 4-methylumbelliferone under ultraviolet light after the gels were sprayed with 0.25*M* glycine-carbonate buffer, *p*H 10.

The *p*H activity curve of the Hurler's (type 1) liver β-galactosidase differed from normal (Fig. 1). The enzyme had two *p*H optimums between *p*H 3 and 7—a major one at *p*H 4 to 5 and a minor one at *p*H 6.6. Although the total activity of the Hurler's β-galactosidase was greatly reduced (to one-tenth that of normal in the patient studied in Fig. 1), the Hurler's enzyme had nearly normal or only slightly reduced activity at *p*H 6.6 but a very large loss of activity at *p*H 4 to 5. The peak at *p*H 4 to 5 was shifted slightly in the Hurler's tissue; the *p*H optimum for this peak in three control subjects was 4.3; in two Hurler's (type 1) patients it was 4.8. These abnormalities in *p*H activity curves were demonstrated both with acetate and citrate-phosphate buffers at different buffer concentrations (0.035 and 0.1*M*). Van Hoof and Hers (*5*) have reported a similar shift in *p*H activity of β-galactosidase in the Hurler's syndrome.

The enzyme activity of β-galactosidase from Hurler's (type 1) liver was maximum at a slightly higher temperature than normal (two controls); maximum activity of the Hurler's enzyme occurred at 41°C; that of the normal enzyme, at 37°C. A similar study of *N*-acetyl-β-galactosaminidase demonstrated that this enzyme had a sharp optimum at 50°C, both from normal and Hurler's liver. When the normal and Hurler's liver homogenates (or 100,000*g* supernatant fractions) were incubated at 42°C prior to assay, the Hurler's total β-galactosidase activity was more stable than normal; after 30 minutes of incubation at 42°C, 40 percent of the activity of the Hurler's enzyme was lost, whereas 70 percent of the activity of the normal enzyme was lost. Over graded time intervals, two different thermal inactivation curves were seen (Fig. 2). One interpretation

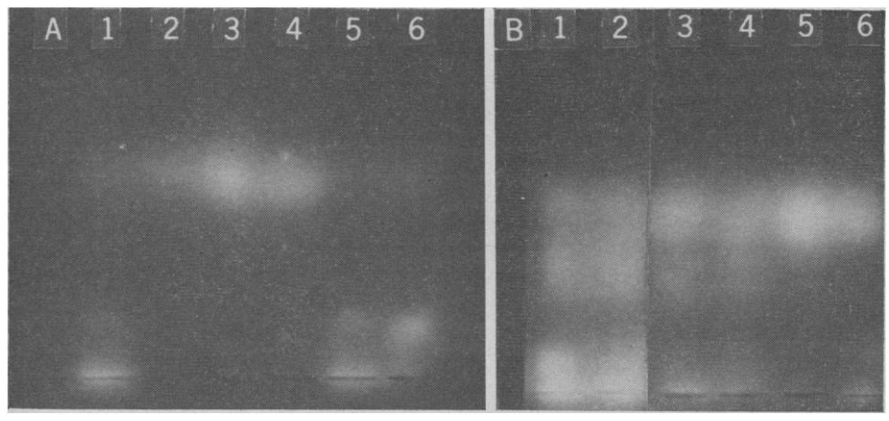

Fig. 3. Starch-gel electrophoresis of β-galactosidase from (A) liver and (B) kidney. (A) Lanes 1, 5, and 6 show controls; lanes 2, 3, and 4 show patients with Hurler's type 1, 2, and 3, respectively. (B) Lanes 1 and 2 show controls; lanes 3 and 4 show patients with Hurler's type 1; lanes 5 and 6 show same sample from a patient with Hurler's type 3. In (B), lanes 1 and 2 are from the same gel as lane 3 to 6, but the photograph has been cut and rearranged for better reproduction. The activity of β-galactosidase applied to the gel was the same for each sample.

of this data is that the Hurler's β-galactosidase is deficient in a thermolabile component (or components).

This suggestion was substantiated by starch-gel electrophoresis studies. Control livers contained three separable β-galactosidase components with activity at *p*H 4 to 5—a fast-moving component and two slow-moving ones. After the preliminary incubation either of homogenates or supernatant fractions from controls, for 30 minutes at 42°C, both slow-moving β-galactosidase components were rendered inactive, whereas the fast component remained active.

A marked deficiency of the slow-moving β-galactosidase components was found in liver tissue from all patients with the Hurler's syndrome (types 1 to 3) (Fig. 3). Repeated freezing and thawing of the normal or Hurler's liver sucrose homogenates, or of the supernatant fractions in distilled water, caused no alterations of β-galactosidase patterns. No deficiency of the slow-moving components was found in liver tissue from a patient with Niemann-Pick disease, an indication that lysosomal storage in itself (in this case of sphingomyelin) does not produce the alteration. In generalized gangliosidosis, a β-galactosidase deficiency disease (*8*), both fast-moving and slow-moving components were nearly absent.

The degree of deficiency of the major slow-moving β-galactosidase in the Hurler's liver tissue was estimated from visual inspection of the gels. This gave only a very rough approximation of the degree of deficiency due to the inaccuracies inherent in the method of estimation. We estimated that, in the Hurler's tissues, the activity of the major slower-moving component was one-tenth to one-twentieth that of normal (*10*).

Examination of the separable β-galactosidase components in the kidney (Fig. 3) demonstrated the presence of four components, two of which were fast moving and two slow moving. In the Hurler's patients (types 1 to 3) both slow-moving components in kidney were markedly deficient but the degree of deficiency was not as great as that in liver, except in a patient with type 3 disease (Fig. 3).

Incubation of the gels with 4-methylumbelliferylglucoside revealed β-glucosidase activity which coincided exactly with β-galactosidase activity of the fastest-moving component in both controls and the patients' liver and kidney tissues. The slow-moving components did not possess β-glucosidase activity. No alteration of β-glucosidase activity was noted in the tissues of patients with Hurler's syndrome.

The mutation in Hurler's syndrome thus may result in preferential loss of specific β-galactosidase isoenzymes. Studies of the fast- and slow-moving β-galactosidases indicate that they are structurally different proteins, probably under separate genetic control, and can be thought of as isoenzymes (*11*). The fact that total β-galactosidase activity in some patients with the Hurler's syndrome is normal or only slightly reduced (*5, 6*) can be explained by a shift in isoenzyme pattern; increases in the fast-moving isoenzyme could give this result. Different isoenzyme patterns in different tissue could also explain the finding of varying degrees of β-galactosidase deficiency in different organs (*5, 6*). For example, the deficiency of β-galactosidase in Hurler's kidney is less than that in liver (*5, 6*); we demonstrate here that kidney normally contains smaller proportions of the slow-moving isoenzymes than liver.

Although the β-galactosidase isoenzyme deficiency could provide an explanation for the ganglioside (and other glycolipid) accumulations, before accepting it as pathogenetically significant it must be demonstrated that (i) the deficient β-galactosidase isoenzyme or enzymes participate in mucopolysaccharide turnover, (ii) the deficiency is genetically transmitted in either autosomal (types 1 and 3) or X-linked (type 2) fashion, and (iii) the nature of the mutation differs in types 1, 2, and 3.

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12. Supported in part by NIH grants NB 06576 and HE 08429.

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Glutathione Reductase: Stimulation in Normal Subjects by Riboflavin Supplementation

Abstract. *Glutathione reductase of hemolyzates from clinically normal subjects is activated by the addition of flavin-adenine dinucleotide. One-half maximum stimulation could be achieved by approximately 0.02 micromolar flavin-adenine dinucleotide; prior addition of adenosine triphosphate, adenosine diphosphate, or adenosine monophosphate prevented activation. Stimulation of glutathione reductase activity of red cells of normal subjects occurred when they were given 5 milligrams of riboflavin daily for 8 days. The degree of stimulation in vitro by flavin-adenine dinucleotide and in vivo by riboflavin was inversely proportional to dietary intake of riboflavin. The variety of clinical disorders which have been associated with glutathione reductase deficiency may have, as a common denominator, abnormalities in flavin-adenine dinucleotide formation.*

Most vitamins serve as substrates for the synthesis of coenzymes. When frank vitamin deficiencies occur, insufficient quantities of coenzyme may be synthesized, and the rates of the corresponding enzymatic reactions are slowed. For example, thiamine deficiency causes a decrease in the activity of transketolase (*1*), an enzyme which has thiamine pyrophosphate as a cofactor. Similarly, pyridoxine deficiency results in a decrease in the activity of