HURLER'S SYNDROME, AN α-L-IDURONIDASE DEFICIENCY

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SUMMARY

The activity of α-L-iduronidase was found to be deficient in extracts prepared from livers, cultured fibroblasts and urine of patients with Hurler's syndrome. Extracts from livers and fibroblasts of Hunter's and Sanfilippo's syndromes did not show such a deficiency. There was a diminution of activity in extracts of "I-cell" fibroblasts. α-L-Iduronidase is believed to be the underlying enzymic defect of Hurler's disease.

α-L-Iduronidase activity has been demonstrated in extracts of human livers, leucocytes, cultured fibroblasts, and urine using desulfated dermatan sulfate and a disaccharide prepared from heparin as substrates (1-3). In these assays, the yield of iduronic acid was low, however, the activity of α-L-iduronidase in extracts of Hurler fibroblasts was strikingly decreased. Weissmann and Santiago have confirmed the presence of this enzyme in lysosomal extracts of rat liver using phenyl α-L-iduronide as substrate (4). The use of such a synthetic substrate simplifies the quantitative assay for L-iduronidase. With phenyl α-L-iduronide as substrate, α-L-iduronidase was found to be deficient in extracts of cultured skin fibroblasts and liver as well as in urine

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of Hurler patients, while the activity was in normal range in extracts of cultured fibroblasts derived from patients with the Hunter and the Sanfilippo syndromes and in extracts of normal amniotic cells.

MATERIALS AND METHODS

Fibroblast cultures from skin biopsies were established and maintained as previously described (5). Enzyme extracts were prepared from 2-3 week old confluent cultures containing 1.4 - 1.6 x 10^7 cells per 100 mm Falcon tissue culture dish. Cells from 10 plates were washed with a 0.05 M sodium acetate buffer containing 0.15 M NaCl, pH 4.5 and 0.1% Triton X-100, and then disrupted with a Dounce glass homogenizer. Livers obtained at autopsy were extracted by homogenizing 2 gm of tissue in 5 ml of the same acetate buffer. Homogenates were centrifuged at 10,000 xg for 10 min and the supernatant fractions were used for assay of enzymic activity. Protein was precipitated from freshly collected urine by addition of $(NH_4)_2SO_4$ to 70% saturation. The precipitate was dissolved in H_2O and dialyzed against the sodium acetate buffer at pH 4.5. All preparative procedures were carried out at 4°. The substrate for quantitation of α-L-iduronidase activity was the sodium salt of phenyl α-L-iduronide synthesized by Friedman and Weissmann (6).

Incubation mixtures contained 0.1 ml of extract and 300 or 60 mcg of the α-phenyl L-iduronide in acetate-NaCl buffer, adjusted to pH 4.0. The reaction mixture was layered with toluene and incubated for 24 hr at 37°, then extracted three times with 0.2 ml of toluene and to the pooled extracts 0.2 ml of 0.05 M NaOH was added. The mixture was centrifuged and frozen in a solid CO_2-acetone mixture. The toluene was decanted and drained as described (7) and phenol was determined by addition of 0.1 ml Folin-Ciocalteau reagent and 1.5 ml of 0.4 M Na_2CO_3. After 30 min, optical density was determined at 650 nM.

β-Galactosidase and β-N-acetylhexosaminidase were determined with the p-nitrophenyl derivatives as substrates (8).

RESULTS AND DISCUSSION

Extracts of normal liver, cultured skin fibroblasts, cultured amniotic cells and normal urine demonstrate α-L-iduronidase activity when incubated with phenyl α-L-iduronide as shown in Table 1. Enzyme activity was also demonstrated in extracts of fibroblasts cultured from patients with Sanfilippo, Hunter, and "I-cell"diseases and extracts of livers of Sanfilippo and "I-cell" diseases. The level of activity in extracts of "I-cell" fibroblasts is somewhat diminished as has been found true of several other lysosomal enzymes (9). In contrast to other preparations, extracts of Hurler liver fibroblasts and Hurler urine showed little or no α-L-iduronidase activity. The highest activity obtained

Table 1. α-L-Iduronidase Activity of Liver, Cultured Fibroblast Extracts and Urine Protein

	Extract	Phenol μM/mg prot/24 hr
Fibroblasts*	Normal	0.260
	Hunter	0.310
	Sanfilippo	0.180
	"I-Cell"	0.080
	Hurler	0.020
Liver†	Normal	0.042
	Sanfilippo	0.067
	"I-Cell"	0.033
	Hurler	0.002
Urine†	Normal	0.137
	Hurler	0.007
Amniotic Cells†	Normal	0.120

* Assays performed with 300 mcg of phenyliduronide per assay.

† Assays performed with 60 mcg of phenyliduronide per assay.

in extracts of Hurler fibroblasts is approximately 10% of normal. These findings confirm the previous observations made with desulfated dermatan sulfate and a disaccharide obtained from heparin (1-3). The phenyl α-L-iduronide substrate permits rapid and accurate quantitation.

Mixing of extracts gave the results described in Table 2. These data show no evidence of the presence of an inhibitor in extracts of Hurler liver or fibroblasts. The somewhat higher than expected activity in mixtures of Hurler fibroblast extracts and Hunter, and Hurler and "I-cell" liver extracts requires further investigation.

Table 2. Effect of Mixing on α-L-Iduronidase Activity

	Extract	Phenol μM/mg prot/24 hr
Fibroblasts*	Normal	0.115
	Hunter	0.116
	Sanfilippo	0.078
	Hurler	0.002
	Normal and Hurler	0.057
	Hunter and Hurler	0.084
	Sanfilippo and Hurler	0.042
Liver*	Normal	0.100
	"I-Cell"	0.048
	Hurler	0.001
	Normal and Hurler	0.083
	"I-Cell" and Hurler	0.048

* Assays performed with 60 mcg of phenyliduronide per assay.

In order to be certain that extracts of Hurler fibroblasts and liver had not been inactivated, all extracts were assayed for β-galactosidase and β-N-acetylhexosaminidase. The results shown in Table 3 indicate high levels of activity of both enzymes. Diminished β-galactosidase in Hurler tissues has been previously noted (10).

The results described indicate that α-L-iduronidase activity is

Table 3. Lysosomal Enzyme of Tissue Extracts*

		β-N-acetylglucosaminidase. p-nitrophenol μM/mg prot/hr	β-galactosidase. p-nitrophenol μM/mg prot/hr
Fibroblasts	Normal	2.50	0.70
	Hunter	3.50	0.60
	Sanfilippo	1.50	0.50
	"I-Cell"	1.25	0.15
	Hurler	2.00	0.40
Liver	Normal	2.25	0.40
	Sanfilippo	1.70	0.30
	"I-Cell"	1.50	0.07
	Hurler	2.00	0.15

* All assays were performed with p-nitrophenol derivatives as substrates.

strikingly diminished in extracts of Hurler liver and fibroblasts, and Hurler urine but in the normal range in Hunter and Sanfilippo fibroblasts. There is some dimunition of activity in extracts of "I-cell" fibroblasts. These data confirm that absence of α-L-iduronidase activity represents the specific gene product defect in Hurler's disease. These findings have obvious implications for diagnosis, therapy and antenatal diagnosis.

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