

## FUCOSIDOSIS: DEFICIENCY OF ALPHA-L-FUCOSIDASE IN CULTURED SKIN FIBROBLASTS\*

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(Received for publication 25 April 1972)

Fucosidosis is a newly recognized inborn error of metabolism involving the accumulation of fucolipids, fucoglycoproteins, and fucomucopolysaccharides. Three families have been reported in the literature (1, 2).<sup>1</sup> The enzymic defect in fucosidosis has been established by Van Hoof and Hers (3) as a specific, nearly complete deficiency of the enzyme  $\alpha$ -L-fucosidase.

Thusfar, no reports have appeared in which  $\alpha$ -fucosidase has been shown to be deficient in cultured skin fibroblasts from affected patients, nor have normal values been given for the activity of the enzyme in amniotic cells. In this study we demonstrate deficiency of  $\alpha$ -fucosidase in cultured skin fibroblasts from two brothers with fucosidosis and give normal values for the activity of  $\alpha$ -fucosidase in amniotic cells.

### *Materials and Methods*

The clinical picture of the patients from whom skin biopsies were taken will be reported in detail elsewhere. We are indebted to Dr. George Donnell who sent fibroblasts to us. The initial diagnosis of fucosidosis in both brothers was made using the serum assay of  $\alpha$ -fucosidase which we reported (4).

Fibroblasts were cultured as described previously (5-7). They were harvested for enzyme assay at 14 days after subculture by mechanical scraping from flasks and centrifuging the cells into a pellet.

Alpha-L-fucosidase activity was determined as follows. The cell pellet was weighed, diluted in 5 volumes of distilled water, and homogenized in a ground glass homogenizer. 20  $\mu$ l of homogenate were taken and to this was added 50  $\mu$ l of *p*-nitrophenyl  $\alpha$ -L-fucopyranoside (1.5 mM, Pierce Chemical Co., Rockford, Ill., in citrate-phosphate buffer, 0.1 M with respect to phosphate, pH 5.8), and the sample was incubated at 37°C for 2 and 4 hr. The reaction was stopped by adding 100  $\mu$ l of 3% trichloroacetic acid. Samples were then centrifuged at 3000 g for 10 min, the supernatant was aspirated, taking care not to disturb the sediment, and 350  $\mu$ l of glycine-carbonate buffer (0.25 M, pH 10) was added to the supernate. Optical density was then read at 420 m $\mu$  on a spectrophotometer and *p*-nitrophenyl was quantified using a standard curve. Tissue blanks for each sample were prepared in the same manner, substituting distilled

\* This work was supported by a fellowship from the Deutsche Forschungsgemeinschaft, Bad Godesberg, Germany, to Klaus Zielke and by the following granting agencies: National Institutes of Health Program Project GM 17702, NIH Grant NS 08682, National Genetics Foundation, and National Cystic Fibrosis Foundation.

<sup>1</sup> Voelz, C., M. Tolksdorf, F. Freitag, and J. Spranger. 1971. Personal communication.

water for the substrate. Final optical densities were obtained by subtracting tissue blank readings from sample readings. Under conditions of assay described above,  $\alpha$ -fucosidase activity was linear between 0 and 4 hr. A pH activity curve for  $\alpha$ -fucosidase in cultured skin fibroblasts demonstrated a single optimum between pH 5.5 and 6.2.

Fibroblast cultures used as controls were obtained from clinically normal children and adults of both sexes as well as from patients with various storage diseases. Amniotic fluid cell cultures were obtained from women undergoing amniocentesis for a variety of reasons during mid-pregnancy and were selected from subjects in which a phenotypically normal baby was delivered.

Assays of other lysosomal hydrolase enzymes, including  $\beta$ -galactosidase,  $\beta$ -glucosaminidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase,  $\alpha$ -galactosidase, and  $\alpha$ -mannosidase were also carried out in fibroblast cultures using 4-methylumbelliferyl substrates as described previously (5-7). Assay of  $\alpha$ -D-N-acetylglucosaminidase activity was carried out using the *p*-nitrophenyl substrate, described elsewhere (8). Protein estimations were made by the Lowry method (9).

#### RESULTS AND DISCUSSION

Activity of  $\alpha$ -fucosidase in cells from 14 control subjects demonstrated a wide variation in activity, even though all cultures were harvested at 14 days after subculture (Table I). However, when ratios of  $\alpha$ -fucosidase to  $\alpha$ -glucosaminidase activity were calculated, the two enzymes maintained a reasonably constant relationship to one another. Cultured fibroblasts from a variety of patients with various lysosomal storage disorders, including Sandhoff's disease, Fabry's disease, metachromatic leucodystrophy, Niemann-Pick disease, and Hurler's disease gave activity of  $\alpha$ -fucosidase which fell within the normal range (Table I).

TABLE I  
*Enzymatic Activity in Cultured Cells*

|                            | $\alpha$ -Acetylglucosaminidase | $\alpha$ -Fucosidase |
|----------------------------|---------------------------------|----------------------|
| Skin fibroblasts           |                                 |                      |
| Controls (17)              | 5.4 (2.1-12.6)                  | 18.4 (2.3-41.9)      |
| Fucosidosis (M.Z.)         | 11                              | 0.05                 |
| Fucosidosis (G.Z.)         | 12                              | 0.08                 |
| Hurler's                   | 4                               | 19                   |
| Hunter's                   | 6                               | 22                   |
| Sanfilippo, type A         | 7                               | 52                   |
| Cystic fibrosis            | 12                              | 21                   |
| Niemann-Pick               | 6                               | 9                    |
| Generalized gangliosidosis | 6                               | 38                   |
| Sandhoff's disease         | 4                               | 32                   |
| Amniotic cultures          |                                 |                      |
| 1                          | 2                               | 35                   |
| 2                          | 3                               | 57                   |
| 3                          | 4                               | 74                   |
| 4                          | 5                               | 81                   |

Activities are expressed as  $\mu$ moles of substrate cleaved per milligram of protein per hour at 37°C.

The activity of  $\alpha$ -fucosidase in cultured skin fibroblasts from the two brothers with fucosidosis ranged between 3 and 6% of normal. When these cells were harvested at varying intervals many months apart, the same degree of deficiency was maintained, demonstrating the persistence of the defect over many cellular generations. The assay is of differential diagnostic value since patients with related storage diseases, such as those with the genetic mucopolysaccharidoses, do not have a deficiency of  $\alpha$ -fucosidase (Table I).

The activity of  $\alpha$ -fucosidase in cultured amniotic cells was higher than that in cultured skin fibroblasts (Table I). The fact that activity for  $\alpha$ -fucosidase is present in normal amniotic fluid cells taken in mid-pregnancy indicates that it may be possible to diagnose fucosidosis prenatally by amniocentesis.

We are indebted to Professor Bernard Weissmann for the *p*-nitrophenyl- $\alpha$ -D-N-acetylglucosaminide used as substrate for  $\alpha$ -acetylglucosaminidase assay. Fibroblast cultures from the fucosidosis patients were sent by Dr. George Donnell.

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