

Fucosidosis in a native-born Briton

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SUMMARY This investigation reports the biochemical findings in a native-born Briton suffering from the adult form of fucosidosis. α -L-fucosidase, α -L-iduronidase, and β -D-galactosidase were studied in cultured fibroblasts and leucocytes from the patient with fucosidosis, her maternal grandfather, and several normal controls. A complete lack of α -L-fucosidase activity was found in the patient's fibroblasts and leucocytes while the grandfather exhibited a heterozygous level of α -L-fucosidase activity in his leucocytes. Excessive excretion of what is very likely to be a fucose-containing sphingolipid was demonstrated in the patient's urine by thin-layer chromatography. Compared with five isoenzyme forms of α -L-fucosidase activity in normal leucocytes, cellulose acetate electrophoresis of the patient's leucocytes produced evidence of a single band of slight activity associated with one of the isoenzymes. This residual activity probably accounts for the survival of such patients into adolescence and beyond.

Fucosidosis is an inborn error of metabolism displaying progressive neurological degeneration and skeletal abnormalities. Durand *et al.* (1966) were the first to report this disease in two siblings of Italian origin but it was not until Van Hoof and Hers (1968) established the main biochemical defect as a deficiency of the enzyme α -L-fucosidase that the name fucosidosis (Durand *et al.*, 1968) was given to the condition. It is a rare disease of which nearly 30 cases have been discovered although not all have been reported in the literature (Durand, 1975). In the affected patients reported, a deficiency of α -L-fucosidase in tissues, leucocytes, cultured fibroblasts, serum, and urine has been found (Durand *et al.*, 1969; Loeb *et al.*, 1969; Voelz *et al.*, 1971; Patel *et al.*, 1972; Zielke *et al.*, 1972; Kousseff *et al.*, 1973; Matsuda *et al.*, 1973; Borrone *et al.*, 1974). Excessive storage of fucose-containing glycolipid and glycoprotein occurs in the tissues with excretion in the urine.

The clinical symptoms of the disease show individual variations (Van Hoof, 1973) and can often be confused with Hurler's syndrome, metachromatic leucodystrophy or mucopolysaccharidosis. However, it is now known that there is genetic heterogeneity in fucosidosis (Gatti *et al.*, 1973; Kousseff *et al.*, 1973). One form causes death in childhood whereas the other shows a slower progression of the disease. The

adult form differs from the fatal juvenile form in that survival to adolescence has been reported only in cases with 'angiokeratoma corporis diffusum', a peculiar skin condition previously thought to be characteristic of Fabry's disease.

A preliminary report has already been published (MacPhee *et al.*, 1975) on this new case of fucosidosis, the first diagnosed in a native-born Briton. In this paper further biochemical findings are reported.

Subjects

The subjects involved in this study were the patient with fucosidosis, her maternal grandfather, and several normal controls. A full clinical report of the affected patient has been given previously (Primrose, 1972).

Clinical résumé

The patient, a white female child weighing 3.6 kg, was born in 1951 after a normal pregnancy and delivery. The mother who was aged 22 years was unmarried and lived with her parents on an isolated farm. The grandmother died when the child was aged 2 and the mother and maternal grandfather reared the child until the age of 16 when the mother died from subarachnoid haemorrhage. The father of the child was never identified and the maternal grandfather cannot be excluded. His own parents were first cou-

sins and he was the last born of seven children and the only one to survive beyond infancy.

The patient's early development was slow but as a baby she learned to sit and to walk. She learned a few words of speech but never attended school.

A mild skin rash was noticed at the age of about 5 years. In 1962, at the age of 11, she was referred to an orthopaedic surgeon because of deterioration in her ability to walk. Bilateral club feet and small generalised capillary telangiectasia were noted on examination. Following triple arthrodesis of both tarsal joints in 1964, she was able to walk again despite the development of flexion contractures of the hips and knees.

At the present time the patient is virtually helpless with moderate flexion contractures of the legs. She is incapable of feeding herself. There is severe dorsal scoliosis but no gross kyphosis. Her tongue is large and coarse but the palate is normal. There is tortuosity of some of the bulbar conjunctival vessels with aneurysmal dilatations. Vision is present and she readily recognises individuals although there is pigmentary degeneration of both maculae. She has some understanding of what is said to her and occasionally utters single words of indistinct speech. There is wasting of the small muscles of the hands and the muscles of the legs. The skin is soft and there is an extensive red maculopapular rash which is most pronounced around the pelvis and thighs and across the breasts. She has a gargoyle-like appearance and has displayed progressive mental and neurological degeneration.

Material and methods

WHITE BLOOD CELLS

Leucocytes were isolated by dextran sedimentation (Kampine *et al.*, 1966) from samples of heparinised venous blood. The sedimented leucocyte-erythrocyte mixture was washed once with isotonic saline and, after recentrifugation, the remaining erythrocytes were lysed with distilled water. After a further 20 seconds, isotonicity was restored by the addition of an equal volume of double strength isotonic saline. Following another wash with isotonic saline the cells were counted in isotonic saline, recentrifuged and reconstituted with distilled water, and stored at -30°C until used for enzymatic determination. Immediately before use the leucocyte preparations were thawed and frozen six times.

CULTURED FIBROBLASTS

Tissue obtained by skin biopsy was set up in culture in Ham's F-10 medium supplemented by 20% calf serum and containing penicillin (250 units/ml) and streptomycin (250 $\mu\text{g}/\text{ml}$). Fibroblast cells were

grown in Petri dishes in 5% CO_2 and air, harvested by trypsinisation, washed twice in Hank's balanced salt solution, and stored at -70°C . When required for analysis, the cell pellets were suspended in distilled water and frozen and thawed 10 times.

PROTEIN ESTIMATION

Protein was measured in the leucocyte and fibroblast extracts according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

ENZYME STUDIES

α -L-fucosidase A modification of the method of Van Hoof and Hers (1968) was used. A mixture of 2 mM p-nitrophenyl- α -L-fucopyranoside, 0.1 M acetic acid-sodium acetate buffer, pH 5.4, and fibroblast or leucocyte preparation (30-100 μg protein) in a final volume of 60 μl was incubated for two hours at 37°C along with the appropriate enzyme and substrate blanks. The reaction was terminated by 500 μl of 0.4 M glycine-NaOH buffer, pH 10.4, and the p-nitrophenol released was measured by determining absorption at 400 nm and comparing this with corresponding standards.

α -L-iduronidase The method of Hall and Neufeld (1973) was used to measure the α -L-iduronidase activity in the fibroblasts and leucocytes (70-170 μg protein per assay). The phenyl iduronide substrate was kindly gifted to us by Dr E. Neufeld.

β -D-galactosidase β -D-galactosidase was used as a reference enzyme. 20 μl of fibroblast or leucocyte preparation (30-100 μg protein) was added to 100 μl of 2 mM p-nitrophenyl- β -D-galactopyranoside in 0.1 M citrate-phosphate buffer, pH 4.3, and incubated for one hour at 37°C . The reaction was terminated by adding 1 ml of 0.4 M glycine-NaOH buffer, pH 10.4, and the p-nitrophenol released was estimated by measuring the optical density at 400 nm. The blank for the enzyme was an enzyme blank with substrate added at the end of the incubation.

CELLULOSE ACETATE ELECTROPHORESIS

The α -L-fucosidase isoenzymes were examined by zone electrophoresis of 10 μl (15-50 μg protein) of supernatant from freeze-thawed leucocyte preparations spotted on cellulose acetate membranes. The electrophoresis buffer was 0.02 M citrate-phosphate, pH 6.2, the same as used for the detection of hexosaminidase isoenzymes (Westwood and Raine, 1974), and the system was run at 200 volts for 60 minutes. The membranes were then incubated for 30 minutes at 37°C on filter paper soaked with a solution of 1 mM 4-methylumbelliferyl- α -L-fucopyranoside in 0.05 M acetic acid-sodium acetate buffer,

pH 5.4. After incubation the membranes were exposed to a second filter paper soaked with 0.4 M glycine-NaOH buffer, pH 10.4, and the α -L-fucosidase isoenzymes were examined under long wavelength ultraviolet light.

URINE ANALYSIS

Glycosaminoglycans were measured in the urine of the patient and her grandfather by the method of Duncan *et al.* (1973). An examination of the urine for abnormal oligosaccharides was performed by thin-layer chromatography (Humbel and Collart, 1975).

Results

ENZYME ACTIVITIES IN CULTURED FIBROBLASTS AND LEUCOCYTES

The Table shows the results of the enzyme activities for α -L-fucosidase, α -L-iduronidase, and β -D-galactosidase in cultured fibroblasts from the patient and two normal subjects and in the leucocytes of the patient, her grandfather, and four normal subjects.

When cultured fibroblasts were used for the determination of α -L-fucosidase activity it was found that the patient exhibited no enzyme activity but the reference β -D-galactosidase activity was normal as were the enzyme measurements in the controls. α -L-iduronidase levels were essentially normal in the controls but the level of activity in the fucosidosis fibroblasts was increased above the normal range.

Leucocyte α -L-fucosidase in the patient was absent whereas the grandfather had a heterozygous level of activity (Patel *et al.*, 1972; Borrone *et al.*, 1974). α -L-iduronidase activity was normal in the leucocytes of the patient and controls, and the reference β -D-galactosidase levels were normal in all the leucocytes tested.

α -L-FUCOSIDASE ISOENZYMES

Five bands of enzyme activity were produced on the cellulose acetate membranes from the normal leucocyte extract (Fig. 1), bands 1 and 4 exhibiting more fluorescence than the other bands. The isoenzyme pattern of the fucosidosis heterozygote (the grandfather) produced a strongly fluorescent band 1 but a weak fluorescence in band 4 with no other fluorescence visible. The homozygote exhibited no fluorescent bands other than a barely detectable fluorescence in band 1.

URINE ELECTROPHORESIS

Electrophoresis of the urine from the patient and her grandfather for glycosaminoglycan material showed normal amounts of chondroitin sulphates A and C with no abnormal bands. Previously, the urine of the patient had shown on electrophoresis an overall normal level of glycosaminoglycans with an abnormally high heparan sulphate band (Primrose, 1972).

URINE CHROMATOGRAPHY

Thin-layer chromatography for the detection of abnormal oligosaccharides showed the presence of an excessive excretion of a compound in the urine of the fucosidosis patient (Fig. 2). The compound had an Rf lower than that of lactose and stained brown-yellow with orcinol. The control and the grandfather's urine showed the presence of compounds with higher Rf values than lactose. These findings compare with the chromatograms of urine specimens from fucosidosis patients described by Humbel and Collart (1975). It is probable that the abnormal compound excreted is a fucose-containing compound.

Table Enzyme activities in cultured fibroblasts and leucocytes in patient, grandfather, and controls

Subject	Enzyme activity		
	α -L-fucosidase (nmol p-nitrophenol released/h/mg protein)	α -L-iduronidase (nmol phenol released/18h/mg protein)	β -D-galactosidase (nmol p-nitrophenol released/h/mg protein)
Cultured fibroblasts			
Patient	0	1598	841
Control 1	92	569	1030
Control 2	79	359	735
Normal range	38-176 (Beratis <i>et al.</i> , 1975)	360-1300 (Hall and Neufeld, 1973)	300-1100*
Leucocytes			
Patient	0	347	168
Grandfather	8	—*	157
Control 3	26	310	133
4	43	—*	208
5	28	—*	224
6	27	—*	131
Normal range	25-56 (Borrone <i>et al.</i> , 1974)	100-350*	60-220*

* = own data; * = not estimated

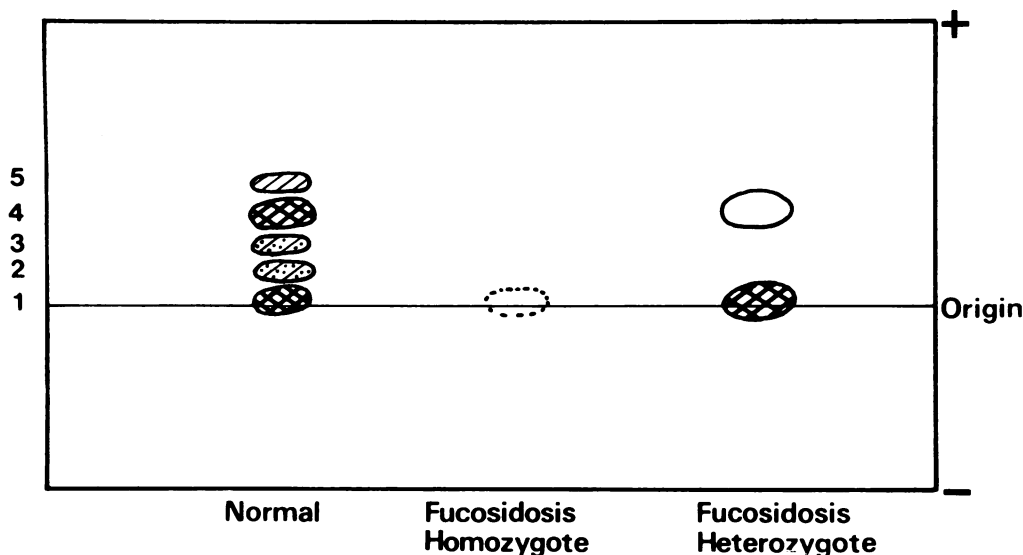


Fig. 1 Schematic representation of fluorescence exhibited by α -L-fucosidase isoenzymes in leucocyte extracts from the affected patient, heterozygote, and normal subjects after cellulose acetate electrophoresis and incubation with 1 mM 4-methylumbelliferyl- α -L-fucopyranoside.

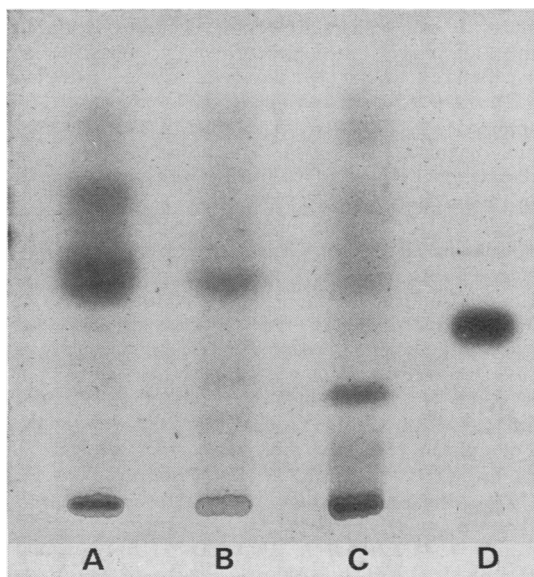


Fig. 2 Thin-layer chromatogram of urinary oligosaccharides for detection of patients with glycoprotein storage disease: (A) 20 μ l normal urine; (B) 20 μ l urine from fucosidosis heterozygote; (C) 20 μ l urine from fucosidosis homozygote; (D) 10 μ g lactose.

IMMUNOCHEMICAL DETERMINATION OF α -L-FUCOSIDASE

A sample of plasma from the affected patient was examined by Professor D. Robinson and Dr R. Thorpe (University of London) for the presence of α -L-fucosidase. Using a specific antiserum to the human enzyme, they were unable to detect any antigenically competent α -L-fucosidase in the patient's plasma. Plasma from both the grandfather and normal control subjects contained easily demonstrable α -L-fucosidase.

Discussion

The deficiency of α -L-fucosidase in both the peripheral leucocytes and cultured fibroblasts from the affected patient must be considered together with the finding of urinary excretion of a probable fucose-containing compound and the clinical features. It seems beyond reasonable doubt that the patient is suffering from the adult form of fucosidosis. Gel chromatography has shown two forms of α -L-fucosidase in human organs (Robinson and Thorpe, 1973; Wiederschain *et al.*, 1973). They can be distinguished by differing pH activity profiles and heat stability. Further investigations with the use of the fluorogenic substrate, 4-methylumbelliferyl- α -L-fucopyranoside, has shown up to nine forms of the

enzyme by isoelectric focusing (Alhadeff *et al.*, 1974; Thorpe and Robinson, 1975) and up to six forms by starch gel electrophoresis (Alhadeff *et al.*, 1974; Turner *et al.*, 1974).

This present study demonstrated five bands of α -L-fucosidase activity in normal leucocyte extracts using cellulose acetate electrophoresis. The subject homozygous for deficiency of α -L-fucosidase activity did, however, exhibit a very faint band of enzyme activity. The survival of the present patient into adolescence and beyond has probably depended on the existence of the residual activity. Previous investigators, using gel electrophoresis to separate the isoenzymes (Alhadeff *et al.*, 1974; Turner *et al.*, 1974) of α -L-fucosidase present in liver, plasma, leucocytes, cultured fibroblasts and lymphoid cells from patients with fucosidosis, could show no isoenzyme activity. In the sera of patients with the adult form of fucosidosis, however, using isoelectric focusing a single major acidic peak has been observed in comparison with four major peaks in normal sera (Durand, 1975). It is of interest to note that, while the immunochemical technique failed to detect any antigenically competent α -L-fucosidase, minimal enzyme activity was found by electrophoresis. The exact polypeptide structure responsible for the antigenicity of α -L-fucosidase is not known and it is quite possible that an abnormality in the enzyme molecule which removes antigenicity need not totally remove biological activity.

This case has proved that a gene abnormality causing fucosidosis is present in the British population and there may be a relatively high heterozygous carrier rate as postulated in the Italian population (Gatti *et al.*, 1973). The need for effective screening for fucosidosis is apparent in all patients exhibiting Hurler-like appearances without excess urinary glycosaminoglycans. Eventually reliable prenatal diagnosis of fucosidosis using cultured amniotic cells should become available, although recently an incorrect diagnosis was made for a pregnancy monitored for fucosidosis (Matsuda *et al.*, 1975).

We wish to thank the Advisory Committee for Medical Research (Scotland) for provision of a grant which enabled the study to be undertaken. Drs E. F. Neufeld and B. Weissmann kindly supplied the phenyl iduronide substrate (NIH-NIAMDD 73-2205). Dr Primrose was the physician who referred the samples for biochemical investigation, and the Department of Medical Genetics at Yorkhill undertook culture of the fibroblasts used for enzyme determinations. The assistance of Mrs F. Hall in performing the glycosaminoglycan determinations is gratefully acknowledged.

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