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Biochemical and Genetic Studies of Plasma and Leukocyte α-L-Fucosidase

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Fucosidosis is an inherited metabolic disorder in which a deficiency of α-L-fucosidase activity results in accumulation of fucosyl compounds in the lysosomes [1, 2, 3]. The clinical manifestations include progressive motor and mental deterioration, coarseness of facial features, weight loss, cardiomegaly, and bony deformities (kyphoscoliosis and beaking of the lumbar vertebrae) [2, 4–6]. Hepatomegaly, corneal opacities and other manifestations have also been mentioned [7]. Initially the patients exhibit hypotonia, but progressive spasticity develops with time. Some patients have died in early childhood, while in others the progression has been slow [8]. In some cases, angiokeratoma corporis diffusum has been described, and it has been suggested that this may be a feature distinguishing one form of fucosidosis from another [7, 9]. Whether variability in the clinical manifestations encountered represents genetic heterogeneity remains undetermined.

Deficiency of α-L-fucosidase has been demonstrated in plasma, leukocytes, and cultured fibroblasts obtained from patients with fucosidosis [8, 10–12]. In the present study, absence of plasma and of leukocyte α-L-fucosidase activity was demonstrated in three affected individuals. However, several normal adults also were found to have extremely low plasma α-L-fucosidase activity, but normal activity in their leukocytes [13]. The low plasma enzyme activity exhibited by normal individuals appears to be an inherited characteristic [14].

MATERIALS AND METHODS

Blood samples were obtained from normal newborns (cord blood), 3-day-old normal infants, hospitalized children, normal adults and family members, three fucosidosis patients in two families, and the parents in one of these families.

Two of the fucosidosis patients are male siblings [15]. The parents are first cousins of Italian ancestry. The older sibling was born in 1961, the younger in 1963. The older was first seen in our hospital at the age of 2 years because of muscular weakness. Muscular dystrophy was suspected but not confirmed. There were further admissions for a variety

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of reasons, including bilateral pansinusitis, otitis media, and progressive weakness. In 1966, when 5½-years-old, he was studied for a possible storage disease, but no diagnosis resulted. Polyps were removed from the right nasal antrum in 1971 and 1972. Histological examination of the polyps revealed a vacuolate cytoplasm suggesting a lysosomal storage disease.

Throughout the period since his first admission he exhibited increasing muscular weakness and mental deterioration. At 12-years-old, grand mal seizures began. His weight was 18.2 kg, height 118 cm, and head circumference 53.3 cm. He could stand with support but was unable to walk or talk. He had a marked kyphosis, a dull facies, protruding tongue and heavy eyebrows. Upon examination of the skin at age 14, telangiectatic lesions were noted similar to those seen in patients with Fabry disease (angiokeratoma corporis diffusum).

The younger sibling was first seen at 3 years of age in our hospital. Generalized muscular weakness was evident. His course was progressive, like that of his brother. By 10 years of age he was unable to walk and required complete nursing care. Skin lesions similar to those seen in the older brother, but less pronounced, were present upon examination at age 12.

The coarse features, progressive mental retardation, kyphosis, and the presence of cytoplasmic vacuoles in cells of a number of biopsied tissues suggested fucosidosis among other possible lysosomal storage diseases. The diagnosis of fucosidosis in both children was established by demonstrating absence of α-L-fucosidase activity in plasma and in cultured fibroblasts [10, 12].

By 10 years of age, both children had diffusely abnormal electroencephalograms. Roentgenologic studies of the skeleton showed trabeculation of the ribs, increased medullary spaces of the long bones and anterior-inferior beaking of the lumbar vertebrae.

The third fucosidosis patient, unrelated to the other two, was found in the course of a survey of patients in an institution for the retarded. He was born in 1958.

Plasma Enzyme Assay

Plasma (or serum) α-L-fucosidase activity was measured by the method of Zielke et al. [10] with minor modifications. The unit of enzyme activity is expressed as nanomoles of *p*-nitrophenyl-α-L-fucoside hydrolysed per hour/ml of plasma. In some instances, enzyme activity with 4-methylumbelliferyl-α-L-fucopyranoside as substrate (Koch-Light, England) also was determined. The incubation mixture consisted of 0.05 ml of 1:10 diluted plasma and 0.10 ml of a solution which is 0.001 M in fluorogenic substrate in 0.5 M citrate, 0.1 M phosphate buffer, pH 5.0 [16]. The incubation period was 1 hour at 37°C. The amount of 4-methylumbelliferone liberated was determined according to O'Brien et al [17]. The enzyme specific activity with the fluorogenic substrate is about two times higher than that for the corresponding *p*-nitrophenyl derivatives [18].

Leukocyte Enzyme Assay

Leukocytes were separated by dextran sedimentation of an 8–10 ml sample of heparinized blood [19]. Residual erythrocytes were removed either by hemolysis in 0.82% NH_4Cl or by rapid hemolysis with water. The leukocyte extracts were made by sonication (MSE) in 1–2 ml of 0.005 M acetate buffer, pH 4.9 for 1 min in an ice bath. The assaying condition was similar to that for plasma. The protein content of the crude extract was determined by the method of Lowry et al. [20]. The unit of enzyme activity is defined as nanomoles of *p*-nitrophenyl-α-L-fucoside hydrolyzed per hour/mg protein.

In the cases in which comparative activity for platelet, lymphocyte and granulocyte α-L-fucosidase was determined, the more sensitive assay using 4-methylumbelliferyl-α-L-fucopyranoside as substrate was employed. The incubation condition is similar to that described for plasma. Activity is expressed as nanomoles of 4-methylumbelliferone liberated per hour/mg protein.

Separation of Platelets, Lymphocytes, and Granulocytes

Whole blood from normal adults was obtained by venipuncture (35–40 ml). To isolate platelets 9 ml of whole blood was added to 1 ml of 3.8% trisodium citrate. Immediately the blood was mixed well, and centrifuged at 200 *g* for 15 min at room temperature to obtain platelet-rich plasma (PRP) in the supernatant fraction. After platelet, erythrocyte and leukocyte counts were made, the PRP was centrifuged at 1000 *g* for 10 min. The platelet button was washed once with 5 ml of 1% disodium EDTA in 0.9% NaCl. Under the experimental conditions employed, the suspension typically contained 1.5×10^9 platelets, 5×10^4 leukocytes (both lymphocytes and granulocytes), and 4×10^5 erythrocytes. After centrifugation, the button was suspended in 1 ml of 0.005 M acetate buffer, pH 4.9, and the mixture was sonicated.

Mixed leukocytes were prepared by the dextran sedimentation procedure from 24 ml of heparinized blood. One-third of this preparation was employed as a control for activity of the mixed cells. The remainder was subjected to a stepwise albumin gradient centrifugation. The gradient consisted of successive 1.5 ml layers of albumin solutions (bovine serum albumin, Fraction V, Sigma Chemical Co., St. Louis, Mo.) of the following specific gravities: 1.116, 1.076, 1.067, and 1.057. Centrifugation took place at 5000 *g* at 4°C in a swinging bucket rotor for 90 min. The yield of cells was variable due to clumping of leukocytes prior to gradient separation. From freely suspended cells, four clear layers of cells were obtained. The upper two consisted primarily of lymphocytes (more than 90%) and the bottom two layers contained predominantly granulocytes (more than 90%). There was a small contamination, primarily of the granulocyte layers, by platelet aggregates. The fractions (top two combined, bottom two combined) were washed twice with 5 ml of saline and then were sonicated in 0.4 ml of 0.005 M sodium acetate buffer, pH 4.9. The α-L-fucosidase activity was determined with the fluorogenic substrate.

Cellulose Acetate Electrophoresis

The electrophoretic procedure was carried out using a discontinuous system, employing cellulose polyacetate strips (Sepraphore III, 1 × 6 inches, Gelman [21]).

RESULTS

Figure 1 depicts the distribution of plasma α-L-fucosidase activity with *p*-nitrophenyl-α-L-fucoside as the substrate. The range for each group is wide. The activity tends to be higher in normal newborn infants (groups 1 and 2) than in normal adults (group 4). Among hospitalized children (group 3), three patients were found to have activity values $>$ 500 U. Their plasma samples were icteric. Among 42 unrelated adults studied (group 4), three were found to have values of $<$ 25 U (one as low as 4 U). No significant variation in plasma activity per individual was observed upon repeated testing over a period of several months. In fucosidosis patients, no plasma enzyme activity could be measured, and two parents were found to have activity in the high normal range.

Table 1 compares the plasma and leukocyte α-L-fucosidase activity with *p*-nitrophenyl-α-L-fucoside as the substrate. For affected individuals, activity was almost absent in both plasma and leukocytes. The parents had half normal leukocyte activity, but normal plasma activity. In the group of unrelated normal adults the mean activity found was somewhat lower than that reported by Zielke et al. [10], but was comparable to that of Öckerman and Köhlin [22]. Two individuals had a very low plasma activity despite normal leukocyte activity.

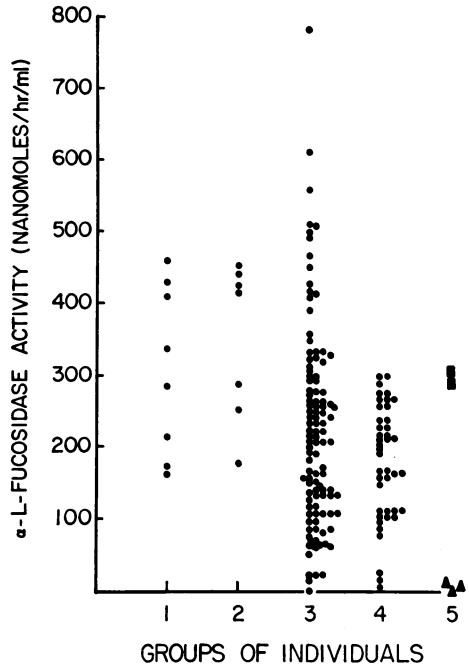

FIG. 1.—Distribution of plasma α-L-fucosidase activity: *group 1,* cord blood; *group 2,* 3-day-old infants; *group 3,* children; *group 4,* adults; *group 5,* fucosidosis patients (*triangles*) and parents (*squares*).

The separate contribution to the α-L-fucosidase activity in a leukocyte preparation by platelets, lymphocytes, and granulocytes was examined. The specific activity in the platelets (29 U) was about half that of granulocytes and lymphocytes (60 U and 54 U, respectively). In three separate preparations, there was no appreciable difference between lymphocyte and granulocyte activity. Large numbers of platelets are removed by dextran sedimentation in the preparation of leukocytes, and the contribution of platelets to that total activity was estimated to be less than 10%.

Family studies were carried out to examine the possibility that the occurrence of low plasma α-L-fucosidase values in unaffected individuals might be under genetic control.

Family R was originally studied for the dibucaine-resistant atypical plasma cholinesterase (homozygous propositus, IV-1, fig. 2). The plasma samples from family members were also studied for α-L-fucosidase activity. Five members were found to have low plasma α-L-fucosidase activity (I-4, II-4, II-5, II-6, II-7); 11 to have intermediate values. The pattern of distribution of activity values in this pedigree suggested that the occurrence of low plasma fucosidase activity in clinically normal individuals is an inherited characteristic. This conclusion is based on three assumptions: first, that a normal individual with low plasma fucosidase activity is homozygous for this characteristic; second, that an individual with an

TABLE 1

COMPARISON OF α-L-FUCOSIDASE ACTIVITY IN PLASMA AND IN LEUKOCYTE EXTRACTS

Subjects	Plasma (nmol/hr/ml)	Leukocyte (nmol/hr/mg protein)
Normal adults*	28	20.1
	137	22.1
	254	20.2
	110	29.3
	154	18.2
	24	23.8
	107	20.2
	126	30.5
	202	27.2
	268	31.5
	158	32.4
	184	25.4
	219	24.6
	234	18.0
	242	23.7
	222	26.3
	292	24.6
	150	24.0
Patients with fucosidosis:		
M. Z.	0	0
G. Z.	3	0
J. L.	7	0
Parents of patients:		
A. Z.	237	12.5
F. Z.	242	11.4

* Plasma: mean activity = 173; range, 24–292; SD ± 77. Leukocyte: mean activity = 24.6; range, 18.2–32.4; SD ± 4.2.

activity $\geq$ 200 U is a normal homozygote; and third, that an individual with an intermediate value (90–180) is heterozygous. Leukocyte activity was measured in members of the R Family, and all were found to have normal leukocyte activity, regardless of their plasma value. No correlation could be seen between the distribution in the pedigree of atypical plasma cholinesterase genotypes and the plasma α-L-fucosidase values.

In each of two other families (family To. and family Th.) there was an individual with low plasma α-L-fucosidase activity (fig. 3). In family E, based on activity values, only heterozygotes were presumed to be present. All members of family P were considered to be normal homozygotes since all were found to have a plasma enzyme activity $>$ 200 U (fig. 4). In nine other families studied, the patterns of distribution have been consistent with the hypothesis.

The observation that plasma and leukocyte α-L-fucosidase activity may not correlate in the same individual (table 1 and in family R of fig. 2) suggested that the two enzymes may differ in some respects. In order to examine this possibility, electrophoretic properties of the plasma enzyme were compared to that from leukocytes.

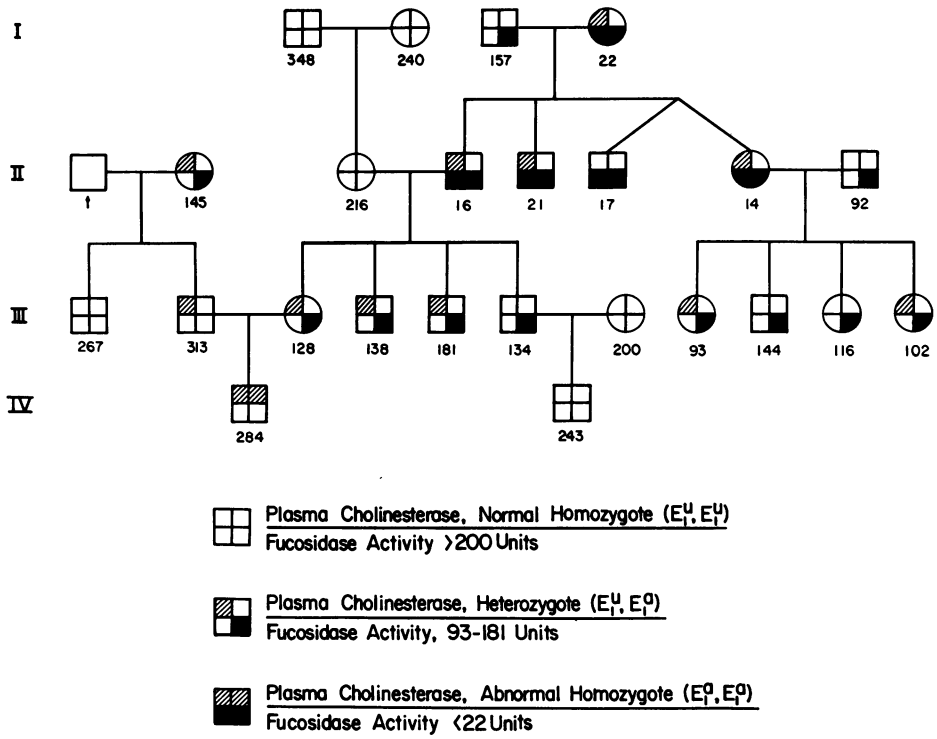

FIG. 2.—Plasma α-L-fucosidase activity and cholinesterase genotypes in members of family R.

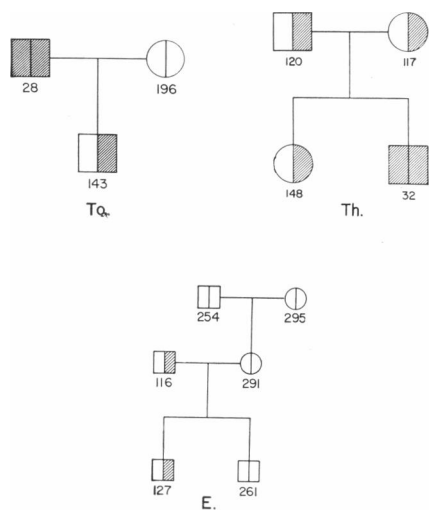

FIG. 3.—Plasma α-L-fucosidase activity in members of three families.

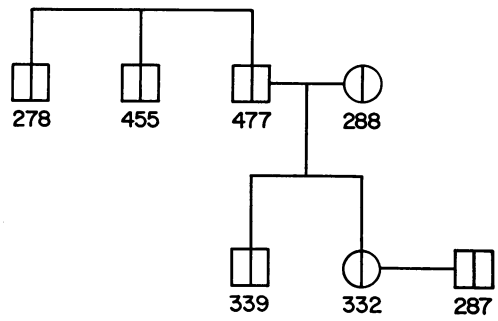

FIG. 4.—Plasma α-L-fucosidase activity.

In cellulose acetate electrophoresis the α-L-fucosidase of the mixed leukocytes from a normal person has a broader band and a slower mobility than the plasma enzyme. The platelet enzyme mobility, and that of granulocytes, is similar to that of a mixed leukocyte preparation. We have not been able to determine the electrophoretic mobility of the lymphocyte enzyme; the number of cells isolated in the albumin density gradient system is too small, and the activity is insufficient to produce a fluorescent band. The electrophoretic mobilities of the fucosidase from autopsy samples of human liver, heart muscle, and of a urine concentrate were found to be similar to that of the mixed leukocyte preparation.

The electrophoretic characteristic of the leukocyte enzyme is the same for all classes of individuals studied. The plasma enzyme has a different mobility from that in leukocyte preparations and those from other tissues, but the mobility is the same for all individuals. The enzyme could not be detected upon electrophoresis of samples from patients with fucosidosis and from normal individuals with very low enzyme activity.

DISCUSSION

The three fucosidosis patients in this study have most features in common with those described by others [2, 4, 6]. They have had a relatively slower progression of the disease as compared to some. As reported by others, our patients with fucosidosis have essentially no α-L-fucosidase activity in plasma or in leukocytes.

Low plasma α-L-fucosidase values are not limited to affected individuals. It has been shown in this study that some clinically normal individuals have an extremely low plasma activity, but normal leukocyte activity. For this reason, measurement of plasma fucosidase activity alone is of little diagnostic value. Confirmation of a diagnosis of fucosidosis should include measurement of the activity of α-L-fucosidase in leukocyte preparations. In addition, the leukocyte procedure appears to have the capability of detecting carriers [8, 11].

Family studies indicate that the plasma enzyme of clinically normal individuals can occur in two different forms ("low" activity and "normal" activity), apparently

genetically determined. In families in which both parents have normal activity, the offspring have normal activity (fig. 4). In families in which low and intermediate values occur, Mendelian segregation is suggested. Since the leukocyte fucosidase activity among the normals is about the same irrespective of plasma enzyme activity, the genetic control for the plasma enzyme may be related to modification of the tissue enzyme in the release process. The enzyme found in the plasma of normal individuals differs from that in leukocytes with respect to electrophoretic mobility. This observation has now been confirmed in another laboratory by a starch gel electrophoretic procedure [23]. The reason for a greater electrophoretic mobility of the plasma enzyme is unknown. Treatment of serum with neuraminidase has resulted in slower mobilities suggesting sialyl group differences. It has now been shown that multiple electrophoretic bands of fucosidase exist in various tissues [23, 24], but the relationship to the plasma enzyme is not understood.

SUMMARY

Deficiency of α-L-fucosidase in plasma and leukocytes has been demonstrated in three patients affected with fucosidosis. The measurement of plasma fucosidase activity alone is of little diagnostic value. Several normal individuals were found to have extremely low plasma α-L-fucosidase activity, but normal activity in leukocyte preparations. The low plasma enzyme activity exhibited by clinically normal individuals appears to be an inherited characteristic. The plasma enzyme was found to be different from that of leukocytes in terms of electrophoretic mobility.

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REFERENCES

1. VAN HOOF F, HERS HG: Mucopolysaccharidosis by absence of α-L-fucosidase. *Lancet* 1:1198, 1968
2. DURAND P, BORRONE C, DELLA CELLA G: Fucosidosis. *Lancet* 1:1198, 1968
3. DURAND P, BORRONE C, DELLA CELLA G: Fucosidosis. *J Pediatr* 75:665–674, 1969
4. LOEB H, TONDEUR M, JONNIAUX G, MOCKEL-PHOL S, VAMOS-HURWITZ E: Biochemical and ultrastructural studies in a case of mucopolysaccharidosis "F" (fucosidosis). *Helv Paediatr Acta* 36:519–537, 1969
5. BORRONE C, GATTI R, TRIAS X, DURAND P: Fucosidosis: clinical, biochemical, immunologic and genetic studies in two new cases. *J Pediatr* 84:727–730, 1974
6. DURAND P, BORRONE C: Fucosidosis and mannosidosis, glycoprotein and glycosylceramide storage diseases. *Helv Paediatr Acta* 26:19–27, 1971
7. GATTI R, BORRONE C, TRIAS X, DURAND P: Genetic heterogeneity in fucosidosis. *Lancet* 2:1024, 1973
8. PATEL V, WATANABE I, ZEMAN W: Deficiency of α-L-fucosidase. *Science* 176:426–427, 1972
9. KOUSSEFF BG, BERATIS NG, DANESINO C, HIRSCHHORN K: Genetic heterogeneity in fucosidosis. *Lancet* 2:1387–1388, 1973
10. ZIELKE K, OKADA S, O'BRIEN JS: Fucosidosis: diagnosis by serum assay of α-L-fucosidase. *J Lab Clin Med* 79:164–169, 1972

11. MASTUDA I, ARASHIMA S, ANAKURA M, OKA Y: α-L-fucosidase and α-D-mannosidase activity in the white blood cells in the disease and carrier state of fucosidosis. *Clin Chim Acta* 48:9–13, 1973
12. ZIELKE K, VEATH ML, O'BRIEN JS: Fucosidosis: deficiency of alpha-L-fucosidase in cultured skin fibroblasts. *J Exp Med* 136:197–199, 1972
13. NG WG, DONNELL GN, KOCH R: Serum α-L-fucosidase activity in the diagnosis of fucosidosis. *Pediatr Res* 7:391, 1973
14. NG WG, DONNELL GN, KOCH R, BERGREN WR: Biochemical and genetic studies of human plasma α-L-fucosidase. 13th International Congress of Genetics, Berkeley, California, August, 1973
15. DURAND P: Progress and problems on fucosidosis and mucolipidosis, in *Glycolipids, Glycoproteins and Mucopolysaccharides of the Nervous System,* edited by ZAMBOTTI V, TETTAMANTI G, ARRIGOUI M, New York, Plenum, 1972, pp 293–303
16. GOMORI G: Preparation of buffers for use in enzyme studies, in *Methods in Enzymology,* vol. 1, edited by COLOWICK SP, KAPLAN NO, New York, Academic Press, 1955, pp 138–146
17. O'BRIEN JS, OKADA S, CHEN A, FILERUP D: Tay-Sachs disease: detection by serum hexosaminidase assay. *N Engl J Med* 283:15–20, 1970
18. WOOD S: A sensitive fluorometric assay for α-L-fucosidase. *Clin Chim Acta* 58:251–256, 1975
19. NG WG, BERGREN WR, DONNELL GN: A new variant of galactose-1-phosphate uridyltransferase in man: the Los Angeles variant. *Ann Hum Genet* 37:1–8, 1973
20. LOWRY OH, ROSENBROUGH NJ, FARR AL, RANDALL RJ: Protein measurement with the foline phenol reagent. *J Biol Chem* 193:265–275, 1951
21. NG WG, DONNELL GN, KOCH R, BERGREN WR: Urinary α-L-fucosidase. *Birth Defects: Orig Art Ser.* In press, 1974
22. ÖCKERMAN PA, KÖHLIN P: Glycosidases in skin and plasma in Hunter's syndrome. *Acta Paediatr Scand* 57:281–284, 1968
23. TURNER BM, BERATIS NG, TURNER VS, HIRSCHHORN K: Isozymes of human α-L-fucosidase detectable by starch gel electrophoresis. *Clin Chim Acta* 57:29–35, 1974
24. ALHADEFF JA, MILLER AL, WENGER DA, O'BRIEN JS: Electrophoretic forms of human liver α-L-fucosidase and their relationship to fucosidosis (mucopolysaccharidosis F). *Clin Chim Acta* 57:307–313, 1974