Amer J Hum Genet 24:237–249, 1972

Biochemical and Electrophoretic Studies of α-Galactosidase in Normal Man, in Patients with Fabry's Disease, and in Equidae

E. BEUTLER[1] AND W. KUHL[1]

Fabry's disease is a glycolipid storage disorder characterized by the accumulation of ceramide trihexoside and by deficiency of α-galactosidase [1–3]. Pedigrees of patients with Fabry's disease clearly show X-linkage of the disorder [3]. X-linkage is further supported by the demonstration that clones of fibroblasts of heterozygotes display a bimodal population with respect to α-galactosidase activity [4]. It has been suggested that normal plasma infusions may result in enzyme production in patients with this disease [5, 6], which suggests that a disorder of regulation of enzyme production could be involved. The sex-linked nature of the enzyme deficiency could be explained on the basis of either the existence of an X-linked regulator gene or X-linkage of the structural locus for α-galactosidase. If the residual α-galactosidase activity in the cells of patients with Fabry's disease were abnormal, this would prove that the disorder is due to a structural gene mutation. On the other hand, if no abnormal protein could be found, a regulatory mutation would be a possibility. Localization of the structural gene for α-galactosidase to an autosome would indicate that the basic abnormality in the disorder was of a regulatory nature, since the gene for deficiency is sex-linked. For these reasons, we have investigated some of the biochemical characteristics of α-galactosidase in normal leukocytes and fibroblasts and in leukocytes and/or fibroblasts from three patients with Fabry's disease. We have also developed electrophoretic techniques which permit the detection of α-galactosidase activity on starch gels and have attempted to obtain information regarding linkage of α-galactosidase using both human subjects and interspecific hybrids of Equidae. These studies reveal that at least two isozymes of α-galactosidase are present in normal cells, and that one of these isozymes, which we have designated α-galactosidase A, is absent from the cells of patients with Fabry's disease. Although we have not been able to obtain any information regarding the location of the structural gene for α-galactosidase in man, this enzyme was found to be autosomally linked in Equidae.

MATERIALS AND METHODS

To obtain white blood cells, a 10 ml sample of blood was mixed with 3.3 ml of 5% polyvinylpyrollidone (PVP) containing 3% sodium citrate. The sample was permitted to

Received June 21, 1971; revised August 26, 1971.

This work was supported in part by grants AM 14755 and HD 01974 from the Department of Health, Education and Welfare, National Institutes of Health.

[1] City of Hope National Medical Center, Duarte, California 91010.

stand in a test tube at 37° C for 20–30 min to allow the red blood cells to settle. The plasma layer, which contains white blood cells and platelets, was removed and centrifuged at 100 *g* for 10 min. The erythrocytes in the sedimented cells were shock-lysed by rapidly adding 2.0 ml of ice cold water, followed in 50 sec by 2.0 ml of 2% sodium chloride solution. The cell suspension was centrifuged at 1,000 *g* for 10 min, and the white cell pellet was resuspended in 0.15–0.2 ml of 0.9% sodium chloride solution. A white blood cell count was done using standard techniques, and the cell suspension was frozen and thawed two times followed by centrifugation at 5,000 *g* for 10 min. The supernatant, which contains approximately 65% of the total enzyme activity, was then used for assay. To prepare extracts for electrophoresis, the same procedure was followed except for omission of shock lysis with water, since hemoglobin does not interfere with the electrophoretic pattern; for thermal stability studies the final preparation was made in 0.9% sodium chloride solution buffered at *p*H 7.0 with .01 M potassium phosphate buffer.

Fibroblasts were grown in minimum essential medium (MEM; Flow Laboratories, Inglewood, Calif.) buffered with .01 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer at *p*H 7.2–7.5 and supplemented with 4 mM glutamine and 20% fetal calf serum. Confluent cultures were trypsinized, washed, and resuspended in saline buffered at *p*H 7.0 with .01 M potassium phosphate buffer. The suspension was frozen and thawed two times and centrifuged at 5,000 *g* for 10 min. The supernatant, which contains approximately 95% of the enzyme, was used for enzyme assay or electrophoresis. Protein determinations [7] were done on the saline suspension before freezing and thawing.

Vertical starch gel electrophoresis [8] was performed using 52 g electrostarch (Otto Hiller, Madison, Wis.) in 500 ml of a 1:10 dilution of 0.01 M potassium phosphate buffer, *p*H 7.0. The 0.01 M phosphate buffer was used undiluted in the electrode chambers. Electrophoresis was carried out for 20 hr in the cold at 180 v (measured across the electrodes; approximately 2 v/centimeter measured across the gel) giving a current of 4.5 ma. Upon completion of electrophoresis, the gel was sliced and stained by overlaying with a thin film of 10 mM 4-methylumbelliferyl-α-D-galactoside (Sigma Chemical Co., Saint Louis, or Koch-Light, England) in 0.5 M sodium citrate buffer, *p*H 4.0. The gel slice was incubated in a humid atmosphere at 37° C for 30 min, after which filter paper generously soaked in 0.5 M sodium hydroxide was placed on the gel. In 2–3 min the filter paper was removed and the gel was examined and photographed through a Tiffin Aero no. 1 filter under long-wave ultraviolet light for fluorescent bands representing the location of α-galactosidase activity.

The α-galactosidase activity was quantitated in a reaction mixture containing 0.1 M sodium citrate buffer at *p*H 4.0, 4 mM 4-methylumbelliferyl-α-D-galactoside, and 20 μliter of cell extract in a total volume of 0.1 ml. After incubation at 37° C for 30 min, 4 ml of 0.2 M glycine buffer, *p*H 10.7, was added, and the fluorescence of the mixture was measured using a Turner 110 or 111 fluorometer with 7-37 and 0-51 (Corning) primary filters and a 3-73 (Corning) secondary filter. A standard consisting of 0.1325 μM 4-methylumbelliferone (Koch-Light) in glycine buffer was read with each set of determinations. One μunit of enzyme will hydrolyze 10^{-12} moles of substrate per minute under these conditions.

Assays for other acid hydrolases were carried out in the same way by substituting different substrates in the reaction mixture as follows: β-glucosidase using 0.5 mβ mM 4-methylumbelliferyl-β-D-glucoside with 60 min incubation; β-galactosidase using 0.5 mM 4-methylumbelliferyl-β-D-galactoside with 15 min incubation; β-N-acetyl glucosaminidase using 0.5 mM 4-methylumbelliferyl-β-N-acetylglucosaminide with 15 min incubation. The α-glucosidase activity was determined in a reaction mixture containing 0.1 M sodium citrate buffer at *p*H 4.0, 25 mM p-nitrophenyl-α-D-glucoside, and 50 μliter of cell extract

in a final volume of 0.2 ml. After incubation at 37° C for 1 hr, 0.2 ml of 4% perchloric acid was added. The tubes were mixed and centrifuged. Then 0.3 ml of the supernatant was placed in a small tube, and 0.05 ml of 2 M K_2CO_3 was added to precipitate the perchlorates. After 0.7 ml of 0.2 M glycine buffer at *p*H 10.7 was added, the optical density of the clear supernatant was determined in a Gilford Model 2400 spectrophotometer at 420 nm against a reagent blank containing water in place of cell extract. Sample blanks were also determined by substituting water for substrate and subtracting these readings from the assay readings. The α-glucosidase activity was calculated from a standard curve of 0.005–0.05 μmoles/milliliter p-nitrophenol in glycine buffer.

RESULTS

Kinetic and Thermostability Studies

As had previously been shown by Kint [1], the α-galactosidase activity of the leukocytes of a patient with Fabry's disease was greatly diminished. In a whole-cell suspension the average activity of normal leukocytes was 438 μunits/10^7 WBC; the activity in Fabry's disease was 75 μunits/10^7 WBC.

The thermostability of the normal leukocyte enzyme at 45° C revealed that the bulk of the leukocyte extract was thermolabile but that an appreciable amount of enzyme activity remained even after several hours heating at 45° C (fig. 1). To confirm that the residual, thermostable activity was due to enzymatic hydrolysis of substrate rather than a nonenzymatic component of the extract, the extracts from leukocytes and fibroblasts were boiled for 2 min before assay. No activity was observed in boiled extracts.

These studies suggested strongly that two enzymes with α-galactosidase activity were present. Since this was confirmed in further investigations (see below), these enzymes are designated as α-galactosidase A (the thermolabile component) and α-galactosidase B (the thermostable component).

When leukocyte extract from a patient with Fabry's disease was examined, no thermolabile component (α-galactosidase A) was found. All of the enzyme was thermostable. The K_m of the residual leukocyte enzyme from a patient with Fabry's disease was measured with respect to the substrate, 4-methylumbelliferyl-α-D-galactoside. It differed clearly from that of normal leukocytes, averaging 19.1 mM compared with the normal value of 4.9 mM. However, the K_m of the residual activity of heat-treated normal leukocyte extracts (α-galactosidase B) was identical with the residual enzyme in leukocyte extracts from a patient with Fabry's disease (fig. 2).

Normal fibroblast extract was found to contain an average of 1,150 μunits α-galactosidase activity per milligram fibroblast protein. Extracts of fibroblasts from three unrelated patients with Fabry's disease contained only 62 μunits, 323 μunits, and 196 μunits of α-galactosidase per milligram protein. The heating of normal fibroblast extracts also indicated that a thermolabile component (α-galactosidase A) and a thermostable component (α-galactosidase B) were present (fig. 3). When fibroblast extract from a patient with Fabry's disease was tested, only thermostable (α-galactosidase B) enzyme was present. Normal fibroblasts were found to retain an average of 3.7% of their α-galactosidase activity after

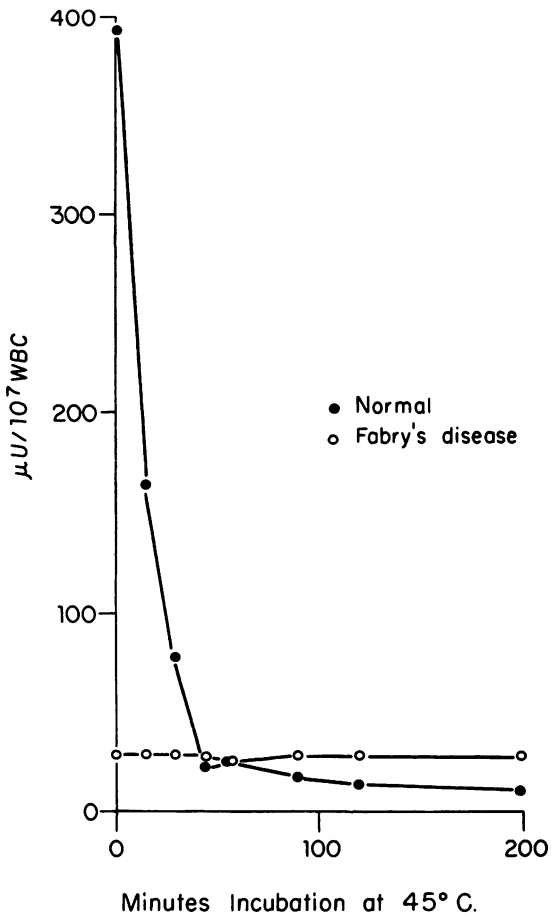

FIG. 1.—Thermal stability of α-galactosidase at 45° C, *p*H 7.0, in white blood cells of a normal subject and a patient with Fabry's disease.

heating at 45° C for 120 min. After similar treatment, the fibroblasts of three patients with Fabry's disease retained 72%, 65%, and 55% of their α-galactosidase activity.

The possibility that the thermostable activity (α-galactosidase B) did not represent the activity of an α-galactosidase but that of another hydrolase which has some galactosidase activity was investigated by studying the thermal stability of leukocyte extract β-galactosidase, α-glucosidase, and β-N-acetyl glucosaminidase in the same system used for α-galactosidase. Of these three enzymes, only α-glucosidase was thermostable. Beta-glucosidase was eliminated from consideration, since the leukocyte enzyme is quite insoluble under the conditions used for investigation of α-galactosidase thermal stability. Indeed, if β-glucosidase is solubilized by the use of deoxycholate, the solubilized enzyme is found to be thermolabile.

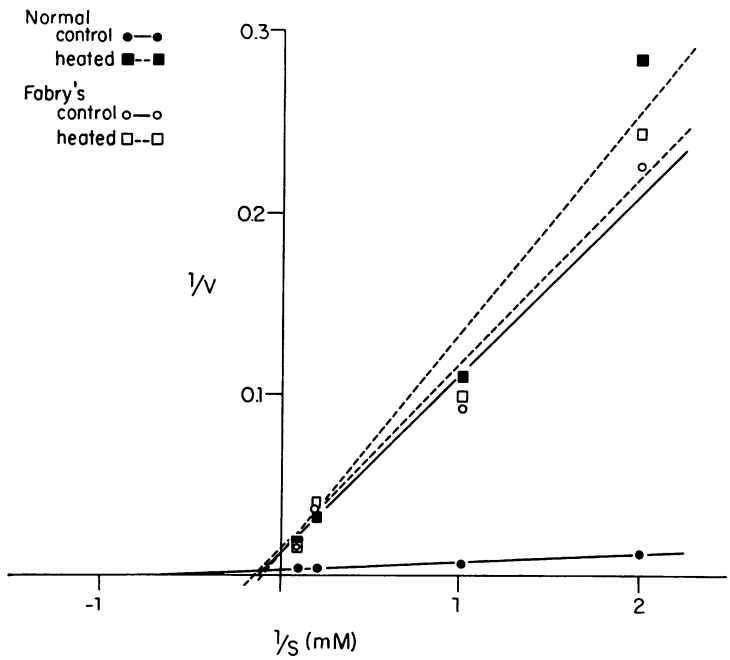

FIG. 2.—Lineweaver-Burke plot of the relationship between substrate concentration and α-galactosidase activity of heated and untreated (control) white blood cell extracts. Extracts from a normal subject and a patient with Fabry's disease were investigated without heating and after heating for 2 hr at 45° C.

Normal fibroblast β-galactosidase, β-glucosidase, and β-N-acetylglucosaminidase were also found to be thermolabile.

Since of all the hydrolases studied only α-glucosidase was thermostable, the possibility that α-galactosidase B actually was α-glucosidase with slight α-galactosidase activity was studied. Extracts of fibroblasts from a patient with Pompe's disease, deficient in α-glucosidase activity, were also heated at 45° C for 2 hr. The same pattern of residual α-galactosidase activity was found as in normal fibroblasts (fig. 3). This indicated that the thermostable activity attributed to α-galactosidase B was not α-glucosidase.

Electrophoretic Studies

In order to find an electrophoretically detectable structural variant of α-galactosidase, electrophoresis of the enzyme was carried out on the leukocytes of 93 males and 132 females (197 Caucasian, 14 Negro, five Oriental, and nine of unknown race). No electrophoretic variants were detected. The normal two- or three-banded electrophoretic pattern seen on starch gel is shown in figure 4. Efforts to carry out electrophoresis of the residual leukocyte enzyme of a patient with Fabry's disease were unsuccessful, in that no band of activity could be visualized. Electrophoresis of fibroblast enzyme revealed the presence of a slowly moving

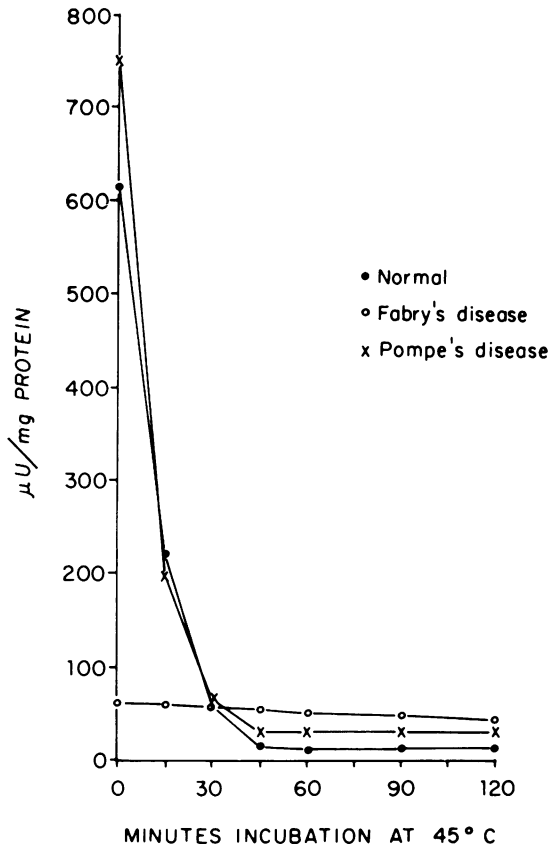

FIG. 3.—Thermal stability of α-galactosidase activity of fibroblast extracts prepared from cells of a patient with Fabry's disease, a patient with Pompe's disease (α-glucosidase deficiency), and a normal control. Residual α-galactosidase activity was as great in the extracts from fibroblasts of the patient with Pompe's disease as in control cells, indicating that the heat-stable activity was not α-glucosidase.

component which did not appear to be present or was only faintly visible in normal extracts of comparable α-galactosidase activity. However, this band corresponded exactly in position to the thermostable component of normal fibroblast extract when the same concentration of residual enzyme activity was adjusted for electrophoresis. Figure 5 shows the result of electrophoresis of α-galactosidase of untreated and heat-treated fibroblast extracts from normal subjects and subjects with Fabry's disease.

A family consisting of a horse (father), donkey (mother), and male offspring (hinny) was also investigated using starch gel electrophoresis. The results of these studies are shown in figure 6. It is apparent that the male hinny inherited both the horse (fast) and the donkey (slow) phenotype. Thus the structural gene for α-galactosidase in a horse must be autosomally inherited. No other family groups of horses and donkeys were investigated, but four other horses, three donkeys, and

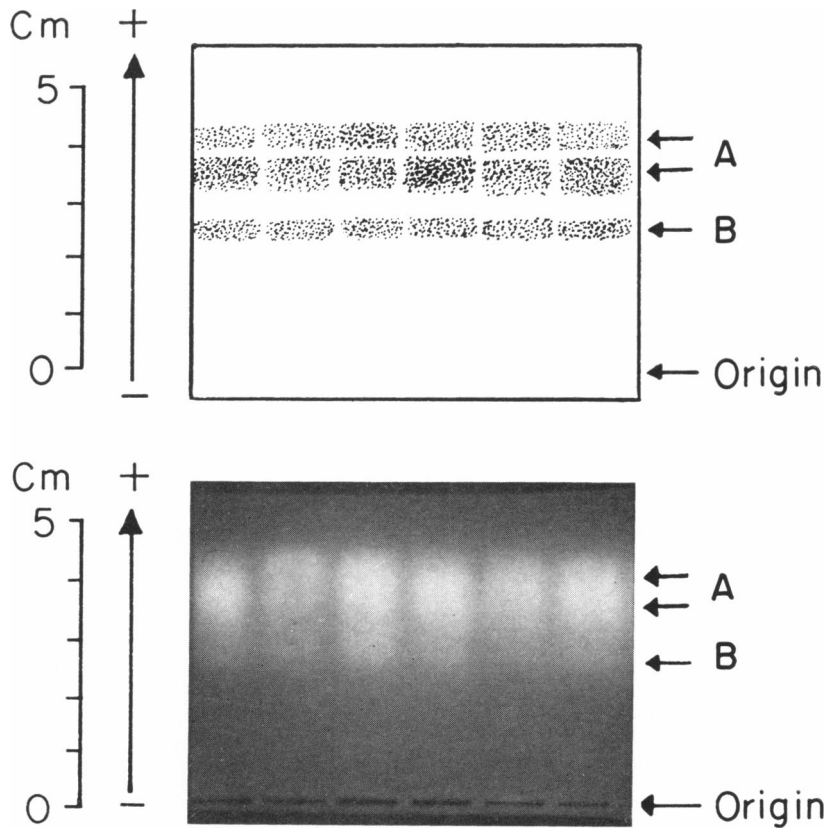

FIG. 4.—Starch gel electrophoresis at *p*H 7.0 of human white blood cell α-galactosidase. Details of electrophoresis are described in text. The rapidly moving, thermolabile α-galactosidase is labeled A. The more slowly moving, thermostable enzyme is labeled B. The A band is usually resolved into two bands, which may be designated A_1 (fast) and A_2 (slow).

four male mules were studied. All of the horses' leukocytes showed only the fast band of enzyme activity. The electrophoretic pattern observed in donkeys was somewhat more variable in that a rapid band, indistinguishable from the enzyme present in horse leukocytes, was present in each of the samples studied in addition to the slow band which is found only in donkeys. The proportion of the two bands differed considerably from animal to animal. Both bands were present in all of the male mules investigated, again indicating the contribution from the paternal parent (donkey), a finding contrary to sex-linkage. When leukocyte enzyme from a mule was heated at 45° C, both fast and slow components were thermolabile.

DISCUSSION

Fabry's disease is clearly a sex-linked disorder. The pattern of transmission in families [3] and the bimodality of enzyme activity in cloned cultured fibroblasts

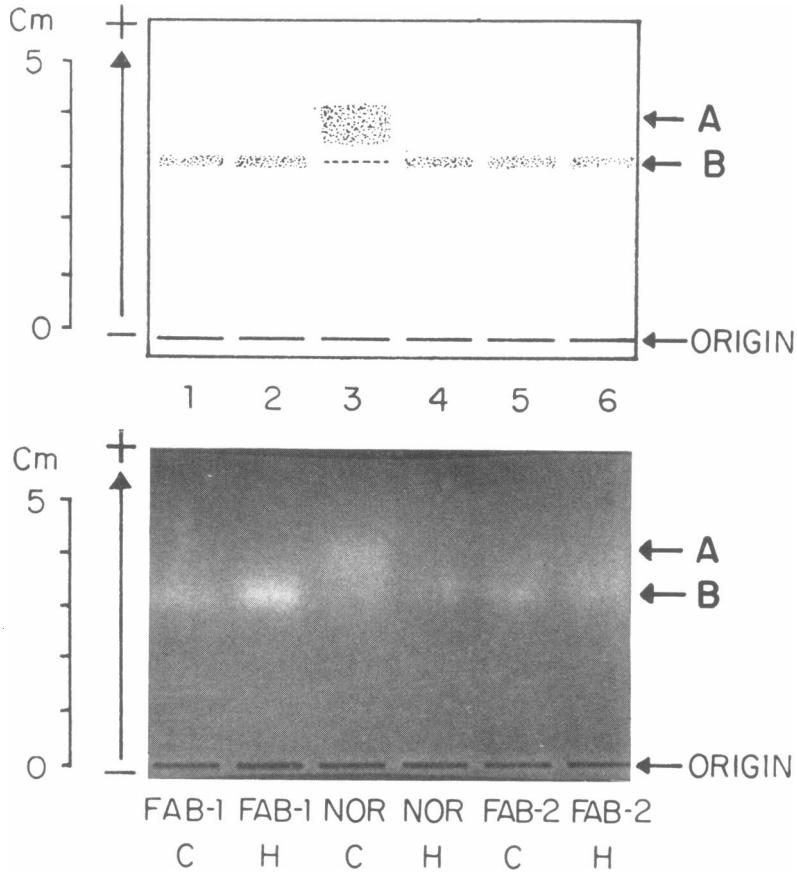

FIG. 5.—Starch gel electrophoresis, pH 7.0, of fibroblast α-galactosidase, untreated (control) and heated at 45° C for 2 hr, from a normal subject and two patients with Fabry's disease. Details of electrophoresis are given in the text, except that 20 mM 4-methylumbelliferyl-α-galactoside was used in staining. Channel 1: Fabry's disease fibroblasts (no. 1), unheated sample. Channel 2: Fabry's disease fibroblasts (no. 1), heated sample. Channel 3: Normal fibroblasts, unheated sample. Channel 4: Normal fibroblasts, heated sample. Channel 5: Fabry's disease fibroblasts (no. 2), unheated sample. Channel 6: Fabry's disease fibroblasts (no. 2), heated sample. Each channel except channel 4 received approximately 180 μunits of enzyme; channel 4 received only about 100 μunits. See legend to figure 4 for explanation of bands.

from heterozygotes [4] both clearly indicate that the gene which causes α-galactosidase deficiency in man is located on the X chromosome. The location of the structural gene for α-galactosidase has not been established, however, and the suggestion that normal plasma infusions may result in enzyme induction in patients with Fabry's disease [5, 6] suggested to us the possibility that Fabry's disease may represent a regulator mutation. The possibility that Fabry's disease is a regulatory mutation could be ruled out by demonstrating an abnormal residual enzyme protein. At first glance it seemed as if the α-galactosidase activity of leukocytes and fibroblasts of patients with Fabry's disease was abnormal with respect to thermal

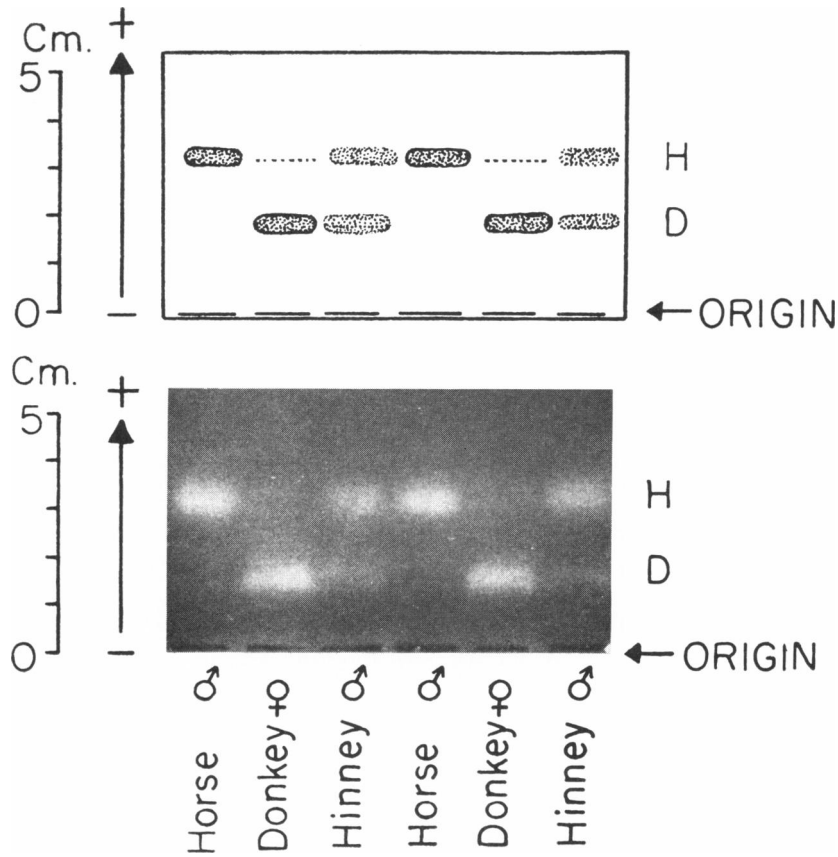

FIG. 6.—Starch gel electrophoresis at *p*H 7.0 of horse, donkey, and hinny white blood cell α-galactosidase. Details of electrophoresis are described in text.

stability, K_m, and electrophoretic mobility. However, our studies indicate that normal fibroblasts and leukocytes contain two isozymes of α-galactosidase, which have been designated α-galactosidase A and α-galactosidase B. The properties of these enzymes are summarized in table 1. The residual enzyme activity found in the leukocytes and fibroblasts of patients with Fabry's disease does not appear to represent an abnormal α-galactosidase A. Rather, it seems to represent a slightly increased quantity of the normal thermostable α-galactosidase isozyme, α-galactosidase B.

The terminal galactose residue of ceramide trihexoside is now known to be bound in the α configuration [9–11]. This finding is consistent with the fact that the enzymatic defect in Fabry's disease involves α-galactosidase activity. Since only α-galactosidase A, as measured with the umbelliferone derivative, is missing in Fabry's disease, and it has been shown that ceramide trihexosidase activity is virtually absent [12], it seems certain that α-galactosidase A has ceramide trihexosidase activity. However, not all α-galactosidases have the capacity to hydrolyze

TABLE 1

PROPERTIES OF α-GALACTOSIDASE A AND B FROM FIBROBLASTS AND LEUKOCYTES

	α-GALACTOSIDASE	
	A	B
Thermostability	Labile	Stable
K_m (4-methylumbelliferyl-α-galactoside)	4.9 mM	19.1 mM
Electrophoretic mobility	Rapidly anodal at *p*H 7.0	Slowly anodal at *p*H 7.0
Occurrence in normal fibroblasts and leukocytes	Present	Present
Occurrence in Fabry's disease fibroblasts and leukocytes	Not detectable	Present in slightly increased amount

this glycolipid. It is quite possible that the leukocyte and fibroblast α-galactosidase which is not affected in Fabry's disease, α-galactosidase B, may, like α-galactosidase from coffee bean [11] or *Moriterella vinacea* [9], lack the capacity to hydrolyze ceramide trihexoside. Further studies are required to define the activity of α-galactosidase A and α-galactosidase B with respect to ceramide trihexoside.

In many instances, attempts to establish the existence of regulatory mutations in man have been based merely on the fact that the residual enzyme protein in genetically deficient subjects appears to be normal. However, in most instances more detailed studies of residual protein revealed that what was believed to be a regulatory mutation was, in fact, a structural mutation. Thus, while our failure to demonstrate the presence of an abnormal enzyme in α-galactosidase deficiency (Fabry's disease) permits further consideration of the possibility that this disorder is a regulatory mutation, it is by no means proof that a regulator gene is involved. It is entirely possible, for example, that immunologic methods will reveal that in patients with Fabry's disease there is, indeed, an abnormal α-galactosidase A protein with little or no catalytic activity.

A more powerful approach in attempting to distinguish between mutations of regulatory genes and those of structural genes would be the demonstration of lack of linkage between the genetically determined deficiency of an enzyme and structural variability. Such an approach has been used by Ohno and his co-workers [13–15] in his elegant study of an X-linked regulator gene which in mice modifies the activity of autosomally inherited alcohol dehydrogenase and certain other enzymes. In searching for regulatory mutations in man, the use of X-linked markers seems to be most promising because of the scarcity of other well-defined linkage groups and the relative ease of distinguishing autosomal from sex-linked genes by pedigree analysis. Thus Fabry's disease is the type of disorder in which it might be possible to obtain strong evidence for a mutation involving a regulatory locus. Since α-galactosidase deficiency (Fabry's disease) is known to be sex-linked, demonstration that the structural locus for α-galactosidase is autosomal would indicate that Fabry's disease is not due to a structural gene mutation. The only

exception to this would be the possibility that α-galactosidase had both sex-linked and autosomally determined subunits. We do not know of the existence of any such enzyme.

The development of an electrophoretic method for the study of peripheral blood leukocyte α-galactosidase made it possible to study a population of human subjects. Although 225 individuals were examined, no clearly identifiable structural variant was found. Interspecific hybrids have been found to be very useful in studying the X-chromosomal linkage group. When localization of the gene has been possible through either the existence of polymorphisms within the species or the use of interspecific hybrids, genes known to be X-linked in man have been found, without exception, to be X-linked in other mammals [16]. It is significant, therefore, that α-galactosidase is autosomally inherited in Equidae. Male hinnies and male mules were both found to inherit paternal enzyme (horse and donkey, respectively). These results suggest that the gene for α-galactosidase may not be X-linked in man, but exceptions to the stability of the X-chromosomal linkage group among mammals may exist, and this may be one. Further evidence regarding the possible sex-linkage of α-galactosidase in humans might be obtained by studying interspecific cell hybrids. Preliminary studies in the mouse indicate that in the system we have described it would be difficult to distinguish the mouse enzyme from the human enzyme by electrophoresis.

SUMMARY

Alpha-galactosidase activity has been investigated in fibroblasts and peripheral blood leukocytes from normal subjects, from three subjects with Fabry's disease, and from horses, donkeys, mules, and hinnies. As reported previously, leukocytes and fibroblasts of patients with Fabry's disease were deficient in α-galactosidase activity. Two types of α-galactosidase activity were found in fibroblasts and leukocytes. A thermolabile, electrophoretically fast component with a low K_m was designated as α-galactosidase A. A slower, thermostable component with a higher K_m was designated as α-galactosidase B. The residual enzyme in the cells of patients with Fabry's disease contained no detectable α-galactosidase A activity, and the residual activity was identical, in all respects studied, with that of α-galactosidase B from normal cells. It is concluded that Fabry's disease is due to α-galactosidase A deficiency. No electrophoretic variants of α-galactosidase were found in the leukocytes of 225 human subjects.

Electrophoretic mobility of horse α-galactosidase was found to differ markedly from that of donkey α-galactosidase. Interspecific hybrids were examined, and it was found that the paternal type of enzyme was present in male mules and male hinnies. This clearly indicates that the structural gene for α-galactosidase is autosomal in Equidae.

It was not possible to obtain any clear evidence regarding X-linkage of the structural gene for α-galactosidase in the genome of humans.

ACKNOWLEDGMENTS

The authors thank Drs. Henry Nadler, John O'Brien, Giovanni Romeo, and Barbara Migeon for supplying us with fibroblast cultures from patients with Fabry's disease and Pompe's disease, and Drs. John Melnyk, Robert Dexter, and Bart Baker for making available to us blood samples from horses, donkeys, and hinnies.

Addendum

We have recently found that the electrophoretic pattern of thermolabile α-galactosidase from various normal human tissues, such as liver, spleen, kidney, placenta, heart, and intestinal mucosa, all differ from one another. Indeed, even the patterns from normal human leukocytes and fibroblasts seem to be different. Because of the recent suggestion that acid hydrolase isozymes may differ from one another by virtue of different numbers of neuraminic acid residues (Goldstone et al., *FEBS Letters* 13:68–72, 1971), we treated extracts prepared from various human tissues with *Clostridium perfringens* neuraminidase. This resulted in a decrease in the electrophoretic mobility of α-galactosidase from all tissues studied except for small intestine mucosa. Although the bands of activity obtained after neuraminidase treatment of normal tissues were very similar to one another, some differences were noted. This implies that carbohydrate residues other than neuraminic acid may also influence the electrophoretic mobility of α-galactosidase.

In contrast with its effect on human leukocyte α-galactosidase, neuraminidase treatment had no effect on the α-galactosidase of horse and donkey leukocytes. Nonetheless, the fact that the electrophoretic mobility of thermolabile α-galactosidase is strongly dependent upon secondary factors must be considered in interpreting our results on horse and donkey enzyme. It is interesting, in this respect, that Hamerton et al. (*Nature* 232:312–315, 1971) have recently reported inability to distinguish between horse and donkey enzyme prepared from fibroblasts. Thus, it may be that the autosomal factor which determines electrophoretic mobility of horse and donkey α-galactosidase is not the structural locus for α-galactosidase itself but rather a glycosyl transferase enzyme which modifies the electrophoretic mobility of horse and donkey leukocyte α-galactosidase.

The effect of neuraminidase on thermostable α-galactosidase (α-galactosidase B) is much more difficult to determine, since the amount of this enzyme is relatively small. A partially purified α-galactosidase B containing no α-galactosidase A has been prepared from placenta by ammonium sulfate fractionation and DEAE chromatography. The electrophoretic mobility of this enzyme is not altered by neuraminidase, nor is its thermostability affected by treatment with this enzyme.

REFERENCES

1. KINT JA: Fabry's disease: alpha-galactosidase deficiency. *Science* 167:1268–1269, 1970

2. BRADY RO: Genetics and the sphingolipidoses. *Med Clin N Amer* 53:827–839, 1969
3. SWEELEY CC, KLIONSKY B: Glycolipid lipoidosis: Fabry's disease, in *The Metabolic Basis of Inherited Disease*, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, New York, McGraw-Hill, 1966, pp 618–634
4. ROMEO G, MIGEON BR: Genetic inactivation of the α-galactosidase locus in carriers of Fabry's disease. *Science* 170:180–181, 1970
5. MAPES CA, ANDERSON RL, SWEELEY CC, et al: Enzyme replacement in Fabry's disease, an inborn error of metabolism. *Science* 169:987–989, 1970
6. KRIVIT W, DESNICK RJ, MAPES C, et al: Recent advances in Fabry's disease. *Trans Ass Amer Physicians* 83:121–132, 1970
7. LOWRY OH, ROSEBROUGH NY, FARR AL, et al: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
8. SMITHIES O: An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. *Biochem J* 71:585–587, 1959
9. HAKOMORI S, SIDDIQUI B: Anomeric structures of globoside and ceramide trihexoside of human erythrocytes and hamster fibroblasts. *J Biol Chem* 246:2271–2277, 1971
10. HANDA S, ARIGA T, MIYATAKE T, et al: Presence of α-anomeric glycosidic configuration in the glycolipids accumulated in kidney with Fabry's disease. *J Biochem* (Tokyo) 69:625–627, 1971
11. BENSAUDE I, CALLAHAN J, PHILIPPART M: Fabry's disease as an α-galactosidosis: evidence for an α-configuration in trihexosyl ceramide. *Biochem Biophys Res Commun* 43:913–918, 1971
12. BRADY RO, GAL AE, BRADLEY RM, et al: Enzymatic defect in Fabry's disease. *New Eng J Med* 276:1163, 1967
13. OHNO S, LYON MF: X-linked testicular feminization in the mouse as a non-inducible regulatory mutation of the Jacob-Monod type. *Clin Genet* 1:121–127, 1970
14. OHNO S, DOFUKU R, TETTENBORN U: More about X-linked testicular feminization of the mouse as a non-inducible (i^s) mutation of a regulatory locus: 5α-androstan-3α-17β-diol as the true inducer of kidney alcohol dehydrogenase and β-glucuronidase. *Clin Genet* 2:128–140, 1971
15. DOFUKU R, TETTENBORN U, OHNO S: A testosterone-regulon in the mouse kidney: an X-linked non-inducible mutation of the presumptive regulatory locus and presumptive operator mutation of the β-glucuronidase locus. *Nature New Biol* 232: 5–7, 1971
16. OHNO S: *Sex Chromosomes and Sex-Linked Genes*. Berlin, Springer-Verlag, 1967