Am J Hum Genet 38:137–148, 1986

Galactosialidosis: Molecular Heterogeneity among Distinct Clinical Phenotypes

SILVIA PALMERI,[1,2] ANDRÉ T. HOOGEVEEN,[1] FRANS W. VERHEIJEN,[1] AND HANS GALJAARD[1]

SUMMARY

The lysosomal storage disorder galactosialidosis has been recognized as a distinct genetic and biochemical entity, associated with a combined β-galactosidase and neuraminidase deficiency that is due to the lack of a 32-kilodalton (kDa) glycoprotein. The molecular basis of different clinical variants of galactosialidosis has been investigated.

In the early-infantile form, the synthesis of the 52-kDa precursor of the 32-kDa "protective protein" is markedly reduced and the absence of the latter protein explains the severe neuraminidase deficiency. In the juvenile-adult form, there is relatively more 52-kDa precursor but no 32-kDa protein can be detected.

Cells from the late-infantile form have in comparison with controls, besides a small amount of the 32-kDa glycoprotein, an accumulation of the 52-kDa precursor. Apparently, this protein is genetically altered in such a way that its further processing is impaired. Furthermore, in this mutant, the residual neuraminidase activity is stimulated four- to sixfold upon leupeptin treatment together with an increase of the 32-kDa glycoprotein.

INTRODUCTION

During the last few years, about 30 patients with galactosialidosis have been described (for reviews, see [1–3]). Somatic cell hybridization studies have shown that this autosomal recessive disease, associated with a combined defi-

Received April 15, 1985; revised June 24, 1985.

This work was supported in part by the University of Siena and the Council of Clinical Genetics Rotterdam.

[1] Department of Cell Biology and Genetics, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

[2] On leave from Department of Neurological Sciences, Prof. G. C. Guazzi and Prof. A. Federico, University of Siena, Italy.

© 1986 by the American Society of Human Genetics. All rights reserved. 0002-9297/86/3802-0003$02.00

ciency of lysosomal β-galactosidase and neuraminidase, is a genetic entity distinct from G_{M1} -gangliosidosis and sialidosis [4].

D'Azzo et al. [5] elucidated the responsible molecular defect in galactosialidosis that appears to be a lack of a 32-kDa glycoprotein, subsequently shown to be required for the aggregation of β-galactosidase monomers [6]. This conformation was found to be essential to protect the 64-kDa β-galactosidase monomers against intralysosomal proteolytic degradation. Also, the 32-kDa protective protein is essential for the activation of neuraminidase that forms a complex with β-galactosidase [7, 8].

As in most other lysosomal storage diseases, different clinical phenotypes of galactosialidosis have been recognized. Patients with an early-infantile form, showing severe edema, ascites, skeletal dysplasia, and ocular abnormalities, die shortly after birth [9, 10]. In the late-infantile form, patients present symptoms after 6–12 months and the main features are dysmorphism, dysostosis, and skeletal dysplasia, visceromegaly, macular cherry-red spot, and mild mental retardation [3, 11, 12]. The largest number of patients, mainly of Japanese origin, are those with the juvenile/adult form [2, 13, 14], where symptoms have appeared between infancy and adulthood. The major features are skeletal dysplasia, dysmorphism, corneal clouding, cherry-red spot, angiokeratoma, neurological manifestations, and mental retardation.

In our present studies on the molecular background of these different clinical phenotypes, we found marked differences in the biosynthesis of the 32-kDa "protective protein." These findings also explain the discrepancy in earlier studies on the effect of leupeptin on the residual neuraminidase activity in galactosialidosis [12, 14, 15].

MATERIALS AND METHODS

Cell Culture

Fibroblasts from normal individuals and patients with an early-infantile form [9] and a juvenile/adult form of galactosialidosis [16] were derived from the Rotterdam Cell Repository (Prof. M. F. Niermeijer). Cells from patients with the late-infantile form were provided by Dr. G. Andria [3] and Dr. L. Pinsky [11]. Early passages from all cell strains were used and were maintained in Ham's F-10 medium (Flow Laboratories, McLean, Va.) supplemented with antibiotics and 10% fetal calf serum.

Radioactive Labeling, Immunoprecipitation, and Electrophoresis

One week prior to radioactive labeling, the medium was replaced by Dulbecco's modification of Eagle's medium (Flow Laboratories) supplemented with 10% fetal calf serum and antibiotics and cells were grown in 25-cm^2 falcon flasks. One hour prior to labeling, this medium was replaced by Dulbecco's medium free of leucine, fetal calf serum, and antibiotics.

Biosynthetic labeling was performed using the same medium plus 2% of dialyzed fetal calf serum and 0.15 ml of L [4-5^{3}H]leucine (0.15 mCi; 135 Ci mmol; Amersham Radiochemical Centre, England). After 48 hrs, cells were harvested and comparable amounts of cell material, based on the protein content, were immunoprecipitated. A polyclonal antiserum raised against β-galactosidase purified from human placenta was used [5]. Since the enzyme exists in a complex with its 32-kDa "protective protein," the antiserum reacts with β-galactosidase, its protective protein, and their precursor forms

[6]. Polyacrylamide gel electrophoresis, in the presence of sodium dodecyl sulfate, was performed according to Laemmli [17] with minor modifications. The radioactive bands were visualized by fluorography and exposure for 1 week at −70°C using X-Omat R film (Eastman Kodak, Rochester, N.Y.). The [^{14}C]methyl-labeled protein molecular weight standards used were: phosphorylase B, 92,500; bovine serum albumine, 69,000; ovalbumin, 46,000; and carbonic anhydrase, 30,000 (Amersham Radiochemical).

To study the effect of inhibition of lysosomal proteases, leupeptin, at a final concentration of 0.02 mM, was added to the medium. In addition to immunoprecipitation studies, as described above, the activities of β-galactosidase and neuraminidase were measured 4–5 days after leupeptin administration using 4-methylumbelliferyl substrates according to Galjaard [18].

For the pulse-chase study, five 25-cm^2 flasks were prepared for every strain, and after labeling for 30 min, the cells were subjected to chase with fresh unlabeled Dulbecco's modification of Eagle's medium for 0, 15, 30, 60, and 120 min, respectively.

Analysis in the Medium of NH_4Cl-induced Secretions

The cells were prepared as described above, but at the moment of labeling, the medium was supplemented with 10 mM NH_4Cl for 48 hrs. Then, the medium was collected and prepared for immunoprecipitation as in previous studies [5, 19]. For the study of glycoprotein phosphorylation, 200 μCi $^{32}P_i$-carrier free was added to a 25-cm^2 Falcon flask, in the presence of NH_4Cl, and the medium was prepared as described [20].

Uptake studies. Confluent fibroblasts from the late-infantile galactosialidosis form were maintained in 75-cm^2 Falcon flasks for 48 hrs in Dulbecco's modification of Eagle's medium containing [^{3}H]leucine and NH_4Cl. The medium from two falcon flasks was collected, and the (precursor) glycoproteins secreted by the cells were precipitated with $(NH_4)_2SO_4$. The pellet was dissolved in 400 μl water and desalted on a small column of Sephadex G_{50} that was equilibrated with phosphate-buffered saline [21]. Subsequently, the sample was added to 5 ml Ham's F-10 medium, which was then added to control fibroblasts in a 25-cm^2 falcon flask and incubated for 48 hrs. After harvesting, immunoprecipitation studies were performed. Reversely, the same procedure was carried out with medium containing secretions from control cells, which was added to mutant cells.

RESULTS

Biosynthesis in Normal and Mutant Cells

Immunoprecipitation studies were performed with conventional antiserum reacting with β-galactosidase, protective protein, and their precursor forms. The results for normal fibroblasts and cells derived from the three clinical phenotypes of galactosialidosis are shown in figure 1. In all cell lines, the 85-kDa precursor of β-galactosidase is synthesized, but in the three mutant lines, the amount of mature 64-kDa β-galactosidase is decreased compared with the control (fig. 1*A*). In cells from both patients with the late-infantile form of galactosialidosis (fig. 1*B* and *E*), there is a large amount of a 52-kDa protein and a band at the 32-kDa position. In the two other mutant cell types, no 32-kDa band is visible and the amount of 52-kDa protein is less than in the control or in the late-infantile form. The juvenile/adult form (fig. 1*D*) shows more 52-kDa protein than the early-infantile form (fig. 1*C*).

The pulse-chase experiments, illustrated in figure 2, indicate that the 52-kDa protein is the precursor of the 32-kDa protective protein. In control fibroblasts, this precursor is visible after 30-min labeling and the 32-kDa band appears after

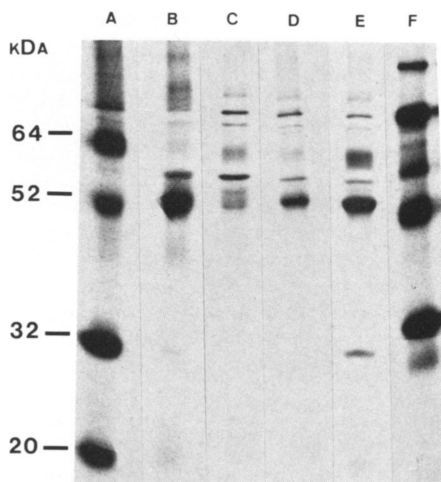

FIG. 1.—Immunoprecipitation of β-galactosidase and 32-kDa protective protein and their precursors in normal and mutant fibroblasts, after [^{3}H]leucine labeling for 48 hrs followed by SDS-PAGE. *Lane A:* control; *lane B:* late-infantile galactosialidosis (Andria et al. [3]); *lane C:* early-infantile form; *lane D:* juvenile/adult form; *lane E:* late-infantile form (Pinsky et al. [11]); *lane F:* standards.

15-min chase; after 60-min chase, the 52-kDa band has disappeared whereas the 32-kDa protective protein remains visible.

In cells from the late-infantile form of galactosialidosis, the 52-kDa precursor remains during the whole chase period and no 32-kDa band appears. These results suggest that the processing of 52-kDa precursor into 32-kDa protective protein is impaired. In the early-infantile form, hardly any 52-kDa precursor is detectable, and in the juvenile/adult form, the amount of precursor is somewhat higher. The 32-kDa protective protein is detectable in neither of these two mutants.

Secretion and Uptake of Precursor

Immunoprecipitation studies on medium above [^{3}H]leucine-labeled cells after NH_4Cl stimulation show equal amounts of the secreted 88-kDa precursor of β-galactosidase in normal and mutant cells (fig. 3*A–D*). Medium above control cells also contains the 54-kDa precursor of the protective protein (fig. 3*A*). This precursor can also be demonstrated in the medium above late-infantile galactosialidosis cells (fig. 3*B*), although much less than in controls. No 54-kDa protein band is seen in the medium above the two other mutant cell types (fig. 3*C* and *D*). Labeling with $^{32}P_i$-carrier free showed that the secreted precursors in the medium are all phosphorylated (results not shown).

To investigate whether the 54-kDa precursor secreted by the late-infantile galactosialidosis cells can be processed into 32-kDa protective protein, the following experiment was carried out. Medium above [^{3}H]leucine-labeled mutant cells was collected after 48 hrs treatment with NH_4Cl (see MATERIALS AND METHODS) and added to control fibroblasts. Immunoprecipitation studies of these latter cells were performed after 48 hrs, and the results are shown in

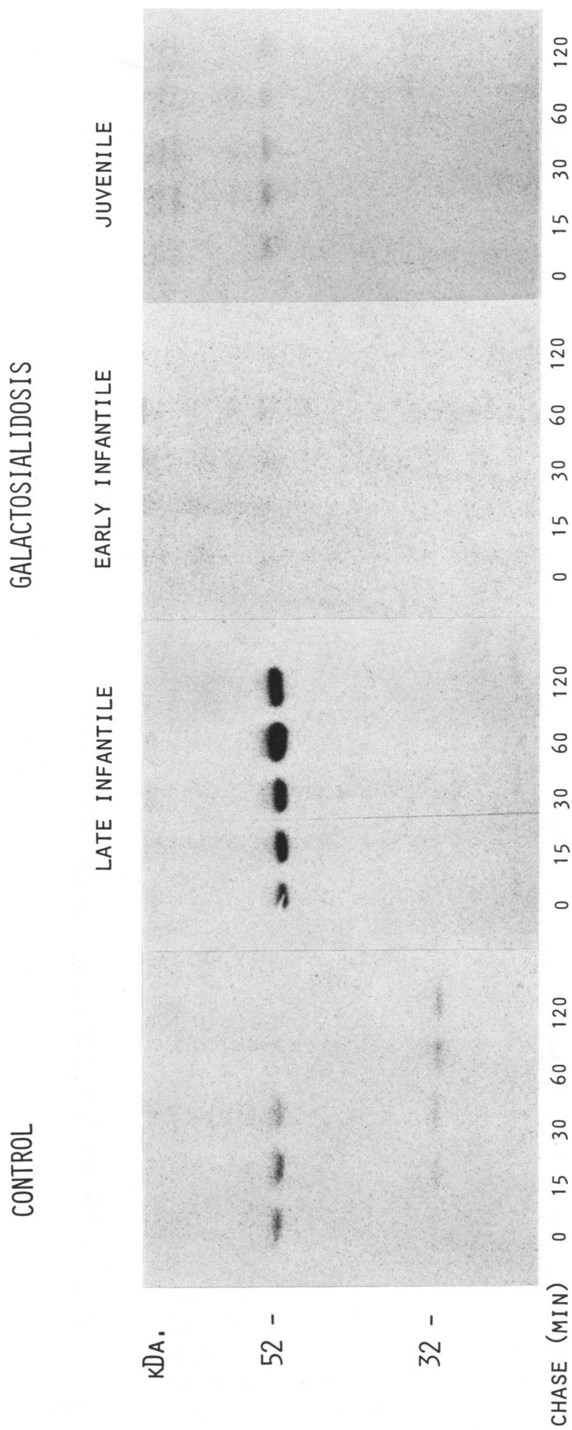

FIG. 2.—Pulse labeling of normal and different mutant fibroblasts (30 min), followed by different periods of chase

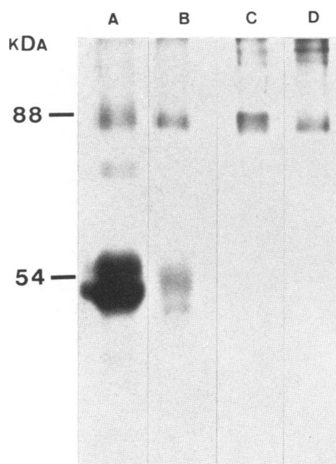

FIG. 3.—Immunoprecipitation of secreted precursor glycoproteins in the medium above normal and mutant fibroblasts after NH_4Cl treatment in the presence of [^{3}H]leucine. *Lane A:* medium above control cells; *lane B:* medium above cells from late-infantile galactosialidosis; *lane C:* medium above cells from early-infantile form; *lane D:* medium above cells from juvenile/adult galactosialidosis.

figure 4*A*. It is clear that the 54-kDa precursor secreted by the mutant cells has been taken up by the control fibroblasts but only a very faint 32-kDa band is visible.

In the reverse experiment (fig. 4*B*), the 54-kDa precursor secreted by normal cells is taken up by the mutant cells and is rapidly processed into 32-kDa protective protein; also, a clear 20-kDa band appears, which is derived either from the 54-kDa or the 32-kDa protein. These results indicate that the cells from late-infantile galactosialidosis are capable of processing normal 54-kDa precursor. The uptake experiments also show that the mutation in this clinical phenotype alters the 54-kDa precursor in such a way that its processing is impaired.

Inhibition of Intralysosomal Proteolytic Degradation

Since the β-galactosidase deficiency in galactosialidosis is due to enhanced intralysosomal proteolytic degradation [5, 14, 15], we investigated the effect of inhibition of lysosomal cathepsins by leupeptin in the different clinical phenotypes of galactosialidosis. The results in table 1 show that the activity of β-galactosidase increases three- to sevenfold in the three mutant cell types after 4 days treatment with leupeptin. The effect on neuraminidase activity, however, is different among the mutants. Leupeptin leads only to an increase of neuraminidase activity in the late-infantile form.

To study the molecular background of this effect, we performed immunoprecipitation studies after leupeptin treatment (fig. 5). In the late-infantile form of galactosialidosis, leupeptin treatment results in an increased amount of the 85-kDa precursor of β-galactosidase (as in controls) and to an increased amount

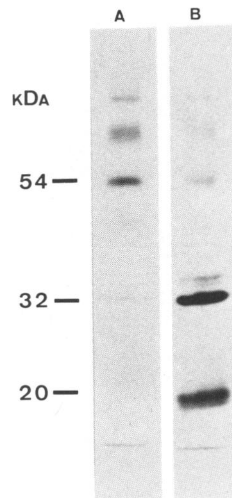

FIG. 4.—Uptake of radiolabeled glycoprotein precursors secreted into the medium. *Lane A:* uptake and processing of secreted precursors from late-infantile mutant fibroblasts by control cells; *lane B:* uptake from control fibroblasts by late-infantile galactosialidosis cells.

of the 32-kDa protective protein. The latter effect was not seen in the two other mutant cell lines (results not shown).

DISCUSSION

The accumulation of knowledge about the biosynthesis of human β-galactosidase [5], its different forms [6, 22, 23], and its relationship with lysosomal neuraminidase [7, 8, 24] made our present study on the molecular background of different clinical variants of galactosialidosis feasible.

The results of immunoprecipitation studies with conventional antiserum reacting with β-galactosidase and its 32-kDa protective protein show that in all three types of galactosialidoses there is little 64-kDa β-galactosidase monomer. This is reflected in the 10% residual enzyme activity found in a variety of cells in these patients [1–3, 23, 25]. Previous studies [5] indicated that the reduced amount of 64-kDa β-galactosidase is due to enhanced degradation of the monomeric form of the enzyme. In the lysosomes of normal cells, β-galactosidase exists for at least 80% as a high molecular weight aggregate [6, 22, 23], and, more recently, it was demonstrated that the 32-kDa "protective protein" is needed for this aggregation process [6].

The results of our present study show that the intracellular 52-kDa protein is the precursor form of this 32-kDa "protective protein" and a 20-kD protein that is present in minor amounts in the high molecular weight aggregate and that probably is a degradation product. The amount of the 52-kDa precursor varies markedly among the different clinical phenotypes (figs. 1 and 2). In the late-infantile form of galactosialidosis [3, 11], the 52-kDa band is even stronger than in control cells but the amount of 32-kDa "protective protein" is much smaller. In the early-infantile form [9], there is very little 52-kDa precursor, and in the

TABLE 1

EFFECT OF LEUPEPTIN ON THE ACTIVITY OF β-GALACTOSIDASE AND NEURAMINIDASE IN CONTROL CELLS AND IN DIFFERENT TYPES OF GALACTOSIALIDOSIS

TYPE OF CELLS	β-GALACTOSIDASE ACTIVITY		NEURAMINIDASE ACTIVITY	
	$^{-}$Leupeptin	$^{+}$Leupeptin	$^{-}$Leupeptin	$^{+}$Leupeptin
Early-infantile galactosialidosis [9]	33.3	207	0.4	0.6
Late-infantile galactosialidosis [3]	27.0	181	2.6	9.0
Late-infantile galactosialidosis [11]	73.0	357	1.2	7.1
Juvenile/adult galactosialidosis [16]	38.6	132	0.5	0.8
Control	706 (Range 350–1,050)	675	106 (Range 40–130)	149

NOTE: Enzymatic activities are expressed in nmol/hr/mg protein. Each value is the mean of three or four separate experiments.

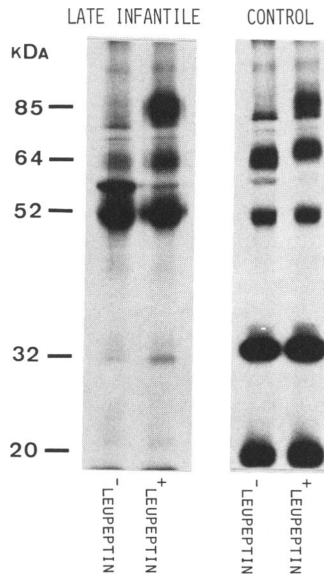

FIG. 5.—Immunoprecipitation of β-galactosidase and 32-kDa protective protein in fibroblasts from controls and from a patient with the late-infantile form of galactosialidosis; [^{3}H]leucine labeled for 4 days with and without leupeptin.

juvenile/adult form [13], only some more is present. No 32-kDa protein can be detected in both forms.

In the pulse-chase experiment, the labeling period is too short to demonstrate the 85-kDa precursor of β-galactosidase and the 32-kDa protective protein in the late-infantile form of galactosialidosis, but these bands are visible after 48 hrs labeling. Precursor forms secreted into the medium after NH_4Cl stimulation show a 2,000–3,000 higher molecular mass than the intracellular forms, an observation made earlier for other lysosomal proteins [19].

The immunoprecipitation studies of the medium after NH_4Cl stimulation indicate equal amounts of secreted 88-kDa precursor β-galactosidase in normal and mutant cells, which points to a normal synthesis of this protein in all forms of galactosialidosis. The secreted 54-kDa precursor of the protective protein is seen only in medium above controls and above cells from the late-infantile form. The discrepancy between the large amount of 52-kDa precursor intracellularly in this mutant and the smaller amount (compared with control) of the secreted 54-kDa precursor suggests that the mutation interferes with normal intracellular routing from the Golgi to the lysosome so that NH_4Cl does not effectively stimulate secretion.

The uptake studies (fig. 4) prove that 54-kDa precursor secreted by normal cells can be processed into 32-kDa "protective protein" by cells from the late-infantile form of galactosialidosis. Consequently, the mechanism needed for this processing is not affected in the mutant. On the other hand, the 54-kDa precursor secreted by the mutant cells is hardly processed after uptake by normal cells. This indicates that the gene mutation in this clinical phenotype

affects the 54-kDa precursor protein in such a way that it cannot be effectively processed into functional 32-kDa protective protein. This might be related to aberrant intracellular routing.

The residual amount of 32-kDa protective protein in the late-infantile form explains the discrepancy between previous studies on the effect of leupeptin. After addition of this inhibitor of proteolytic degradation by cathepsins, our group found in galactosialidosis cells a marked increase of the activities of both β-galactosidase and neuraminidase [15]. Others [14], however, did not find an effect of leupeptin on neuraminidase activity. The explanation is the use of different mutant cell types in these studies. In fibroblasts from the juvenile/adult form, studied by Suzuki et al. [14], or in cells of a similar patient reported earlier by Loonen et al. [16], there is no effect of leupeptin on the neuraminidase activity (see also table 1), which seems to be related to the absence of 32-kDa protective protein in these patients. A four- to sixfold increase of neuraminidase activity occurs, however, in the two patients with the late-infantile form, both of which also have residual 32-kDa protective protein. Recent experiments [8] explain this relationship on a molecular basis, because the 32-kDa protective protein appears to be an essential subunit for lysosomal neuraminidase activity.

Our present study provides a molecular basis for some differences among patients with galactosialidosis. The three main clinical forms have in common a 10%–15% residual β-galactosidase activity that is based on the monomeric form of the enzyme. The differences between the clinical phenotypes must be due to differences in lysosomal neuraminidase activity and in the 32-kDa protective protein and its precursor. In the late-infantile form, there is a normal synthesis of 52-kDa precursor of the protective protein but its processing is impaired. The small amount of 32-kDa protein is sufficient for some 2% residual neuraminidase activity, which might be sufficient to explain the relatively mild clinical course [3].

In the early-infantile form, the mutation results in a marked reduction of the amount of 52-kDa precursor and no 32-kDa protein, essential for neuraminidase activity, can be detected. The pulse-chase experiments suggest a reduced rate of synthesis rather than an enhanced degradation of the precursor.

In the juvenile/adult form, there is relatively more 52-kDa precursor than in the early-infantile form, but the amount of 32-kDa protective protein is below detection level even after 48 hrs labeling. We do not yet know whether the 52-kDa precursor may play any functional role, nor can we as yet relate the molecular observations and the clinical features in the juvenile/adult form. It should, however, be mentioned that these molecular studies are limited to fibroblasts and defective processing might have fewer consequences in other cells and organs. Also, the neuraminidase activities have been measured with artificial substrates under in vitro conditions. It may well be that the differences between the early-infantile form and the juvenile/adult forms are based on small differences in residual neuraminidase activity toward their natural substrates under in vivo conditions [26].

ACKNOWLEDGMENTS

We thank Dr. L. Pinsky (McGill University, Montreal Children's Hospital Research Institute), Dr. J. A. Lowden (Hospital for Sick Children, Toronto), Dr. G. Andria (Dept. of Pediatrics, Naples), and Prof. M. F. Niermeijer (Dept. of Clinical Genetics, University Hospital, Rotterdam) for providing the mutant cell strains used in this study. The illustrations were kindly made by Mr. T. van Os.

REFERENCES

1. LOWDEN JA, O'BRIEN JS: Sialidosis: a review of human neuraminidase deficiency. *Am J Hum Genet* 31:1–18, 1979
2. SUZUKI Y, SAKURABA H, YAMANAKA T, ET AL.: Galactosialidosis: a comparative study of clinical and biochemical data on 22 patients, in *The Developing Brain and Its Disorders,* edited by ARIMA M, Tokyo, Univ. of Tokyo Press, 1984, pp 161–175
3. ANDRIA G, STRISCIUGLIO P, PONTARELLI G, SLY SW, DODSON WE: Infantile neuraminidase and β-galactosidase deficiencies (galactosialidosis) with mild clinical courses, in *Perspectives in Inherited Metabolic Diseases,* vol 4, Milan, Ermes, 1981, pp 379–395
4. GALJAARD H, REUSER AJJ: Genetic aspects of lysosomal storage diseases, in *Lysosomes in Biology and Pathology,* edited by DINGLE JT, DEAN RT, SLY W, Amsterdam/New York, Elsevier, 1984, pp 315–345
5. D'AZZO A, HOOGEVEEN AT, REUSER AJJ, ROBINSON D, GALJAARD H: Molecular defect in combined β-galactosidase and neuraminidase deficiency in man. *Proc Natl Acad Sci USA* 79:4535–4539, 1982
6. HOOGEVEEN AT, VERHEIJEN FW, GALJAARD H: The relation between human lysosomal β-galactosidase and its protective protein. *J Biol Chem* 255:4937–4945, 1980
7. VERHEIJEN FW, BROSSMER R, GALJAARD H: Purification of acid β-galactosidase and acid neuraminidase from bovine testis: evidence for an enzyme complex. *Biochem Biophys Res Commun* 108:868–875, 1982
8. VERHEIJEN FW, PALMERI S, HOOGEVEEN AT, GALJAARD H: Human placental neuraminidase: activation, stabilization and association with β-galactosidase and its 'protective' protein. *Eur J Biochem* 149:315–321, 1985
9. KLEIJER WJ, HOOGEVEEN A, VERHEIJEN FW, ET AL.: Prenatal diagnosis of sialidosis with combined β-galactosidase and neuraminidase deficiency. *Clin Genet* 16:60–61, 1979
10. GRAVEL RA, LOWDEN JA, CALLAHAN JW, WOLFE LS, NG YIN KU NMK: Infantile sialidosis: a phenocopy of type 1 G_{M1} gangliosidosis distinguished by genetic complementation and urinary oligosaccharides. *Am J Hum Genet* 31:669–679, 1979
11. PINSKY L, MILLER J, SHANFIELD B, WALTERS G, WOLFE LS: G_{M1}-gangliosidosis in skin fibroblast culture: enzymatic difference between types 1 and 2 and observation on a third variant. *Am J Hum Genet* 26:563–577, 1974
12. STRISCIUGLIO P, CREEK KE, SLY W: Complementation, cross reaction, and drug correction studies of combined β-galactosidase neuraminidase deficiency in human fibroblasts. *Pediatr Res* 18:167–171, 1984
13. LOONEN MCB, VAN DER LUGT L, FRANKE LC: Angiokeratoma corporis diffusum and lysosomal enzyme deficiency. *Lancet* 2:785, 1974
14. SUZUKI Y, SAKARABA H, YAMANAKA T, KO Y-M, OKAMURA Y: Galactosialidosis (β-galactosidase-neuraminidase deficiency): a new hereditary metabolic disease with abnormal degradation of enzyme molecules. *Acta Paediatr Jpn* 25:31–37, 1983
15. GALJAARD H, HOOGEVEEN A, VERHEIJEN F, ET AL.: Relationship between clinical, biochemical and genetic heterogeneity in sialidase deficiency, in *Perspectives in Inherited Metabolic Diseases,* vol 4, Milan, Ermes, 1981, pp 317–333

16. LOONEN MCB, REUSER AJJ, VISSER P, ARTS WFM: Combined sialidase (neuraminidase) and β-galactosidase deficiency. Clinical, morphological and enzymological observations in a patient. *Clin Genet* 26:139–149, 1984
17. LAEMMLI UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680–685, 1970
18. GALJAARD H: *Genetic Metabolic Diseases: Early Diagnosis and Prenatal Analysis.* Amsterdam, New York, Elsevier/North-Holland, 1980
19. HASILIK A, NEUFELD EF: Biosynthesis of lysosomal enzymes in fibroblasts. Synthesis as precursors of higher molecular weight. *J Biol Chem* 255:4937–4945, 1980
20. HASILIK A, NEUFELD EF: Biosynthesis of lysosomal enzymes in fibroblasts. Phosphorylation of mannose residues. *J Biol Chem* 255:4946–4950, 1980
21. PENEFSKY HS: Reversible binding of Pi by beef heart mitochondrial adenosine triphosphatase. *J Biol Chem* 252:2891–2899, 1977
22. NORDEN AGW, TENNANT LL, O'BRIEN JS: G_{MI}-ganglioside β-galactosidase A. Purification and studies of the enzyme from human liver. *J Biol Chem* 249:7969–7976, 1974
23. O'BRIEN JS: The gangliosidoses, in *The Metabolic Basis of Inherited Disease,* 5th ed, edited by STANBURY JB, WYNGAARDEN JB, FREDRICKSON DS, GOLDSTEIN JL, BROWN MS, 1983, pp 945–969
24. WENGER DA, TARBY TY, WHARTON C: Macular cherry red spots and myoclonus with dementia: coexistent neuraminidase and β-galactosidase deficiencies. *Biochem Biophys Res Commun* 82:589–595, 1978
25. GALJAARD H, D'AZZO A, HOOGEVEEN A, VERHEIJEN F: Combined β-galactosidase-sialidase deficiency in man: genetic defect of a "protective protein," in *Molecular Basis of Lysosomal Storage Disorders,* New York, Academic Press, 1984, pp 113–131
26. CONZELMAN E, SANDHOFF K: Partial enzyme deficiencies: residual activities and the development of neurological disorders. *Dev Neurosci* 6:58–71, 1983–84