Ultrastructure of the eye in fetal type II glycogenosis (Pompe's disease)

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Type II glycogenosis is an autosomal recessive storage disease characterized by absence of the enzyme acid α-1,4-glucosidase. The eye of a 16 week fetus, aborted after diagnosis by amniocentesis, was studied by light and electron microscopy. Extensive deposits of lysosomal and cytoplasmic glycogen were present in virtually all ocular tissues examined, with the notable exception of pigment epithelia (iris and retina). The massive glycogen deposits present in this, the youngest case thus far examined histologically, emphasize the involvement of the fetus from its earliest stages and the importance of prenatal diagnosis. (INVEST OPHTHALMOL VIS SCI 22: 25-31, 1982.)

Key words: Pompe's disease, glycogenosis, lysosomal storage disease, silver proteinate, glycogen deposition, acid α-1,4-glucosidase, ocular involvement, fetus

Pompe's disease (type II glycogenosis) is an autosomal recessive, lysosomal storage disease resulting from the deficient activity of the enzyme acid α-1,4-glucosidase (acid maltase). The enzymatic defect leads to an accumulation of cellular glycogen, primarily lysosomal. Although heart and skeletal muscle usually show the most marked pathologic and clinical involvement, glycogen deposition is also evident in virtually every tissue of the body, including brain, liver, pancreas, kidney, skin, and eye.

Three major clinical subtypes of Pompe's disease have been identified: (1) an infantile form, characterized by severe muscle involvement and cardiomegaly, leading to death from cardiorespiratory failure during the first few months of life; (2) a late infantile (or juvenile) form, similar in nature but more slowly progressive—the patients usually dying during childhood; and (3) an adult form, presenting primarily with muscle weakness and characterized by a partial deficiency of acid α-1,4-glucosidase activity. Fetuses with any form of the disease may be diagnosed prenatally. Ultrastructural findings of each form of Pompe's have been reported. Ocular findings in Pompe's disease are those of widespread glycogen deposits.

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This investigation was supported in part by National Institutes of Health grant RR71 of the Clinical Research Centers Program of the Division of Research Resources and grants EY-01867 and GM-25279, by an unrestricted grant from Research to Prevent Blindness, Inc., N.Y., by the National Society to Prevent Blindness, Inc., N.Y., and by grant 1-273 from the National Foundation March of Dimes. Dr. Ritch is the recipient of an N.I.H. Academic Investigator Award EY-00133. Dr. Desnick is the recipient of an N.I.H. Research Career Development Award L-K04-AM 00451.
Submitted for publication Jan. 18, 1980.
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Fig. 1. Micrograph showing basal epithelium of conjunctiva with membrane-delimited accumulations of glycogen (large arrows) and free, cytoplasmic glycogen (small arrows). Architecture of cells appears normal except for these deposits. N, Nucleus; Bm, basement membrane. (Uranyl-lead stain; ×9239.)

We report the ultrastructural findings from the eye of a fetus that was selectively aborted after a 16 week gestation, thus providing information on the early pattern of ocular involvement in this inborn error of metabolism.

Case report

The fetus was the result of a second pregnancy of a woman whose first child died at 2 years of age from enzymatically documented type II glycogenosis. The parents received genetic counseling concerning the risk of this disease in a subsequent child. Fetal diagnosis of Pompe's disease was made by the demonstration of deficient acid glucosidase activity in cultured amniotic cells. The parents requested termination of the pregnancy, which was done at 16 weeks gestation by dilation and extraction.

Materials and methods

Fixation of the eyes for light and electron microscopy was accomplished by use of a trialdehyde fixative containing 1% each of glutaraldehyde, acrolein, and paraformaldehyde in 0.1M sodium cacodylate buffer with 5 mM CaCl₂, pH 7.4, for a minimum of 1 hr in the cold. Light microscopy. After fixation, tissue was processed routinely, embedded in paraffin, and cut at 8 μm. Sections were then stained with periodic acid–Schiff (PAS), Masson's trichrome, or hematoxylin-eosin (H & E). Diastase sensitivity was tested on sections later stained with PAS, with controls.

Electron microscopy. After initial fixation, tissue was rinsed in 0.1M cacodylate buffer containing 0.5% NaCl, it was then postfixed for 1 hr in the cold in 1% osmium tetroxide in 0.1M cacodylate buffer, pH 7.4, dehydrated through a graded ethanol series to propylene oxide, and embedded in Epon. Thick (1 μm) plastic sections were stained for metachromasia and orientation with...
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Fig. 3. Micrograph showing glycogen-filled lysosomes in conjunctival fibroblast. Glycogen particles can be seen bridging the gap through an opening in the membrane. Arrows show cytoplasmic particles of glycogen. (Silver proteinate stain; ×23,600.)

azure II-methylene blue or toluidine blue. Thin sections were stained with uranyl and lead. To enhance ultrastructural localization of glycogen, a modification of the periodic acid-thiocarbohydrazide-silver proteinate reaction was performed, in which thin sections were supported on gold grids and treated for 20 min with 1% periodic acid, after which time they were floated on the thiocarbohydrazide reagent for 1, 12, 24, 48, and 72 hr. This was followed by staining with silver proteinate for 30 min.

Results

Light microscopy. Generalized light histopathologic study revealed development of the eye compatible with 16 week gestation. PAS-positive granules were digested by diastase in sections of extraocular muscle. They were thus identified as glycogen.

Electron microscopy. Glycogen particles (β-type) were observed by routine contrast stains in almost all of the ocular tissue examined (Figs. 1, 5, 8, and 10), including conjunctiva, cornea, iris, retina, choroid, and extraocular muscle. The affinity of the particles for silver proteinate confirmed their identification as glycogen. The glycogen granules were found primarily in membrane-delimited, vacuolar inclusion bodies of various dimensions (from 0.25 to 2 μm or more in length) (Figs. 3, 6, 7, and 10). In addition, large amounts of glycogen were dispersed cytoplasmically (Figs. 1, 2, 5, and 8). The fine structure of the fetal ocular tissue appeared normal in other respects. Some of the inclusion bodies were only partially filled with glycogen, the remainder appearing homogeneously electron-dense (Figs. 2 and 4). In many areas the membrane-delimited sacs were confluent, having barely discernible boundaries (Fig. 7). Conjunctival basal

Fig. 4. Micrograph showing conjunctival fibroblast with glycogen-filled lysosomes (large arrow). Note intermediate stage of lysosomal involvement (small arrows). G, Glycogen; N, nucleus. (Silver proteinate stain; ×9199.)

Fig. 5. Micrograph showing rhabdomyoblast of developing extraocular muscle. There are massive accumulations of cytoplasmic glycogen interspersed among the fibrillar bundles. Arrows show lysosomes that contain glycogen particles. N, Nucleus. (Uranyl-lead stain; ×3682.)
Fig. 6. Micrograph showing rhabdomyoblast of developing extraocular muscle. Massive lysosomal and cytoplasmic deposits of glycogen are present. Large arrow indicates areas of possible rupture of lysosomal membrane leading to confluence of inclusions and dense deposits of glycogen granules. G, Glycogen; N, nucleus; f, fibrils. (Uranyl-lead stain; x6879.)

epithelium and fibroblasts showed marked involvement (Figs. 1 and 4), as did corneal epithelium and keratocytes in the stroma and rhabdomyoblasts of developing extraocular muscle (Figs. 5 and 6). Extraocular muscle showed the greatest concentration of membrane-bound, glycogen-filled inclusions. Cytoplasmic deposits were also found between the latticelike myofibrils. Some cell types in extraocular muscle were difficult to identify because of massive glycogen accumulation and confluence of the inclusion bodies (Fig. 7). Retina displayed glycogen storage, with the remarkable exception of retinal pigment epithelium (Figs. 9 and 10). Developing iris was involved in deposition, although no inclusions were seen in iris pigment epithelium. Capillary endothelium and nerve of various tissues showed some involvement, with sparser involvement of nerve.

Discussion

Prenatal diagnosis, through amniocentesis and enzyme analysis of cultured amniotic cells, now permits the early detection of a number of inborn errors of metabolism. In the present case the total absence of acid α-1,4-glucosidase activity in cultured amniotic cells was diagnostic of Pompe’s disease, and the pregnancy was terminated.

Light and electron microscopic histopathologic findings confirmed the diagnosis. Glycogen deposition, both within membrane-delimited vacuoles and free in the cytoplasm, was consistent with ultrastructural studies on cultured amniotic cells in Pompe’s disease. The positive reaction to silver proteinate, which allows ultrastructural visualization of aldehyde-containing polysaccharides, confirmed the nature of the deposits.

The membrane-bound inclusions were typical of secondary lysosomes or residual bodies and were similar to the lesions usually seen in Pompe’s. Their appearance in virtually all of the tissue that was examined, indicated widespread storage, even at so early a gestational stage. Although fetal tissue is normally glycogen-rich, the carbohydrate is typically found as free particles throughout the cytoplasm. In Pompe’s disease, glycogen is characteristically localized within lysosomes; it is also found in unusually large amounts in the cytoplasm.
Extraocular muscle was the most heavily involved tissue, as indicated by the enormity and confluence of the glycogen-engorged lysosomes. Comparison of random electron micrographs of this fetus with those reported by Libert et al.¹⁶ indicates as much, if not more, glycogen in this 16 week fetus as that in the 22 week one. Skeletal muscle is usually heavily involved in all forms of the disease,³,¹¹,¹⁵,¹⁶ and the massive deposits seen in extraocular muscle here may be responsible for the apparent rupture of lysosomal membranes, resulting in large, confluent, glycogen-engorged bodies. Massive accumulations between myofibrils may be the result of lysosomal saturation, leaving no place for excess glycogen to be stored. Glycogen-degrading enzymes in the cytoplasm are apparently not sufficient to handle the breakdown of excess glycogen so displaced. Accumulations of excess glycogen have been noted in skeletal muscle in the juvenile²¹ and lethal¹⁸ forms.

With the exception of pigment epithelium of iris and retina, all of the tissues examined, including conjunctiva, cornea, iris, extraocular muscle, retina, and choroid, had massive glycogen deposition. The lack of deposition in retinal pigment epithelium is in agreement with the results of Libert et al.¹⁶ in their report of a 22 week fetus and with the results of Goebel et al.,²⁰ who reported preferential involvement of photoreceptor inner segments and inner granular ganglionic cells in a 9-month-old infant with the disease. The lack of glycogen inclusions in iris pigment epithelium is a new finding. The reasons for the apparent absence of glycogen in these pigment epithelia (iris and retina) are unclear and are perhaps caused by some functional difference in lysosomal enzymes of pigment epithelia over other cell types. The involvement of retinal pigment epithelium in phagocytosis may have to do with its lack of glycogen storage. Perhaps the turnover is so rapid in pigment epithelia that the substrate simply does not have time to accumulate. Or it may
simply be caused by an as yet undeveloped system.

The observation of partially filled lysosomes suggests intermediate stages in glycogen segregation within the enzyme-deficient lysosomes. Although acid α-glucosidase normally hydrolyses lysosomally trapped glycogen into oligosaccharides and glucose, no account can thus far be made for the fact that greater-than-normal amounts of glycogen are disseminated throughout the cytoplasm in this disease. Studies on muscle led to the speculation that this was due to the fact that myofibrillar glycogen amassed early in the disease process, penetrating the lysosomes later on. This would explain the presence of some cytoplasmic glycogen. Further overdis-
tension of glycogen-engorged vacuoles could cause disruption of the lysosomes, providing another mechanism for the accumulation of glycogen in the cytoplasmic pool. The accumulation of free cytoplasmic glycogen has also led to speculations that an enzyme other than acid α-1,4-glucosidase is involved, and some investigators presented evidence of decreased neutral maltase activity in the infantile and late onset forms.

Abnormal, glycogen-engorged lysosomes indicative of Pompe’s disease are already formed at this early stage of development, as shown by the extensive involvement of ocular tissue. The marked involvement of the conjunctiva confirms the value of conjunctival biopsy in the diagnosis of inborn errors of metabolism.

We thank Laurie Raisher, Joseph Suhani, and Michalina Lokietko for their excellent and cheerful technical assistance, and we gratefully acknowledge Stemma Askinazi for typing the manuscript.

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