Ocular ultrastructural study in a fetus with type II glycogenosis

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SUMMARY The general pathological and ocular studies in an aborted fetus with type II glycogenosis revealed the widespread lysosomal storage of glycogen. Obvious lesions are observed in the viscera, in the skeletal and ocular muscles, and in all ocular tissues except the pigment epithelium of the retina. Brain and heart are relatively spared. Conjunctival and skin biopsies have a diagnostic importance, since specific alterations are evident early in the course of the disease.

Type II glycogenosis, an autosomal recessive disorder, is characterised by a lack of α -1,4-glucosidase (acid maltase) (Hers, 1963) resulting in the lysosomal storage of glycogen in various tissues (Baudhuin *et al.*, 1964). The muscles are mainly involved, and their abnormal contractile functions explain the symptoms of hypotonia, respiratory insufficiency, cardiomegaly, and cardiac failure leading to death within the first 2 years of life in the classical infantile form (Pompe disease). Glycogen storage has also been demonstrated in the liver, the kidney, the skin, and the nervous system in these cases (Hers and de Barsey, 1973). Less severe phenotypes have been reported, with childhood and adult onset (Engel *et al.*, 1973).

The disorder can be detected in utero by the enzymatic study of cultured amniotic cells. Subsequent therapeutic abortion permitted the investigation of the early biochemical and histopathological changes (Hug *et al.*, 1973; Nadler and Messina, 1969; Schaub *et al.*, 1974).

Toussaint and Danis (1965) observed by light microscopy the widespread storage of glycogen in the eyes of an infant with Pompe disease. Ultrastructural changes of the ocular muscles have been studied by Smith and Reinecke (1972), but the ocular lesions have not, to our knowledge, been reported by electron microscopy.

The purpose of this paper is to describe the lysosomal storage of glycogen in most of the ocular tissues in an aborted fetus with acid maltase deficiency. The presence of abnormal glycogen deposits

within the conjunctival cells is important for the diagnosis of the disease by conjunctival biopsy, which was demonstrated in an unrelated case.

Case reports

CASE 1

This female fetus resulted from the fourth pregnancy of unrelated parents. Their first child died at 2 days of age because of oesophageal atresia. Their second child, a boy, presented a type II glycogenosis with prolonged survival to the age of 4 years 4 months. Pathological and biochemical findings have been previously reported (Martin *et al.*, 1976). Their third pregnancy ended with a spontaneous abortion, but the fetus was biochemically unaffected, with normal α -1,4-glucosidase activity.

During the 15th week of the fourth gestation a diagnostic amniocentesis was performed. Alpha-1,4-glucosidase activity was reduced to 1% of the normal range in the cultured amniotic cells. At the parents' request a therapeutic abortion utilising prostaglandin was performed at 22 weeks of gestation.

CASE 2

This male infant, the first child of unrelated parents, was admitted to hospital at the age of 3½ months because of muscular weakness, respiratory insufficiency, and heart failure. The diagnosis of classical infantile Pompe disease was based on the clinical picture and on enzymatic, biochemical, and histopathological studies of a muscle biopsy (Dr de Barsey). A conjunctival biopsy was performed at the age of 4 months. At this time the eyes were clinically normal.

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Fig. 1 *Biceps femoris muscle. A large membrane-bound glycogen vacuole (V) occupies the centre of a muscle fibre (uranyl acetate, lead citrate. $\times 15\ 000$)*

Material and methods

The fetal tissues were obtained within 1 hour of abortion. The eyes, the nervous system, the visceral organs, and the skeletal muscles were fixed and processed for light and electron microscopy according to previously reported methods (Libert *et al.*, 1975; Martin *et al.*, 1973). The conjunctival biopsy of the second case was obtained under topical

anaesthesia from the upper bulbar conjunctiva and prepared for electron microscopy by the same technique. After orientation of the specimens on semithin sections, ultrathin sections were cut with a diamond knife on a Reichert OmU 2 microtome, stained with uranyl acetate and lead citrate (Reynolds, 1963) or with silver proteinate after periodic acid oxidation (Thiéry, 1967), and observed with a Philips EM300 electron microscope. Compari-

Fig. 2 *Liver. Numerous glycogen-filled vacuoles (arrow) are present in a hepatocyte (uranyl acetate, lead citrate. $\times 15\ 000$)*

son was made with 3 normal fetuses aborted at similar gestation ages.

Results

AFFECTED FETUS

(a) General pathological examination (case 1): Lysosomal storage of glycogen was demonstrated

Fig. 3 Cornea. The corneal endothelial cells contain free glycogen granules and storage lysosomes, which are markedly stained with silver (silver proteinate. $\times 10\,000$)

both by histochemical and by ultrastructural methods in four different skeletal muscles (Fig. 1) (quadriceps, triceps suralis, biceps femoris, and biceps brachii), in the liver (Fig. 2), in the skin (fibroblasts, endothelial cells, Schwann cells), in the peripheral nerves (Schwann cells of myelinated and unmyelinated axons), and in the kidneys (distal convoluted tubules). The heart and central nervous system were relatively spared: a few glycogen inclusions were found in myocardial fibres and in neurons of the anterior horns and of the mesencephalic root of the trigeminal nerve. The other neuronal groups were devoid of any glycogen storage. Only the ganglion cells of the spinal ganglia were loaded with glycogen.

(b) Ocular examination: Glycogen could be identified by light microscopy in the corneal epithelium and endothelium, in the lens epithelium, in the ciliary epithelium, and in the endothelium of the capillaries. The fetal retina seemed to be normal, though immature as in controls. The fibres of the oculomotor muscles contained many glycogen granules, but the general architecture was apparently conserved. These granules did not appear in salivary amylase-treated preparations.

In addition to confirming the lesions observed by light microscopy the ultrastructural studies demonstrated a more generalised storage process. Almost all examined cells displayed 0.2 to 1.5 μm membrane-bound inclusions, which contained innumerable small glycogen particles, moderately contrasted by routine techniques, and markedly stained by silver proteinate.

Fig. 4 Choroid. Several glycogen engorged inclusions are evident in the endothelial cell of a choroidal capillary (uranyl acetate, lead citrate. $\times 22\,000$)

Fig. 5 Retina. The immature photoreceptors contain some inclusions (I). The mitochondria are particularly numerous in this portion of the cell, near the external limiting membrane (arrows) (uranyl acetate, lead citrate. $\times 25\,000$)

Fig. 6 Optic nerve. The lysosomal glycogen (arrows) can be more or less contrasted by the routine staining technique in the same structure or even in the same cell, as in this glial cell (uranyl acetate, lead citrate. $\times 34\,000$)

These inclusions were observed in the conjunctival and corneal epithelial cells, in the keratocytes, the sclerocytes, the corneal endothelial cells (Fig. 3), the fibroblasts of the conjunctiva, iris, and the choroid, in the capillary endothelial cells of the conjunctiva, the uvea, and the retina (Fig. 4), in the ganglion cells, the Müller cells, and the photoreceptors (Fig. 5). The glial cells of the optic nerve were also involved (Fig. 6). In contrast, the retinal pigment epithelium was unaffected.

The fibres of the extrinsic ocular muscles contained a variable number of storage inclusions. They were sometimes packed closely together and displayed a marked tendency to confluence (Fig. 7). Their dimensions varied from 0.2 to 5 μm . Some fibres were much disorganised, stuffed with membrane-bound vacuoles and large areas of apparently free glycogen deposits in which disrupted membrane residues were recognisable.

CONTROL FETUS

Isolated glycogen granules were observed in the ocular fetal cells. These particles were particularly numerous in the corneal endothelium, but they were lying free in the cytoplasm and never included within a unit membrane.

CONJUNCTIVAL BIOPSY

In the conjunctival biopsy of case 2 at 4 months of age the glycogen inclusions had the same ultrastructural aspect and dimension as in the fetal eyes, but they were much more numerous. The epithelial cells, the fibroblasts (Fig. 8), and the blood capillary endothelial cells were considerably distended by the storage vacuoles. The Schwann cells of the sensory nerves were also involved, while the axons and the myelin sheaths were normal.

Discussion

Alpha-1,4-glucosidase deficiency was demonstrated on cultured amniotic cells of a high-risk pregnancy

Fig. 7 Ocular muscles. The lysosomes (arrows) show a marked tendency to confluence and sometimes occupy large areas of the cytoplasm (asterisk) (uranyl acetate, lead citrate. $\times 16\,000$)

and resulted in the therapeutic abortion of a fetus with type II glycogenosis. This biochemical technique is now widely used for the prenatal diagnosis of lysosomal diseases and allowed histopathological and biochemical analysis of affected fetal tissues (Adachi *et al.*, 1974; Schneider *et al.*, 1972), including the eyes (Howes *et al.*, 1975). In our case these investigations indicated the early lysosomal storage of glycogen in skeletal muscles, liver, Schwann cells of peripheral nerves, skin, kidney, and to a much less extent in heart and central nervous system, strictly paralleling, although at a much earlier age,

our previous findings in the affected sibling thought to represent a childhood phenotype rather than a classical example of Pompe disease (Martin *et al.*, 1976). It can be hypothesised that the relative sparing of heart and central nervous system accounted for the prolonged survival of the index case.

Although no ocular abnormalities except strabismus (Smith and Reinecke, 1972) have been reported clinically, the eyes are markedly involved in type II glycogenosis. Toussaint and Danis (1965) demonstrated on necropsy material the accumulation of glycogen in the mural cells of the retinal capillaries,

Fig. 8 Conjunctiva. The fibroblasts of this conjunctival biopsy of case 2 display numerous and characteristic glycogen-engorged inclusions (uranyl acetate, lead citrate. $\times 28\,000$)

in the ganglion cells, and in the smooth and striated muscle fibres of the eyes. Our light microscopic studies of an aborted fetus also suggest a widespread storage process. However, this might be a false conclusion because the fetal eye tissues are physiologically rich in glycogen, as shown in the controls.

Electron microscopy demonstrates clearly the generalised lesions. All ocular cell types, except the pigment epithelium of the retina, display membrane-bound inclusions which contain innumerable granules packed close together. The ultrastructural aspect of the inclusions and their staining properties with silver proteinate are characteristic of glycogen-engorged lysosomes, while the glycogen particles of the control fetal cells lie free and disseminated in the cytoplasm, and the lysosomes have the normal appearance of very small dense bodies.

The storage is much more important in the oculomotor muscles, in which the inclusions reach enormous size and sometimes disorganise the general architecture of the cells. Extensive areas of free glycogen particles containing residual membrane fragments suggest mechanical rupture of the distended lysosomes. However, it seems impossible to determine whether it was the result of an ante- or post-mortem process. These findings are consistent with ultrastructural studies on muscle biopsy and necropsy specimens in the brother of the present case (Martin *et al.*, 1976) and in other reported patients (Engel *et al.*, 1973; Martin *et al.*, 1973).

Smith and Reinecke (1972) examined by electron

microscopy the inferior oblique muscle of a patient with type II glycogenosis. The tissues appeared less severely involved with deposits of free glycogen in generally well preserved muscle fibres, but the case seemed to belong to a milder clinical phenotype.

In case 2 the presence of many glycogen-engorged lysosomes in the conjunctival epithelial cells, fibroblasts, and capillary endothelial cells confirmed the diagnosis of type II glycogenosis by conjunctival biopsy. Skin biopsy also showed specific alterations in this disease (Martin *et al.*, 1976). These morphological diagnoses are certainly possible just after birth, as the tissues are already affected in the fetus.

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