Type III A Glycogenosis
A Biochemical and Ultrastructural Study*

FRANCO MINIO PALUELLO, CARMELO B. BRUNI** and HERMINA SPIELE
Istituto di Patologia Medica, Università di Roma, Rome, Italy

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Summary. A combined biochemical and ultrastructural study, including cytochemical and negative staining techniques, has been made of three tissues, liver, muscle and leucocytes, in a case of type IIIA glycogenosis.

The electron microscopic studies revealed an increased accumulation of glycogen in the liver, in the skeletal muscle and in the leucocytes.

The hepatic glycogen, either isolated or within the hepatocytes, is mostly represented in the form of monoparticulate granules and of rosettes with fewer component units, whereas the typical rosettes are rare. The isolated and intracellular glycogen of muscle cells appears composed of monoparticulate granules, as in normal muscle cells.

The biochemical studies confirm the diagnosis of type IIIA glycogenosis and seem to exclude the simultaneous occurrence of the enzymatic defect responsible for type II glycogenosis.

Type III glycogenosis (CORN, 1957) or limit dextrinosi is a glycogen storage disease characterized by the congenital absence of the debranching enzyme, amylol-1,6-glucosidase (ILLINGWORTH et al., 1956). In its most frequent type, limit dextrinosis A, a complete lack of the enzyme occurs in both liver and muscle (HERS, et al., 1964). Amylo-1,6-glucosidase is absent also in leucocytes of patients affected by such disease (WILLIAMS et al., 1963). Biochemical studies (ILLINGWORTH and BROWN, 1964) revealed that in type III glycogenosis the polysaccharide has shorter outer chains and a higher end-group percentage than normal.

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** Present address: Istituto di Patologia Generale, Università di Napoli, Naples, Italy. Dr. MINIO PALUELLO and Dr. BRUNI are fellows of Consiglio Nazionale delle Ricerche.
glycogen. Previous studies (SALOMON, et al., 1961; HUG et al., 1966) of patients affected by type III glycogenosis are mainly concerned with the ultrastructural changes in the liver and in the muscle.

This report deals not only with the ultrastructure of liver, skeletal muscle and leucocytes in a patient affected by type IIIA glycogenosis, but also includes cytochemistry, and negative staining of the glycogen particles. Glycogen, amylo-1,6-glucosidase and acid and neutral maltase activities were evaluated in homogenates of liver and muscle. The acid and neutral maltase activities were studied according to ILLINGWORTH (1961) observation of the possible simultaneous occurrence of type II and type III glycogenosis.

The fine structure of intracellular and isolated glycogen particles was studied in order to investigate a possible relationship between the biochemical and morphologic changes.

**Materials and Methods**

**Clinical Data**

In the patient, an 11-years-old-boy, the first manifestations of the disease date back to the age of 1 year and 4 months when muscular weakness, hypoglycemic shocks and hepatomegaly became evident. During his last admission in November 1966 surgical biopsies of the liver and of the muscle rectus abdominis were performed under total anesthesia. The tissues obtained were employed for the present study.

**Morphologic Techniques**

Fragments of tissues were partly fixed in 2% osmium tetroxide for 90' and partly fixed in 2.5% glutaraldehyde for 2 hours with and without osmium postfixation for 30'. Both fixatives were buffered at pH 7.2 according to MILLONI (1961). The tissue blocks were embedded in Araldite (Durcupan ACM). Heparinated blood samples were centrifuged at 200 r.p.m. for 10'. The supernatant, containing leucocytes and platelets, was partly fixed in osmium tetroxide for 30' and partly in glutaraldehyde for 60' with osmium postfixation for 15' and embedded in Araldite. Thick sections (0.5—1 μ) were stained with toluidine blue (TRUMP et al., 1961) and observed in the light microscope. Thin sections were stained with lead hydroxide (KARNOVSKY, 1961), with uranyl acetate and lead hydroxide, and with 10% phosphotungstic acid (PTA) (WATSON, 1958).

Other fragments of liver and muscle were immediately frozen in a carbon dioxide bath, kept at —20° and then processed according to ORBELL and BEEDING (1958) for the glycogen extraction. For "negative staining" (DUBCHANS, 1962) a distilled water suspension of isolated glycogen was mixed with an equal volume of 2% PTA adjusted to pH 7.2 with 1 N KOH, and then collected on carbon coated grids.

**Biochemical Techniques**

For determination of the glycogen content in fragments of liver and muscle, the tissues were immediately frozen after biopsy and then weighed and thawed. Samples of 100 mg were minced with scissors and homogenized in a mortar with distilled water, the final concentration being 5% w/v. The glycogen content of the homogenates was measured with the anthrone method (SEIFTEl et al., 1950) as modified by HERS (1964). The values of glycogen concentration were expressed as per cent of the weight of the frozen tissue. The glycogen of the leucocytes was extracted and measured according to WILLIAMS and FIELD (1961) and to the "negative staining" (DUBCHANS, 1962) a distilled water suspension of isolated glycogen was mixed with an equal volume of 2% PTA adjusted to pH 7.2 with 1 N KOH, and then collected on carbon coated grids.