Amniotic cell 4-methylumbelliferyl- α -glucosidase activity for prenatal diagnosis of Pompe's disease

Summary. Using a simple fluorometric assay for α -glucosidase activity of cultured amniotic cells, we have monitored two pregnancies from families at risk for Pompe's disease. The fetus was judged to be affected in one, the pregnancy being terminated and unaffected in the other. The accuracy of these predictions was confirmed. These results suggest that this assay allows accurate prenatal diagnosis of Pompe's disease, three weeks after diagnostic amniocentesis.

Pompe's disease (glycogenosis type 2) is an autosomal recessive disorder presenting with muscular hypotonia and cardiac failure in infancy leading to early death. There is glycogen accumulation in all tissues, which has been attributed to deficiency of lysosomal α 1,4-glucosidase (α -glucosidase) (Hers, 1963). The disorder has been detected *in utero* by demonstration of amniotic cell α -glucosidase deficiency. Two methods have been used for the enzyme assay, both of which rely on measurement of glucose released from maltose (Nadler and Messina, 1969; Hug, Schubert, and Soukup, 1970). A complication of such methods is that commercial preparations of maltose may be contaminated by free glucose and require purification before they can be used as substrates for α -glucosidase assay (Messer and Dahlqvist, 1966; Benson *et al*, 1972). We have, therefore, investigated the possibility of using the simpler assay (Salafsky and Nadler, 1973) which depends on fluorometric measurement of the aglycone moiety released from the synthetic substrate 4-methylumbelliferyl- α -D-glucoside (MU- α -glu). This assay was reported to be effective for detection of α -glucosidase deficiency of liver and cultured

fibroblasts from a patient with Pompe's disease, but not of the kidney (Salafsky and Nadler, 1973). However, deficiency of the kidney enzyme can be demonstrated when the assay is performed using the natural substrate-glycogen (Brown, Brown, and Jeffrey, 1970). Although 4-methylumbelliferyl- α -glucosidase (MU- α -glucosidase) activity has been reported in amniotic cells from normal pregnancies (Salafsky and Nadler, 1973; Galjaard *et al*, 1973), in view of this tissue specificity towards different substrates, no confident prediction can be made as to the behaviour of this enzyme in amniotic cells derived from pregnancies where the fetus has Pompe's disease. We, therefore, report our experiences in two pregnancies in families at risk for Pompe's disease which we monitored using both the glucose release and fluorometric assays.

Methods and Patients

Amniotic cells were collected at amniocentesis and cultured as described previously (Fensom, Benson, and Blunt, 1974) for case 1 and for controls. For case 2 culture was initially in Eagles medium supplemented with 20% fetal calf serum, the cells being transferred to TC 199 medium supplemented with 20% human serum before assay. The latter medium was also used for culture of fetal fibroblasts.

Enzyme assay

Amniotic cells were detached from the culture vessels using a solution of edetic acid (0.02%) in Dulbecco A phosphate-buffered saline (Oxoid), washed twice with saline (0.9%), and resuspended (about 5×10^6 cells/ml) in water containing triton X-100 (0.0025%). The cells were disrupted at 4°C using a M.S.E. 150 W ultrasonic disintegrator; two 30-s bursts with a 30-s interval between the bursts were used with the instrument set to low power, amplitude 4. The sonicate was centrifuged for 15 minutes at 2600 *g* and the supernatant fluid taken for enzyme assays. MU- α -glucosidase activity was measured using a reaction mixture containing 10 μ l. 0.5M acetate buffer pH 4.0, 50 μ l 2mM MU- α -glu. (Koch Light) and cell supernatant containing 50 to 150 μ g protein (measured by the method of Lowry *et al*, 1951) in a final volume of 120 μ l. The reaction mixtures were incubated for periods up to 3 hours and the reaction was stopped by addition of 1.5 ml.

0.5M glycine-NaOH buffer, pH 10.7. Fluorescence was measured in a Perkin Elmer MPF3 spectrofluorometer with an excitation wavelength of 360nm and an emission wavelength of 448 nm. α -Glucosidase activity was also measured using maltose as substrate (Benson *et al*, 1972).

Patients

Using these methods, we have monitored two pregnancies in different mothers, both of whom had previously been delivered of infants who had died with proved Pompe's disease.

Case 1. Amniocentesis was carried out at 15 weeks' gestation. Amniotic cells were cultured and assayed after 5 weeks and 2 days. MU- α -glucosidase activity was 4.9% of the control mean of 19.58 nmol of 4-methylumbelliferone released/h per mg protein (units) (control range 10.68 to 33.00 units). The assay was repeated on amniotic cells cultured for a further week when the value was 3.2% of the control mean. Using maltose as substrate, no amniotic cell α -glucosidase activity could be demonstrated. The fetus was judged to be affected and at the request of the parents the pregnancy was terminated at 23 weeks' gestation. The diagnosis of Pompe's disease was confirmed by showing a striking deficiency of 4-MU- α -glucosidase activity in the fetal liver. α -Glucosidase activity in cultured fetal fibroblasts was 1.29 units towards MU- α -glu. (control of same gestation; 10.56 units) and absent towards maltose.

Case 2. Amniocentesis was carried out at 12 weeks' gestation. After 7 weeks of culture, amniotic cells were found to have normal α -glucosidase activity towards both MU- α -glu. (19.80 units) and maltose (93.3 nmol maltose cleaved/h per mg protein), the fetus being judged to be unaffected. This was confirmed by the absence of clinical features of Pompe's disease at 12 months of age.

Conclusion

Our results suggest that the simple fluorometric assay for α -glucosidase activity on amniotic cells collected at amniocentesis and cultured for about three weeks allows accurate prenatal diagnosis of Pompe's disease.

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Addendum

Since this article was written, correct prenatal diagnosis of Pompe's disease has been reported in two other fetuses using 4-methylumbellifer- α -D-glucoside as enzyme substrate (Niermeijer *et al*, 1975; Schaub *et al*, 1974).

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Leucocyte values of α -L-iduronidase activity in mucopolysaccharidosis I*

Summary. Assay of α -L-iduronidase in peripheral leucocytes is a rapid and simple diagnostic aid in mucopolysaccharidosis I. The mean value for heterozygotes is one-half the value of normal controls, but overlap between the

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