0.5*M* glycine-NaOH buffer, *p*H 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-methylumbelliferl-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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two groups occurs. Use of this assay may be helpful in genetic counselling of selected couples.

Mucopolysaccharidosis I has been classified on clinical grounds into three subtypes: MPS-IH (Hurler syndrome), MPS-IS (Scheie syndrome), and MPS-IH/S (Hurler-Scheie compound) (McKusick *et al*, 1972). The deficient lysosomal enzyme in MPS-I is α-L-iduronidase (Bach *et al*, 1972; Matalon, Cifonelli, and Dorfman, 1971). The three subtypes are indistinguishable by enzyme assay and radioactive sulphate kinetics in cultured fibroblasts and quantitative analysis of mucopolysaccharidiuria.

This report describes the results of α-L-iduronidase assays on peripheral leucocytes of affected individuals with each of the three MPS-I subtypes, obligate heterozygotes, normal controls, and homozygotes and heterozygotes of other lysosomal storage disorders.

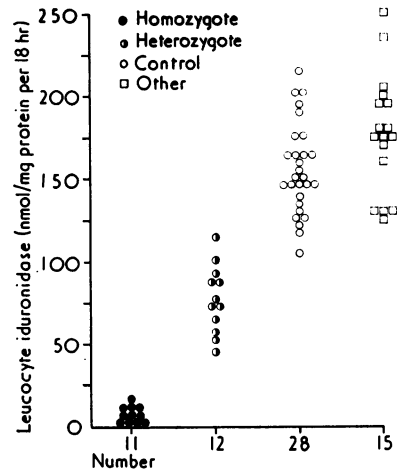

FIG. Distribution of α-L-iduronidase activity in leucocytes, with number of individuals in each group.

Selection of patients

Affected individuals in this series had documentation of the diagnosis of MPS I by α-L-iduronidase assay and radioactive sulphate kinetics studies in cultured skin fibroblasts. Heterozygotes were parents of the probands. Controls were normal adults from the staff of the John F. Kennedy Institute. The fourth group consists of patients having either MPS II (Hunter syndrome), MPS VI (Maroteaux-Lamy syndrome) metachromatic leucodystrophy, mucolipidosis II (I-cell disease), or mucolipidosis III (pseudo-Hurler polydystrophy), and their parents.

Material and methods

Leucocytes were separated from 10 ml heparinized whole blood using dextran (Skoog and Beck, 1956). Fibroblasts were harvested on the seventh day of subculture. Assay of α-L-iduronidase employed 0.01 M phenyl-iduronide,* as previously described (Hall and Neufeld, 1973). Values are expressed as nanomoles phenol liberated per mg protein in 18 hours at ambient temperature. Radioactive sulphate incorporation by cultured fibroblasts and cross correction studies were done using the methods of Fratantoni, Hall, and Neufeld (1968a, b).

Results

Assay of α-L-iduronidase in fibroblast sonicates of the 11 patients with MPS I revealed no detectable activity (less than 10 nanomoles phenol liberated per mg protein in 18 hours). The level of enzyme activity in 12 control fibroblast sonicates was 910 nanomoles phenol liberated (471–1,132).

The MPS I fibroblasts accumulated 4.3 (2.9–5.4) times more intracellular radioactive sulphate than control cell lines. No cross correction was observed when the MPS I cell lines were grown in mixture whereas correction did occur when the MPS I cells were grown with either control or MPS II fibroblasts.

Eleven patients with MPS-I (5 Hurler syndrome, 5 Hurler-Scheie compound, and 1 Scheie syndrome) had a mean leucocyte value of α-L-iduronidase activity of 4.2, ±5.5 SD (range 0–17). Values in 12 heterozygotes were 77.5, ±19.4 SD (range 40–115). Normal control values in 28 subjects were 156, ±28.6 SD (range 104–213). Values for homozygotes and heterozygotes of other lysosomal storage disorders were not significantly different from controls at 178.5, ±35.0 SD (range 132–250).

The results are graphically shown in the Fig.

Discussion

These results indicate the efficacy of leucocyte assay of α-L-iduronidase as a simple and rapid diagnostic aid for mucopolysaccharidosis I. Use of the leucocyte assay will assist in the initial evaluation of the patient suspected of having MPS I, especially the Hurler-Scheie compound and Scheie syndrome where the clinical features are less distinctive than the Hurler syndrome. Skin fibroblast studies of

*The phenyl-iduronide was supplied by Elizabeth F. Neufeld and prepared by Bernard Weissmann under contract NIH-NIAMDD 73-2205.

radioactive sulphate kinetics and enzyme assays require 6 to 8 weeks, but at the present time are still warranted for a definitive diagnosis.

Leucocyte assay of α-L-iduronidase using phenyl-iduronide does not give clear separation of heterozygotes and controls. A similar overlap was obtained in the two groups when α-L-iduronidase was assayed in cultured fibroblasts (Hall and Neufeld, 1973). Because of the rarity of MPS I and the lack of ethnic concentration, prospective screening of the general population for carriers is impractical at the present time. Assay of leucocyte α-L-iduronidase may prove helpful in selected families seeking genetic counselling.

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