Lysosomal Enzyme Activities in Different Types of Amniotic Fluid Cells Measured by Microchemical Methods, Combined With Interference Microscopy

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Summary. In primary amniotic fluid cultures, four distinct types of cells were characterized as epithelioid (E I and E II), fibroblast-like (F), and large cells. Small numbers (1—200) of freeze-dried cells were isolated from colonies of each cell type and analyzed for the activity of three lysosomal enzymes: β-N-acetylgalcosaminidase, β-galactosidase, and α-glucosidase.

When expressed per cell, the activities for each of the enzymes were not significantly different among the small types of cells (E I, E II, and F). However, 5 to 10-fold higher enzyme activities were found in the large cells. The dry mass of individual large cells, as measured by microinterferometry, was also 5 to 10 times higher than that of the smaller cell types.

When expressed per unit of dry mass, the enzyme activities tested, appeared to be independent of the type of amniotic fluid cell. The significance of this observation for the rapid prenatal diagnosis of metabolic diseases is discussed.

Introduction

At present the prenatal detection of about 50 genetic metabolic disorders is possible by the measurement of a specific enzyme deficiency in cultured amniotic fluid cells (Burton et al., 1974; Milunsky, 1975). To reduce the time interval between amniocentesis and diagnosis, micromethods enabling the analyses of small groups of freeze-dried cells (50—100 cells) have been successfully applied (see for review, Galjaard et al., 1975). In the prenatal analysis of glycogenosis Type II (Galjaard et al., 1973; Niermeijer et al., 1975), Fabry's disease (Galjaard et al., 1974), and GM1-gangliosidosis (Kleijer et al., 1976), remarkable differences in enzyme activities per cell were sometimes found between different cell groups isolated from the same culture. Such a heterogeneity might be the result of the presence of different types of amniotic fluid cells with different enzyme activities.
In amniotic fluid cultures several types of cells have been defined on the basis of their morphology (Uhlendorf, 1970; Sutherland et al., 1974; Hoehn et al., 1974). Besides the most common types of epithelioid and fibroblast-like cells, different types of large, often multinuclear, cells have been described.

Melancon et al. (1971) and Gerbie et al. (1972) found no significant differences in the specific activities of a number of enzymes in subcultures of epithelioid and fibroblast-like amniotic fluid cells. Separated cultures of these two cell types were obtained by selective detachment of the fibroblast-like cells using trypsinization. So far no biochemical analyses have been reported on different cell types in primary cultures of amniotic fluid cells. This seemed important to us for a correct interpretation of data obtained by microchemical assays of such cultures.

In the present study we have investigated the activities of enzymes in different morphologic cell types that were isolated by microdissections under microscopic control from primary cultures as described earlier (Galjaard et al., 1973, 1975). The activities of β-galactosidase, β-N-glucosaminidase, and α-glucosidase were analyzed in sub-microliter volumes of 4-methylumbelliferyl substrate and the fluorescence was measured by microfluorometry (Galjaard et al., 1973, 1975).

To determine to what extent the enzyme activities per cell are related to cell size, the dry mass of different cell types was measured by microinterferometry and the enzyme activities per unit of dry mass were determined. The implications of varying proportions of different cell types in primary amniotic fluid cultures for the reliability of prenatal diagnosis of metabolic diseases are discussed.

Materials and Methods

Cultivation and Isolation of Amniotic Fluid Cells. Amniotic fluid samples were obtained by trans-abdominal amniocentesis in the 14th–20th week of pregnancies that were either monitored for chromosomal aberrations or terminated for nongenetic reasons. The amniotic fluid cells were cultured as described earlier (Niermeijer et al., 1975), and the various cell types and the topology of the colonies were examined with an inverted phase-contrast microscope after 9–15 days of growth.

For microchemical enzyme assays, primary cultures of amniotic fluid cells were grown on dishes with a thin plastic bottom, and freeze-dried as soon as a sufficient number of colonies was available. For each specific cell type present in the culture 10–40 groups of cells were isolated according to methods described by Galjaard et al. (1973, 1975). Each dissected piece of plastic foil contained 1–10 cells for the assay of β-N-acetylglucosaminidase, 10–20 cells for β-galactosidase, and 70–200 cells for α-glucosidase. The exact number of cells was usually counted after isolation, but in the case of confluent epithelioid colonies the total cell number per colony was counted before freeze-drying.

Microchemical Assays of Enzyme Activities. The pieces of plastic foil with counted numbers of freeze-dried cells were incubated under hexadecane-paraffin oil (40/60 vol/vol) for 1 h in 0.3–1 μl of the appropriate substrate solution, the exact volume depending on the size of the dissected pieces of plastic foil. The reaction was stopped by addition of 3–5 μl of 0.5 M sodium carbonate buffer, pH 10.7. The amount of liberated 4-methylumbelliferylone in the microdroplets was measured in capillary tubes, using a Leitz MPV microspectrofluorometer. Details of the procedures used have been described elsewhere for the assay of β-N-acetylglucosaminidase (Reuser et al., 1976), β-galactosidase (Galjaard et al., 1975; Kleijer et al., 1976), and α-glucosidase (Galjaard et al., 1975).