AMNIOTIC FLUID CELL CULTURE FOR PRENATAL DIAGNOSIS OF SOME METABOLIC DISEASES

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Primary cultures of amniotic fluid cells were obtained. Optimal conditions of culture were determined and the mean incubation period was 17.9 days. Normal values for the activity of eight glycosidases, deficiency of which causes the development of inborn errors of metabolism, were obtained. On the basis of the results it is concluded that glycolipidoses (Tay-Sachs', Fabry's, and Gaucher's diseases, etc.) can be diagnosed prenatally. Data on the prenatal diagnosis of Tay-Sachs' disease are presented.

KEY WORDS: amniotic fluid cell culture; glycosidases.

The development of cytological and biochemical methods of investigation has now made it possible to undertake the prenatal diagnosis of many hereditary diseases characterized by metabolic disturbances, based on deficiency of specific enzymes. More than 30 recessive hereditary metabolic disorders are due to a deficiency of lysosomal enzymes or enzymes of the glycolytic cycle. These enzymes can be found in cultures of skin fibroblasts and in amniotic fluid cell cultures (AFCC) [2]. Prenatal diagnosis of metabolic diseases enables the birth of an affected child to be prevented. Three possible test objects are available: amniotic fluid (AF), AF cells, and AFCC. Most investigators consider that possession of AFCC is essential for reliable prenatal diagnosis [5]. It should be pointed out that values for the activity of several AFCC enzymes given by different authors vary considerably, possibly on account of the great variety of methods used for cell culture (different culture media, different sera, different numbers of passages), and also the use of different micromodification of biochemical tests. Each laboratory concerned with prenatal diagnosis of metabolic diseases has had to work out its own limits for normal values of enzyme activities in the fetus at the corresponding times of pregnancy (16-20 weeks). Such data are also of great scientific importance for the determination of levels of activity of these enzymes in the population.

The object of the present investigation was to study optimal conditions of culture in order to obtain a suitable culture for biochemical investigation, and also to determine activity of eight lysosomal enzymes, deficiency of which causes the development of certain inborn errors of metabolism: β-D-hexasoaminidase A (Tay-Sachs' disease), α-D-galactosidase (Fabry's disease), β-D-glucuronidase (type VII mucopolysaccharidosis), β-D-galactosidase [GM(1) gangliosidosis], α-D-mannosidase (mannosidosis), α-L-fucosidase (fucosidosis), aryl sulfate A (metachromatic leukodystrophy), and β-D-glucosidase (Gaucher's disease).

EXPERIMENTAL METHOD

AF for subsequent culture was obtained by transabdominal amniocentesis at the 16th-20th week of pregnancy in women attending for medical genetic counseling or for termination of pregnancy on medical grounds. AF cells were cultured by the method described previously, with certain modifications [11]. The cells were incubated in a humid atmosphere with 5% CO_2 in plastic flasks with loose-fitting screw-on lids. The initial cell concentration was 2000-3000/ml. Ham's F-10 medium (75% by volume) and embryonic calf serum (25% by volume)

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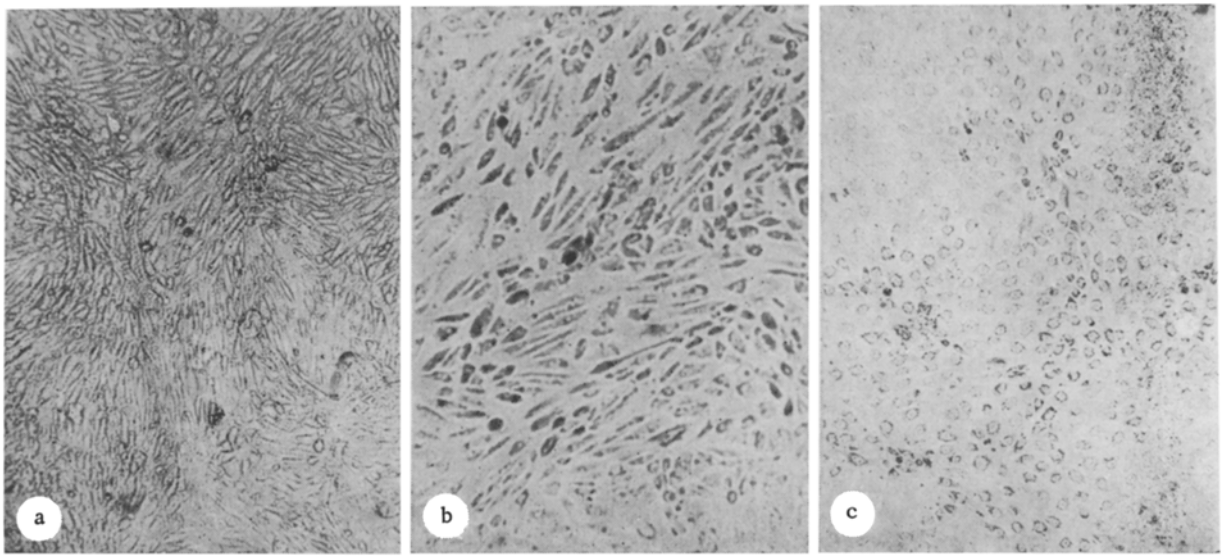

Fig. 1. Types of cell growth. a) Fibroblast-like, ocular 12.5, objective 3.2; b) transitional, ocular 12.5, objective 6.3; c) epithelioid, ocular 12.5, objective 3.2. Unstained cultures seen under inverted microscope.

was used. Activity of the above-mentioned glycosidases could be determined in 50 of the 66 cultures set up. The investigation was carried out on extracts and homogenates of the cells, using 4-methylumbelliferylglycosides as substrates, except in the case of aryl sulfatase A, for which p-nitrocatechol sulfate was used. Activity of the glycosidases was determined in the primary AFCC after 14-24 days (mean 17.9±0.7 days) of culture [1]. By that time the formation of a loose monolayer or of 8-12 colonies from 3 to 12 mm in diameter could be observed. The first change of medium took place 72 h after the beginning of culture, and it was subsequently changed every 5-6 days. Growth was monitored under an inverted microscope.

EXPERIMENTAL RESULTS

The difficulty of prenatal diagnosis of metabolic diseases is associated with the need to obtain a large number of growing cells (of the order of 1-2 million) for subsequent biochemical analysis. This is achieved usually by a series of subcultures, which takes over 30 days. Subculture also is associated with certain difficulties arising during prenatal diagnosis. It has been shown that the activity of some glycosidases can vary considerably depending on the number of passages [3, 8, 12]. Subculture is attended by the risk of bacterial and viral infection of the culture and it takes a long time, which is extremely undesirable, because the late termination of pregnancy may cause problems. Accordingly the possibility of performing biochemical tests on a prolonged primary AFCC was studied. It was important to ensure that the culture did not pass into the stationary phase of growth before sufficient material for analysis had accumulated.

Like other workers, we found morphological heterogeneity of AFCC, with gradual transition from fibroblast-like to the epithelioid type of growth [2, 7]. As a rule a mixed type of growth was observed in our material. Fibroblast-like growth predominated in 31.3% of cultures, epithelioid in 15.2%, and the transitional type in 53.5% (Fig. 1). The morphological heterogeneity of the culture was evidently due to the fact that epithelial cells of varied origin are present in AF. As the culture grows, the epithelioid cells are replaced by the stronger fibroblast-like cells. Similar data were obtained during passage of AAFC, when the long-living fibroblast-like cells displace the short-living epithelioid cells [9]. Activity of some enzymes depends on the morphological composition of the culture [10]. No such dependence could be found for the glycosidases which we investigated [8]. Accordingly we investigated the activity of the above enzymes without regard to the morphological characteristics of the AFCC. Data on glycosidase activity in extracts and homogenates of AFCC are given in Table 1. The results agree with data in the literature [6]. In some cases activity was determined in homogenates containing detergent, for it was often necessary to work with AFCC containing a small number of cells.

TABLE 1. Activity of Glycosidases (in nmoles/mg/h) in Extracts and Homogenates of Amniotic Fluid Cell Culture

Enzyme	Extract	Limits of variation	Homogenate	Limits of variation
β-D-galactosidase	451±106	557—303	675±228	1022—287
α-D-galactosidase	37±7,4	44—28	42±21	80—17,5
α-D-fucosidase	240±123	397—90	168±91	363—77
α-D-mannosidase	235±58	251—75	134±35	291—52
β-D-glucuronidase	95±9	104—38	67±30	112—37
Aryl sulfatase A	208±117	433—121	206±48	266—107
β-D-hexosaminidase (total)	1782±750	3040—940	2478±556	3065—1309
β-D-glucosidase	—	—	29±17	55—10

TABLE 2. Determination of Hexosaminidase A Activity for the Prenatal Diagnosis of Tay-Sachs' Disease (in % of total hexosaminidase activity)

Test object	Control	Patient E
AF	18 (12—29)	17.6
AF cells	31 (17—46)	27
AFCC	44—48	43—50
Placenta	67	48
Child's leukocytes	55—68	60

Analysis of failure in setting up the cultures revealed the following possible causes: 1) too few cells initially in AF (8.2%); 2) bacterial infection of the culture (5.5%); 3) much clotted blood in the sample of AF (3%); 4) too few growing cells when epithelioid cultures are found (5%); 5) other causes: toxicity of the serum, use of nutrient medium after its expiry date, and so on (1.6%).

On the basis of the results obtained for normal values of glycosidase activity, the prenatal determination of enzyme activity for the diagnosis of Tay-Sachs', Fabry's and Gaucher's diseases, etc., appears to be a possibility.

As an example the data for prenatal diagnosis of Tay-Sachs' disease in heterozygous couples, with a previous child affected with Tay-Sachs' disease, confirmed clinically and biochemically, are given in Table 2. In the pregnancy in question the possibility that the fetus was affected was ruled out. This was confirmed by analysis of hexosaminidase A activity in the placenta and in the child's leukocytes.

Investigation of a primary AFCC is an important and promising development, for it enabled informative material for biochemical study to be obtained within a shorter time than by the use of subcultures. Analysis of enzyme activity in the primary AFCC can be used with success for the prenatal diagnosis of glycolipidoses and glycoproteinoses.

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