



Fetal Abnormalities of Metabolic Origin*

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The subject of my discussion is prenatal diagnosis of genetic disorders of a *metabolic* rather than a *chromosomal* nature. Whereas the chromosomal defects are the result of either the transmission of a translocated chromosome from a single parent to its offspring or an error in meiosis or mitosis, almost all *metabolic* disorders are inherited in an autosomal recessive fashion. Therefore, except in rare instances, the only clue to the possible presence of an inborn error of metabolism (IEM) in the fetus is that a previous child of the parents has had the disorder. This history identifies both mother and father as heterozygotes for the deleterious gene and thus forewarns that each subsequent pregnancy is at a 1:4 risk of yielding the disorder again. By studying the amniotic fluid of the at-risk fetus, it is now possible to determine if the disorder is present, and if so, to prevent its occurrence by therapeutic abortion. My main purpose is first to review the status of the art of amniocentesis and biochemical analysis of the material thus obtained, and then to examine the impact this procedure will have on the incidence and management of the IEM.

Amniocentesis in the *second* half of pregnancy for the diagnosis and treatment of erythroblastosis has been widely used for almost two decades with a minimum of morbidity or mortality to the mother or fetus. Theoretical risks of the procedure (fetal abortion, puncture, or induced malformation, and maternal bleeding, infection, or sensitization) had not been reported when the accumulated experience of 500 amniocenteses during the *first* half of pregnancy were reviewed one year ago (Nadler: *BD*, 1971). Prior to the 10th week of gestation there are less than 30 cc. of amniotic fluid present, and the uterus has not yet risen outside of the pelvis. Because of these facts, a transabdominal approach at

this time is usually unsuccessful and the transvaginal route is attendant with a high risk of complications (especially abortion) (Fuchs, 1971). For these reasons the procedure is usually withheld until the 13th to 14th week, at which time approximately 100–120 cc. of amniotic fluid are present, and the uterus can easily be positioned to the anterior midline by bimanual examinations. Most investigators do not use placental localization, and they accomplish the procedure on an out-patient basis. In the combined experience of four investigators only 11% of 353 taps had to be repeated in order to get adequate material; initial failure in the majority of cases was due to contamination by gross blood (Nadler: *BD*, 1971).

Almost all of the cells present in the amniotic fluid are of fetal origin (either from amnion or skin), and some are viable. If the removed fluid is placed in a siliconized container, the cells will not adhere to the walls, and they can be shipped by mail over a 48-hour period or stored overnight in a refrigerator. When these cells are isolated from the amniotic fluid by centrifugation and placed in appropriate tissue culture media, their growth and multiplication rapidly ensues. After two subcultures and a total of four weeks of growth, hopefully a sufficient number of cells is present for analysis. Depending upon the disorder, the biochemical test performed is the measurement of either specific enzyme activity or of possible accumulation of a substance (such as mucopolysaccharides). Unfortunately, tissue sufficient in quantity to perform the desired test can be obtained only 75–90% of the time (Nadler: *SMJ*, 1971); with chromosomal studies, success rates of 95–99% have been achieved (Nadler: *BD*, 1971; *SMJ*, 1971).

The major premise utilized with this procedure is that these cultured amniotic cells accurately reflect the specific characteristics of the disorder in question. Previous studies of skin fibroblast cultures have proven that these cells *do* reflect the deficient state of the whole organism in most disorders.

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When the amniotic fluid cells are first removed they have the morphological appearance of epithelial cells, but in culture they take on the biochemical and morphological characteristics of fibroblasts. However, many factors affecting the enzyme activity of these cells must be considered before proper interpretation of results is possible (for example, stage of gestation, stage of growth of cells, degree of cell confluency). If one also remembers that these techniques must be refined to the point where the investigator can accurately differentiate the heterozygote from the homozygote, then hopefully he is left with the highest regard for the complexities of this diagnostic procedure.

In addition to the use of *cultured* amniotic fluid cells, biochemical analysis of *uncultured* amniotic fluid cells or of the amniotic fluid itself may provide sufficient information to make an accurate prenatal diagnosis. Although presently less than a half-dozen disorders can be detected by these methods (Nadler: *BD*, 1971), we hope further refinements will serve to increase this number, because such progress would obviate the minimum 4-week delay required to culture. Using cultured cells to establish the diagnosis leaves only a precious week or two to effect any needed abortion, as there are substantial medical, if not legal, restrictions to performing a therapeutic abortion much after the 20th gestational week.

Now that we have briefly described the capabilities and limitations of the procedure itself, let us examine how its usage can affect our present management of the IEM. The various modalities of treatment presently utilized (for example, limiting substrate, providing deficient end product), for the most part, do not attack the basic problem—that of decreased enzyme activity. Recently, there has been much discussion regarding the feasibility of gene therapy, that is, the isolation of some of the patient's somatic cells, the alteration of their genetic endowment *in vitro*, and their replacement in the individual. Other possible methods of providing the deficient enzyme include organ transplantation or specific enzyme replacement, but none of these approaches (particularly gene therapy) appears feasible in the next few years.

The only *preventive* measures available in the past were genetic counseling and abortion of male fetuses of mothers carrying a serious sex-linked disease such as muscular dystrophy (the sex of the fetus can be fairly accurately established by a study of the sex chromatin status of uncultured or cultured amniotic fluid cells). Genetic counseling is a

good preventive measure because the majority of parents given a 1:4 recurrence risk will be deterred from future pregnancies, especially where the disorder is lethal or uncontrollable and where there are already one or more normal children in the family. But what of the parents whose *first* child has a serious inborn error of metabolism and who wish a normal child but are rightfully fearful of the 1:4 recurrence risk? This is where the amniocentesis and subsequent determination of the specific enzyme content of fetal cells is especially useful. I presently have two sets of young parents, each with an infant with Hurler's syndrome, who wish to have their first normal child. Only 3–4 laboratories in the country have developed the necessary techniques for the *in utero* diagnosis of this condition. Should one of these laboratories agree to monitor the pregnancy, and if the parents consent to an abortion if studies show an involved fetus, either the amniotic fluid (with the cells) or already cultured cells would be mailed to that laboratory.

At the present time there are less than twelve laboratories active in the prenatal diagnosis of the IEM—most, if not all, of these operate in a research rather than service capacity. A coordination of these various centers is needed because no one laboratory can perform all the available tests. As of one year ago there had been less than 25 therapeutic abortions performed in the United States for proven fetal metabolic disorders (Nadler: *BD*, 1971). This number would have been much higher had the biochemical determinations been more readily available.

What effect will this development have in reducing the actual number of infants born with metabolic disorders? One should remember that at present we must have an index case before knowing that we should monitor future pregnancies. Therefore, the birth of a majority of patients with IEM will not be prevented. Making the assumption that the goal of a family is to have two normal children, Motulsky has calculated that performing therapeutic abortions on all fetuses found to have a metabolic disorder (after one sibling involvement) will only reduce the incidence of that disorder by 12.5–34% (Motulsky, 1971).

In order to substantially reduce the incidence of these disorders we would need to identify which parent pairs are heterozygotic for the same deleterious gene *before* the delivery of their first affected child. It would be impractical to screen all prospective parents for heterozygosity for most of the

over 150 autosomal recessive IEM, because either the disease is so rare, or the test is too difficult to perform on a mass basis. However, it *is* presently feasible to screen certain high-risk groups for certain disorders—for instance, sickle trait testing for Blacks and the measurement of serum hexosaminidase A (for Gaucher's disease) in Ashkenazi Jews. Also, because of its prevalence in Caucasians, mass screening for the gene for cystic fibrosis should be performed once a simple reliable test is developed.

Many ethical, moral, legal, and theological issues are raised with our ability to define the metabolic status of the fetus. Time does not permit any in-depth discussion of these matters, but let me pose two questions as examples:

1. Should one abort a fetus who has galactosemia, pyridoxine-responsive homocystinuria, or phenylketonuria? These disorders are severe if untreated but the prognosis is good with dietary restriction or vitamin supplementation.
2. Should one induce an abortion where only one of nonidentical twins has an untreatable metabolic disorder?

At present it is felt that the final decision must be left to the parents, with the obstetrician and geneticist providing informed but impartial counsel. Per-

haps shortly, each center involved in prenatal diagnosis will establish a board of physicians and lay people to reach a consensus in each given case.

In summary, it is now theoretically possible to diagnose approximately 40 different inborn errors of metabolism in a fetus early enough in gestation to perform a therapeutic abortion. However, most of these disorders are extremely rare, the techniques laborious, and diagnosis possible only after one sibling is already involved. For now, higher priority should be given to the development of procedures for massive heterozygote screening and intrauterine diagnosis of more common recessive diseases such as cystic fibrosis and sickle cell anemia.

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