METHODS IN
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PRENATAL DIAGNOSIS: CELL BIOLOGICAL APPROACHES

EDITED BY

SAMUEL A. LATT
GRETCHEN J. DARLINGTON

ared under the Auspices of the American Society for Cell Biology

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Prenatal Diagnosis
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To our parents, spouses, and children

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PREFACE

Prenatal diagnosis is a rapidly growing field in which increasingly sophisticated analytical tests are used to obtain vital information about a patient, the fetus. The prenatal examination is, at best, a partial or indirect one, without communication from the patient. The volume of prenatal diagnostic tests has escalated dramatically over the past decade. Despite an increased use of antenatal diagnosis and an enhanced public awareness of the availability of the procedures, fewer than 20% of women for whom antenatal diagnosis might be indicated utilize the tests.

Subject, of course, to personal beliefs about the desirability of prenatal diagnosis, the frequency, scope, and complexity of diagnosis *in utero* could easily double over the next few years. This state of affairs has at least two implications for laboratory scientists. First, there will be an increased need for laboratory personnel who are highly competent with state-of-the-art techniques for performing prenatal diagnostic tests. Second, there will be an intense need and challenge for basic scientists to adapt current advances in biomedical instrumentation, molecular biology, and cell biology to the development of better diagnostic methodology.

This book addresses the needs anticipated both of active diagnostic laboratory staff, who wish information about current procedures and an indication of directions for future development, and of basic scientists, who wish to know not only what currently is being done in prenatal diagnosis, but also where opportunities exist for new applications of basic science to the field of prenatal diagnosis.

Following an overview and historical perspective by Hirschhorn, a series of chapters outlines basic information currently necessary to operate a prenatal diagnostic laboratory. These chapters hopefully will serve to increase standardization of techniques in an effort to improve the accuracy and success of antenatal studies. The chapter by Hoehn and Salk describes the types of cells expected in amniotic fluid samples, indicating current and anticipated studies of their biological features, while the chapter by Sandstrom, Beauchesne, Gustashaw, and Latt discusses methods of chromosome analysis as applied to these cells. Basic procedural detail is accompanied by an indication of directions for future development. Similarly, the chapter by Haddow and Miller discusses not only the methods for alpha-fetoprotein (AFP) analysis in the detection of open neural tube defects, but also the possible impact of widespread maternal serum AFP screening and the development of ancillary laboratory tests to reduce diagnostic ambiguity. At present, AFP analysis is performed primarily in a few central referral laboratories. However, with increased availability of diagnostic reagents, this situation might change, and the need for interlaboratory standardization will become more urgent. The chapter by Grabowski and Desnick

details the types of biochemical tests currently available for prenatal diagnosis. These tests are typically spread over numerous specialty centers and will continue to grow in number with the development of new techniques and better definition of the biochemical basis of various inherited diseases.

Prenatal diagnosis typically utilizes ultrasound for both sample acquisition and ancillary examination of the fetus, and Campbell, Griffin, Little, and Allen have written an extensively illustrated chapter acquainting individuals involved with prenatal diagnosis with the types of information that might be forthcoming from ultrasound studies. Fetuses can also be examined by endoscopy and, as discussed in the chapter by Mahoney, direct sampling of fetal tissue can be done for diagnostic studies. The risk and complexity of fetoscopy limits its use, but in isolated situations, it can provide essential information not available by other techniques. Fetoscopy provides small samples that require miniaturized analytical techniques. As described in the chapter by Galjaard, miniaturization of amniotic fluid cell analyses is possible and can speed up the acquisition of diagnostic information. A reduction in the time required for analysis of amniotic fluid cells has long been a goal of the clinician and counselor who deal with expecting parents. As indicated in the chapter by Epstein, the speed and success of prenatal diagnostic studies can also be increased by stimulating cell growth. A burgeoning number of identified growth factors provides ample opportunity for attempts at increasing cellular growth rate.

In the diagnostic sequence, the process of amniotic fluid sample acquisition continues to be the source of greatest risk to the mother and fetus. The chapter by Parks and Herzenberg describes an exciting new approach to sample acquisition, based on the isolation in a cell sorter of fetal cells present in the maternal circulation. This approach requires complex instrumentation and thus far cannot provide cells capable of further division, as needed for metaphase chromosome analysis. However, improvements in both immunodiagnostic reagents and flow cytometric equipment can be expected, and further development in this area as it relates to prenatal diagnosis can be anticipated. Also, procedures for obtaining fetal tissue transcervically, very early in pregnancy, have recently been described, and those may well influence certain prenatal diagnostic strategies.

Finally, it is often desirable to obtain information about differentiated properties or genetic markers not typically expressed by amniocytes. The chapter by Darlington describes how amniocytes can be induced to produce liver-specific proteins by fusion with hepatocytes and indicates a broad area for applying somatic cell genetics to prenatal diagnosis. Similarly, the chapter by Kurnit, Orkin, and White describes how cloned nucleic acid probes provided by recombinant DNA techniques can be used to "read" the genetic information contained in amniocytes and other fetal cells. Ultimately, the presence of restriction endonuclease fragment length polymorphisms, to which mutant alleles can be linked, places virtually any genetic disease within range of DNA linkage

analysis. An explosion in the scope of this approach can be expected as recombinant DNA techniques are applied to prenatal diagnosis.

The chapters included here were chosen to cover the major basic laboratory techniques of prenatal diagnosis presently available and other sources of relevant data utilized in making diagnoses (e.g., ultrasound), and to identify technologies that exploit new research advances. With time, it is anticipated that many of these new technologies will be incorporated into standard diagnostic protocols, while new methodology will continue to emerge. To further both practical and investigative developments in prenatal genetic diagnosis is a goal of this book.

The efforts of the authors of individual chapters, upon which the success of this volume depends, are greatly appreciated. Also, discussions with colleagues and students during the course of this work were extremely valuable. Perhaps the greatest debt of the Editors is to their families and their mentors. Gretchen Darlington remains grateful to Robert S. K. Krooth, Frank H. Ruddle, James V. Neel, and Alexander G. Bearn. Samuel A. Latt is indebted to his teachers, Herman W. Lewis, Elkan R. Blout, Herbert A. Sober, and Bert L. Vallee, to Park S. Gerald for providing him with an entry into human genetics, and to Mary Ellen Avery and Kenneth J. Ryan for encouragement and support essential for continued work in the field of prenatal genetic diagnosis.

SAMUEL A. LATT
GRETCHEN J. DARLINGTON

CONTENTS

8. *Miniaturization of Biochemical Analysis of Cultured (Amniotic Fluid) Cells*
Hans Galjaard

9. *The Use of Growth Factors to Stimulate the Proliferation of Amniotic Fluid Cells*
Charles J. Epstein

10. *Fetal Cells from Maternal Blood: Their Selection and Prospects for Use in Prenatal Diagnosis*
David R. Parks and Leonard A. Herzenberg

Chapter 1

Overview and Historical Perspective of Prenatal Diagnosis

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Of all the advances in human genetics made over the past few decades, the one with the greatest impact on the clinical application of this science has been the development of prenatal diagnosis. The nature of genetic counseling has changed from a recital of statistical risks for disease to the ability to predict accurately whether a fetus is normal or affected as to the abnormality for which it is at risk.

There is a story, which may be apocryphal, as to how prenatal diagnosis began. It is said that someone from the agricultural community in Israel was interested in discovering the sex of early bovine pregnancies because for a variety of economic reasons it is better to have female calves born and, if possible, to abort most male calves. One needs only one bull to fertilize many cows, but the cows give milk. When Barr and Bertram (1949) discovered that the X chromatin body could distinguish female from male cells, it was suggested to Sachs and Danon at the Weitzman Institute that it may be possible to determine the sex of the unborn calf by examining amniotic fluid cells for X chromatin. Apparently, attempts at amniocentesis in the cow frequently lead to spontaneous abortion. This project was therefore abandoned. However, Sachs and Danon maintained their interest in this problem and approached an obstetrician named Serr to try to predict the sex of human fetuses that were going to be aborted in any case for other reasons. In 1955, Serr, Sachs, and Danon described the feasibility of antenatal sex determination by the analysis of sex chromatin in freshly obtained amniotic cells (Serr *et al.*, 1955; Sachs *et al.*, 1956). Soon thereafter Fuchs in Denmark utilized this same technique for the first time for actual prenatal diagnosis of genetic disease (Riis and Fuchs, 1960). A number of women at risk for sons with X-linked recessive diseases, primarily hemophilia and Duchenne mus-

METHODS IN CELL BIOLOGY, VOL. 26

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cular dystrophy, were subjected to amniocentesis and many of these women opted to abort male fetuses.

Since these first attempts, a new and revolutionary field of study of fetal cytogenetics, biochemical genetics, and other genetically related studies has evolved. In 1966, Steele and Breg published their successful technique for the culture and karyotyping of fetal cells obtained through amniocentesis (Steele and Breg, 1966). At about that time it also became possible to perform amniocenteses safely by the transabdominal route rather than by the riskier transvaginal route used in the early studies. The transabdominal approach had been developed for the purpose of third-trimester amniocentesis in the study of pregnancies at risk for Rh sensitization. The transabdominal route obviated the previously relatively common finding of amnionitis following transvaginal amniocentesis. Within a year of the report by Steele and Breg, Jacobson and Barter (1967) described the successful prenatal chromosome diagnosis of three pregnancies at high risk for chromosomal abnormalities. In that article they discussed a number of issues that have still not been completely resolved. These, of course, include a variety of ethical, moral, and legal questions that have been addressed in several publications (Littlefield *et al.*, 1973; Harris, 1974; Milunsky, 1976) and with which we are still struggling. An example of such problems relates back to prenatal sex determination, but for the simple selection of sex rather than in a pregnancy at risk for X-linked disease. Although it has been recommended (Powledge and Fletcher, 1979) that this not be done by reputable laboratories performing prenatal diagnosis, even here differences of opinion exist and have been stated rationally (Fletcher, 1979). Despite some unresolved issues, many thousands of prenatal diagnoses have been performed and the diagnosis of abnormalities has presumably helped many families with their decisions relating to high-risk pregnancies.

Several articles, and reports of large series have been published (Hsu and Hirschhorn, 1974; Galjaard, 1976; Hsu *et al.*, 1978; Golbus *et al.*, 1979; Milunsky, 1979) relating the world experience with cytogenetic and other prenatal diagnosis, and the reader is referred to these for a discussion of results, pitfalls, and problems.

Soon after the utilization of amniocentesis for cytogenetic diagnosis, it became clear that a number of inborn errors would be detectable by enzyme analysis of cultured amniotic fluid cells. Nadler, who was in the forefront of this development (Nadler, 1968), published a review of the early findings (Nadler, 1972), and some of the articles just listed provide compilations of those inborn errors that are currently diagnosable. It is almost impossible to keep up with the constant additions to these inborn errors, because new discoveries of enzyme defects are allowing an ever-increasing number of diseases to be diagnosed prenatally. Means for the detection of genetic diseases in which the specific defect is not known have also been suggested, such as the use of the nitroblue tetrazolium test

in cultured amniotic fluid cells for the prenatal diagnosis of chronic granulomatous disease (Fikrig *et al.*, 1980).

The question of the risks of amniocentesis was addressed in great detail in studies in the United States (NICHD, 1976), Canada (Simpson *et al.*, 1976), and Great Britain (Working Party on Amniocentesis, 1978). In general, the conclusions of these studies are that there is virtually no risk to the mother and that the risk of inducing an unwanted abortion of the fetus may be as high as 0.5% or 1 in 200 attempts. It is likely that in competent hands the actual risk may be lower. All these studies have reported 99% or better accuracy of results. The question of the risk of ultrasound for the purpose of placental localization, determination of gestational age, and the diagnosis of congenital defects has been raised, but until now there is no short-term or long-term evidence of any real damage to the unborn child from this technique.

A number of other methods have come into use for the purpose of prenatal diagnosis. Amniography and fetography, popular in the early 1970s, have not been widely used in recent years. Conventional X-rays have been of some use in the detection of some bony abnormalities, but are not common in the general armamentarium of prenatal diagnosis. The most important of the new techniques in use for some years has been ultrasonography. Perhaps its most important application is the determination of gestational age by the measurement of the fetal biparietal diameter. This technique is crucial not only for studying the growth of the fetus, but also for determining the exact gestational age of the fetus, important for the interpretation of other studies, particularly the level of alpha-fetoprotein in the amniotic fluid. Another common use of ultrasonography is for the detection of multiple pregnancies, of critical importance for accurate prenatal diagnosis because if there are twins, both sacs need to be tapped in order for a correct answer to be given to the family. An important aspect of ultrasonography is the guidance of the obstetrician during amniocentesis. It becomes easy to avoid puncturing the placenta or injuring the fetus, because in most cases it is possible to locate and enter a window into the large pool of amniotic fluid under the guidance of ultrasonography, when performed on the table on which amniocentesis is to be done. A reduction in bloody taps has been reported as a result of this practice (Kerenyi and Walker, 1977). Finally, ultrasonography has been extremely useful in the detection of a variety of congenital malformations, particularly anencephaly and meningomyelocele, as well as a variety of limb and renal abnormalities (Kaffe *et al.*, 1977; Hobbins *et al.*, 1979). It has been demonstrated that ultrasound is capable of diagnosing congenital heart disease and, potentially, cardiac dysrhythmias (Kleinman *et al.*, 1980).

In addition to the biochemical studies of cultured amniotic fluid cells, the last few years have seen advances in such studies of the amniotic fluid itself. The most important advance has been the use of alpha-fetoprotein measurement in the diagnosis of open neural tube defects (Brock and Sutcliffe, 1972). Elevation of

amniotic fluid alpha-fetoprotein is also associated with other anomalies, including omphalocele and intestinal atresia, as well as severe fetal distress or fetal death (Ainbender *et al.*, 1978). Of great interest and potentially wide application are the preliminary studies of Nadler's group (Walsh and Nadler, 1980; Nadler and Walsh, 1980) on the successful intrauterine detection of cystic fibrosis by a combination of enzymatic and separative studies of amniotic fluid.

A number of other innovative methods have led to further exciting advances. The development of narrow-bore fetoscopes has led to direct inspection of fetuses for certain types of anomalies (e.g., Laurence *et al.*, 1975). Much more useful, until now, has been the utilization of fetoscopy for fetal blood sampling (Hobbins and Mahoney, 1976). The application of biochemical techniques and those of molecular biology to such fetal blood specimens has already produced prenatal diagnoses of various hemoglobinopathies (Alter, 1979) and of hemophilia (Firshein *et al.*, 1979). The study of fetal white blood cells from these specimens is promising for the detection of certain immune-deficiency diseases and leukocyte abnormalities (e.g., Newberger *et al.*, 1979). If this technique is as safe as ordinary amniocentesis, prenatal chromosomal analysis may more commonly be performed on fetal blood, as already reported (Cordesius *et al.*, 1980), with answers obtained by 72 hours rather than the usual 2 to 3 weeks' wait, a terribly difficult period of time for the family.

Once a fetoscope is inserted, it is possible to obtain small samples of fetal skin. Utilizing this technique, followed by tissue culture or histopathological examination of the skin sample, a number of diagnoses have recently become possible. These include such primary skin diseases as epidermolysis bullosa letalis and other related diseases, such as several forms of ichthyosis (Elias *et al.*, 1980; Golbus *et al.*, 1980; Rodeck *et al.*, 1980). It has been suggested that such fetal skin fibroblast cultures may become useful for the diagnosis of diseases that show specific sensitivities of the DNA of the cultured cells to environmental agents. For example, it may be possible to diagnose ataxia telangiectasia prenatally by demonstrating the known increased cellular sensitivity to X-rays (Patterson *et al.*, 1979), and it has been suggested, although by no means proven, that fibroblasts from patients with Huntington's disease, and therefore perhaps fibroblasts from fetuses with Huntington's disease, may also be unusually radiosensitive (Moshell *et al.*, 1980). If the latter turns out to be true, it may at last become possible to approach rationally the counseling and prevention of this devastating entity. It has even been proposed that fetal muscle biopsy may be possible. If this is the case, a variety of primary genetic muscle diseases may become diagnosable, as has been suggested for Duchenne muscular dystrophy (Emery and Burt, 1980).

The techniques of modern molecular genetics have recently been applied to the DNA of amniotic fluid cells. With these highly sensitive methods, several forms of thalassemia and, in certain families, sickle cell anemia have become amenable