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44. We thank all of our colleagues who put human and medical genetics at the service of our fellow citizens. Supported by the Ministry of Social Affairs (Quebec), the Department of Health and Welfare (Ottawa, Division of Health Research Grants), the Medical Research Council of Canada, the National Genetics Foundation (New York) and special grants from Peter and Edward Bronfman and Arnold Steinberg. Address correspondence to C.R.S., deBelle Laboratory for Biochemical Genetics, Montreal Children's Hospital Research Institute, 2300 Tupper Street, Montreal, Quebec, Canada H3H 1P3.

Prenatal Diagnosis of Genetic Disorders

Gilbert S. Omenn

Summary. Sampling of amniotic fluid, visualization of the fetus, fetal blood sampling, and screening of maternal blood represent successive approaches to the diagnosis of specific genetic disorders in the second trimester of pregnancy. Clinical and ethical concerns about the appropriateness, safety, and efficacy of the techniques have led to multidisciplinary assessments at an early stage. A major growth in demand for medical and educational genetic services can be anticipated.

A relatively simple procedure, amniocentesis, has revolutionized the practice of clinical genetics and genetic counseling. With this procedure, an increasing array of tests of chromosomes, enzymes, and other proteins can be applied to pregnant women at "high risk" of delivering infants with certain birth defects and other genetic disorders. Previously, the physician could offer only statistical estimates of the likelihood of occurrence or recurrence of these disorders; now prospective parents can learn whether the fetus is affected or not affected. It must be emphasized, however, that these tests cannot guarantee a "normal" baby, since there are many disorders for which no tests are available.

The two most common disorders that can be detected by this approach are Down syndrome (formerly called Mongolism) and neural tube closure defects. Down syndrome accounts for 10 percent of cases of severe mental retardation and can be diagnosed by examination of the fetal chromosomes. There are about 5000 new cases in the United States each year. Neural tube closure defects (open spine) include anencephaly, which is lethal, and meningomyelocele or spina bifida with paraplegia and incontinence and infectious complications. This birth defect, which affects 6000 to 8000 babies per year in this country, can be detected biochemically by the presence of a fetal protein in the amniotic fluid.

In my opinion, the development of the technologies for testing of fetuses during pregnancy represents a good example both of the progressive enhancement of technological capabilities and of the careful assessment of the safety and efficacy of the techniques. I have organized this article into five overlapping chronological stages in the development of techniques for prenatal diagnosis during the past decade and for the foreseeable future.

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Stage 1 Approach: Sampling of Amniotic Fluid and Cells

The procedure. Amniocentesis (*1*) should be performed by an obstetrician skilled in the technique only after the patient has received appropriate genetic counseling and has given her informed consent. Amniocentesis is a simple outpatient procedure for the woman at 15 to 17 weeks of pregnancy. After the overlying skin of the abdomen is anesthetized, a needle is inserted through the abdominal and uterine walls into the amniotic fluid sac surrounding the fetus. The timing is determined by the facts that the uterus enlarges beyond the pelvic bony structures only after about 12 weeks and that the volume of amniotic fluid is too small before 13 to 14 weeks. Figure 1 illustrates the procedure schematically, and Fig. 2 provides data on the actual volumes of fluid as a function of estimated gestational age. The amniotic fluid represents primarily fetal urine and contains cells sloughed from the skin, respiratory tract, and urinary tract. At least two different types of cells have been distinguished, epithelioid cells and fibroblastic cells. Chromosomal or biochemical studies usually require about 3 weeks to complete in the laboratory, and therefore the couple must wait until the 18th to the 20th week of pregnancy for the results. Further delay in performing the test would make termination of the pregnancy less safe, in the event that an affected fetus is detected and the parents elect to terminate the pregnancy.

Safety and efficacy. For the last several decades amniocenteses have been performed in the final 3 months of pregnancy; the procedure was used originally to inject radiopaque dye for fetal x-ray

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studies and later, about 1961, to monitor pregnancies at risk for Rh blood incompatibility, including intrauterine exchange transfusions in some cases.

When amniocentesis was adapted for use at 16 weeks of pregnancy, considerable attention had to be given to the assessment of potential risks of the procedure, both for the mother and for the fetus. Major collaborative studies were undertaken early in the 1970's in the United States, Canada, and the United Kingdom to evaluate the safety and efficacy of amniocentesis and associated laboratory procedures (*2*). In the U.S. study, sponsored by the National Institute of Child Health and Human Development, 1040 prospective subjects and 992 controls were enrolled at nine institutions between July 1971 and June 1973. At the start of this study only a few hundred midtrimester amniocenteses had been done in the United States. Controls were matched for maternal age, family income, week of gestational age, practice setting, and number of previous pregnancies. During the study the match for maternal age had to be abandoned, since most women over 35, and especially those over 40 years of age, elected to have the amniocentesis done when given information about the study. Mother and baby were evaluated immediately after the performance of amniocentesis, at the time of delivery, and when the baby was 1 year of age. Immediate complications of amniocentesis (vaginal bleeding or amniotic fluid leakage) occurred in 2 percent of the women. The rate of spontaneous miscarriage or fetal death was the same in the two groups (3.4 percent). Rates of prematurity, of malformations, and of infant mortality, the growth measures in the first year, and the Denver Developmental Screening Test measures were not significantly different between the two groups. Very similar results were reported from the Canadian Collaborative Study of 990 women who underwent amniocenteses, from 1972 through 1975. There were no major complications of the sort feared in the early days of this procedure; that is, there was no serious infection or hemorrhage in the mother, and, most importantly, there was no evidence of an increased rate of miscarriage and fetal loss. A few cases of skin punctures have now been recognized, and it must be admitted that follow-up studies of the infant beyond the age of 1 year have not been reported.

Despite the remarkable safety of the procedure and an accuracy rate of 99.4 percent, there was considerable concern raised by erroneous diagnoses in the U.S. study. In two cases, a baby with

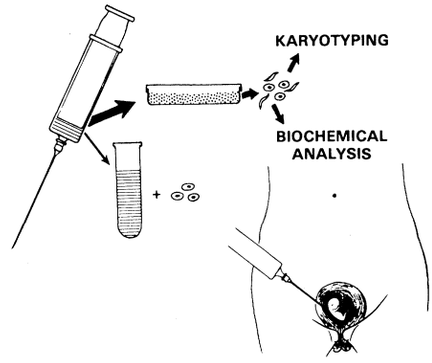

Fig. 1. Schematic illustration of the insertion of the amniocentesis needle, withdrawal of fluid from the amniotic fluid sac around the fetus, and culture of the cells obtained from the fluid to permit analysis of the chromosomes (karyotyping) and of enzymes (biochemical tests).

Down syndrome was born despite a report of normal chromosomes from the amniocentesis sample; in one case, a diagnosis of galactosemia was made on amniotic fluid cells, but the pregnancy was carried forward and an unaffected baby was born; and in three cases, the sex was identified incorrectly, although the chromosomal disorder feared was ruled out. Intensive restudy of the six cases failed to establish the causes of error; contamination with maternal cells or interchange of samples in the clinic or laboratory are the likely explanations. In the Canadian study, there were seven erroneous diagnoses (0.6 percent), of which two were false-positives, three were false-negatives for the still new α-fetoprotein test for neural tube defects, and two were incorrect designations of sex.

Counselors in prenatal genetic diagnosis centers currently inform the woman and her husband of the following potential hazards and sources of errors. In 2 to 5 percent of cases no fluid is obtained, necessitating a second amniocentesis a few days later. Insertion of the amniocentesis needle may be facilitated by ultrasonographic determination of the position of the fetus (see Fig. 3), but experienced obstetricians have a high rate of success even without ultrasound. In 5 to 10 percent of cases, the amniotic fluid cells fail to grow in the laboratory culture media, necessitating a second and sometimes even a third amniocentesis. In a small number of cases the samples are contaminated with skin bacteria and the sample is lost; sterile technique and immediate transport to the laboratory diminish this risk. Since the placenta lies between the abdominal wall and the fetus and amniotic fluid sac in about one-half of cases, it is often unavoidable to pass through the placenta and cause some bleeding, sometimes producing a bloody sample with poor growth in cell culture. Ultrasound is helpful in identifying twin pregnancies (one per 100 pregnancies). A single amniocentesis will be insufficient for twin pregnancies; it is a tour de force to sample both amniotic sacs with certainty (*3*). Just as in other laboratory tests, there is always a risk of clerical or technical error in the performance, interpretation, and reporting of chromosomal, biochemical, and enzymatic results. Finally, all concerned must recognize that about 3 to 4 percent of liveborn babies have serious congenital anomalies and an additional 1 to 2 percent will develop severe mental retardation. Thus, all couples take an approximately 5 percent risk of major anomalies

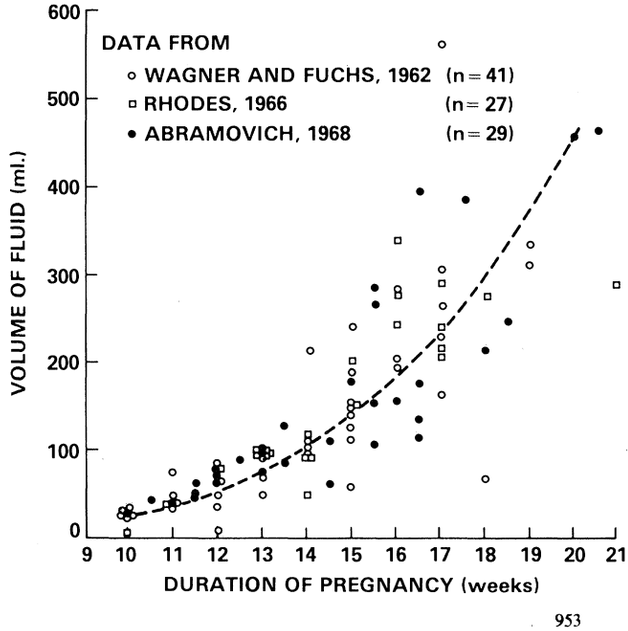

Fig. 2. Data obtained by direct measurements of the volume of amniotic fluid in pregnancies of various times of gestation which had to be terminated by hysterotomy to protect the life of the mother. [From Emery (*1*), with permission]

or mental retardation in their children, from a great many different causes. A normal amniocentesis test for a specific disorder will not guarantee a "normal baby." Prenatal diagnosis of genetic disorders can aim only at reducing the burden for couples with even higher risks to the 5 percent baseline level.

Indications. There are several categories of indications for midtrimester amniocentesis and prenatal diagnosis requiring amniotic fluid and its cells (Table 1). By far the most common are advanced maternal age, a previous child with Down syndrome, and a previous child with neural tube defect.

Cytogenetic disorders. The main genetic risk associated with advanced age of the mother is the occurrence of Down syndrome, due to trisomy 21. Nondisjunction of the two No. 21 chromosomes leads, after fertilization with a normal sperm, to trisomy 21 in the zygote (*4*). The empirical risks for Down syndrome as a function of the mother's age rise sharply from less than 1/1000 before age 30, to 1/500 at about age 35, to 1/100 at age 40, to 1/40 at age 45 and above. Recent prospective data from the U.S. and Canadian collaborative studies suggest that the frequency may already exceed 1 percent in the 35- to 39-year-old group. Given the safety of the procedure and reliability of chromosome testing, most geneticists and many obstetricians now recommend that all women over 35 years of age be offered the amniocentesis procedure. These pregnancies constitute 6 percent of births and more than 25 percent of Down syndrome babies. However, in most states less than 5 percent of the women in this category receive this test. Experience with seeking matched controls for the collaborative study indicates that women who are informed about the test usually elect to have it.

Couples with a previous child affected with Down syndrome are prime candidates for amniocentesis. Already burdened emotionally and financially with the first child, these couples may terminate a subsequent pregnancy unless the prenatal test can be performed to demonstrate that the new baby will be unaffected. The risk of recurrence is about 1 percent, if the first child had the usual trisomy 21. In 5 percent of cases, Down syndrome is due to translocation. Half of these are transmitted by a parent with a balanced translocation. In familial cases, the risk of recurrence is 15 percent if the mother is the carrier and 5 percent if the father is the carrier. Translocations are readily recognized in the cultured amniotic fluid cells.

Other chromosomal abnormalities are not common indications for amniocentesis. However, a previous child with trisomy 13 or trisomy 18 is an indication for testing, since subsequent pregnancies have the same higher risk for trisomy 21 as in a family with a previous trisomy 21. Other kinds of balanced translocations give rise to unbalanced translocations, with severe congenital anomalies. Rarely a detectable chromosome abnormality is responsible for a series of spontaneous miscarriages; the pattern of multiple miscarriages, in addition to a malformed baby and a normal baby, is suggestive of a balanced translocation in one of the parents.

Table 1. Indications for midtrimester amniocentesis.

Cytogenetic studies on cultured amniotic fluid cells
Mother's age >35
Previous child with Down syndrome
Family history or carrier status for chromosomal disorder
Determination of sex in X-linked disorders
Biochemical studies on amniotic fluid
Previous child with neural tube closure defect
Linkage study for myotonic dystrophy
Enzymatic analyses on cultured amniotic fluid cells
Previous child with testable inborn error of metabolism
Couple at risk for Tay-Sachs disease, detected by population screening
Gene studies on cultured amniotic fluid cells
Couple at risk for α-thalassemia

Minor variation in chromosomal appearance is common and generally disregarded. An experienced cytogeneticist is required to make such judgments, and even then uncertainty may persist. Abnormalities of the sex chromosomes (X and Y) are not sources of major congenital anomalies; however, sexual differentiation and mental development are often affected. These sex chromosome syndromes are not rare (>1/1000), so that cases of XXX females, XXY males, and XYY males have been encountered while testing pregnancies for more serious conditions (*5*). Most physicians agree that the patients are entitled to all the information obtained from chromosome studies, not just whether or not the fetus has Down syndrome. In the cases cited, some of the parents elected to continue the pregnancies and accept the birth of children with these particular chromosomal syndromes, while others chose to terminate the pregnancy (*5*).

X-linked disorders. Determination of X and Y chromosome status has special relevance for disorders inherited as X-linked traits, such as hemophilia, Duchenne muscular dystrophy, Fabry disease, and Hunter syndrome. Only boys are expected to be affected, and mothers are carriers. Often the mother has grown up caring for a brother who succumbed to hemophilia or muscular dystrophy. The young woman presents a plaintive plea that she would not risk a pregnancy, let alone a delivery, if there were any chance that her own son would have the very same disease. Sometimes she does not even want to risk passing on the abnormal X-linked gene to a daughter (50 percent likelihood of being a carrier). She and her husband can now exercise the option of having amniocentesis and chromosomal determination of the sex of the fetus, with the plan to go ahead with the pregnancy if it is female (XX) or to terminate the pregnancy if it is male (XY). Much research effort is being directed at development of tests to distinguish affected males from unaffected males. Chromosomal staining tests on the uncultured cells in the amniotic fluid cannot be recommended, since maternal cells too often are present. The use of amniocentesis just to determine the sex of the fetus is not recommended.

Neural tube defects: biochemical studies on the fluid. In the past 6 years (*6*), analysis of α-fetoprotein (AFP) in the amniotic fluid has been developed into an extremely valuable assay for the presence of neural tube closure defect (NTD); this test is based on the leakage of this normal fetal serum protein. By 1976, Milunsky and Alpert were able to summarize results from 2495 midtrimester amniocentesis samples assayed for AFP by electroimmunodiffusion. All samples (22/22) from cases diagnosed to have NTD by other methods (visualization) were positive; of 509 cases with one previous child affected, seven were positive; of 25 cases with two previous children affected, three were positive; and of 1858 routine samples, six were positive and proved to have NTD's. In all, 49 positive diagnoses of NTD were made; the only case missed was a closed lesion with myelomeningocele. These figures are consistent with clinical data indicating a frequency in the general population of approximately one per 500 births (two to three times higher in Irish and Welsh) and recurrence risks in families of 4 percent after one affected child and 8 to 12 percent after two affected children. The mode of inheritance is uncertain, and heterogeneity of underlying mechanisms is strongly suspected. In fact, one particular autosomal recessive syndrome with encephalocele, polycystic kidneys, polydactyly, and other anomalies (known as Meckel syndrome)

may account for some of the recurrences (25 percent recurrence risk). Ultrasound and amniography are important adjuncts to confirm the presence of NTD. The test is relatively nonspecific, since fetal death, spontaneous abortion, stillbirth, twinning, and a variety of major malformations other than NTD are associated with elevations of AFP; with some of these causes, the explanation is fetal blood contamination of the amniotic fluid. A drop or two of fetal blood contains enough AFP to elevate the amount of AFP in the amniotic fluid. Measurement of the fluid content of fetal hemoglobin can be a useful indicator of blood contamination. In the series noted, the use of 2 standard deviations above the mean AFP level as the cutoff of normal led to 122 cases as false positives, with 84 of 110 known outcomes being normal; when 3 standard deviations were used as the cutoff, all 49 open NTD's were still positive, and the false-positive number was reduced to 29, of which 14 had normal pregnancy outcomes. More recently, with a cutoff point of 4 or 5 standard deviations above mean values, several laboratories have achieved better discrimination between true positives (NTD, congenital nephrosis, duodenal atresia, omphalocele) and lesser elevations of AFP associated with normal pregnancies (*7*).

Assay of AFP is clearly indicated for families with previous children with NTD's. Many of these families despaired of having further children, because of the 4 percent recurrence risk and the extreme medical, economic, and social burden of the disorder in affected surviving children. Since the test is inexpensive and can be done directly on frozen and shipped fluid, it is desirable to perform the test on all samples obtained by amniocentesis whatever the primary indication. When AFP levels are minimally elevated however, a decision about what to tell the patient can be difficult; depending upon the level, additional studies to visualize the fetus by ultrasound or radiography may be required.

One of the other causes of elevated AFP in the amniotic fluid is nephrosis. Seppala *et al.* (*8*) have used amniocentesis and AFP testing for nine families in which a previous child was affected with an autosomal recessive form of congenital nephrosis, which is unresponsive to any treatment. Seven were normal, while two showed marked elevation of AFP in the amniotic fluid and evidence of congenital nephrosis, after termination of the two pregnancies, in the fetal kidneys examined (*8*). Of some 200 reported cases, half have been identified in Finland. As is often the case, rare genetic disorders may occur with much higher frequency in certain geographically isolated or inbred populations.

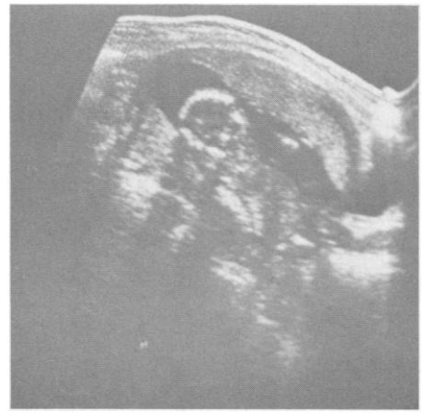

Fig. 3. Photograph of ultrasound recording of fetus just before amniocentesis. The placenta is in the anterior position between the mother's abdominal wall (top) and the amniotic fluid sac containing the fetus. The target for the amniocentesis needle is to the left of the fetus' head (largest volume and thinnest transplacental route). [Courtesy of M. J. Mahoney, Yale University]

A special indirect approach with the use of amniotic fluid takes advantage of linkage between the gene for an otherwise untestable disease and a marker gene. The only current application of this approach for autosomal diseases is the prediction of inheritance of myotonic muscular dystrophy from the pattern of inheritance of the linked gene determining secretion of ABH blood group substances in the amniotic fluid (*9*).

Inborn errors of metabolism. Despite the convenience of direct assays on the amniotic fluid sample, few applications are available, since metabolites rapidly equilibrate with the maternal circulation and since reliable diagnoses of inborn errors of metabolism require assays of enzyme activities within the fetal cells. As a general rule, any autosomal recessive or X-linked recessive metabolic disorder is thought to be due to an enzyme deficiency, and any enzyme deficiency that can be demonstrated in cultured fibroblasts from skin biopsies of children or adults is likely to be demonstrable also in cultured cells from the amniotic fluid. Conversely, enzymes not normally measurable in fibroblasts, such as the liver enzyme that is deficient in phenylketonuria, cannot be tested in amniotic fluid cells. Several dozen inborn errors of metabolism, therefore, can be tested in cultured amniotic fluid cells. Published lists indicate those which have been detected, as well as many additional disorders for which prenatal biochemical diagnosis is feasible. These include various disorders of amino acid, lipid, mucopolysaccharide, and carbohydrate metabolism (*10*). The laboratory work required is often highly sophisticated, since the disorders are rare and the assays complex. The precise biochemical type of a clinically diagnosed metabolic disorder is required to plan the proper assays. Cells with abnormal metabolism often grow poorly in culture media, so that few cells are obtained even after waiting well beyond the usual 3 weeks of culture time. For these reasons, considerable effort is being directed to microanalytical methods (*11*). Research laboratories specializing in these rare diseases must be consulted in advance to arrange for appropriate analyses.

The single disorder best suited to enzymatic assay is Tay-Sachs disease, a devastating condition in which sphingolipid is accumulated because of a block in its degradative pathway. Apparently normal newborns develop blindness at 4 to 6 months of age and begin a progressive neurological deterioration leading to death. Tay-Sachs is particularly frequent among Ashkenazic Jews (100 times more frequent than among non-Jews). As with all of the other autosomal recessive errors of metabolism, the recurrence risk after the birth of an affected child is 25 percent. In Tay-Sachs disease, the responsible enzyme (*N*-acetylhexosaminidase A) can be measured as deficient in many tissues, including skin and amniotic cells, as well as in serum samples. Therefore, about 10 years ago, Kaback initiated what has become a major public health program in metropolitan Jewish populations: screening serum samples from young women to detect those with half-normal enzyme levels indicative of heterozygote (carrier) status, then screening their mates, and finally monitoring pregnancies in which both parents are carriers to detect affected fetuses (the risk is 1/4) in families without any previous child affected. Although the enzyme assays have been complicated by the natural occurrence of several variants of the enzyme, the screening program has been highly successful (*12*).

An exception to the rule that enzyme deficiencies are inherited as recessive metabolic disorders is acute intermittent porphyria (AIP). This disease is inherited as an autosomal dominant and is characterized by a defect in the biosynthesis of heme, with excessive excretion of porphyrin precursors in the urine. Uroporphyrinogen I synthetase has been demonstrated to have approximately half-normal activity in liver, erythro-

cytes, and cultured skin fibroblasts and amniotic cells. Thus, it has been feasible to diagnose a carrier of this dominant gene from midtrimester cells, with confirmation 15 months after birth of the infant (*13*).

Gene studies on cultured cells. The most elegant biochemical diagnosis to date is the detection of α-thalassemia in cultured fibroblasts by the technique of molecular DNA-DNA hybridization (*14*). α-Thalassemia is a lethal disease due to insufficient production of α-globin chains essential for both fetal and adult hemoglobin. The disease is caused by deletion of genes (two pairs) specifying the α-globin polypeptides; when only three of the four genes are absent or two of the four, a progressively less severe hematological disorder results. Hemoglobin is not produced in fibroblastic cells; as noted below, analyses of hemoglobins in the fetus require blood samples. However, the genes for any protein product are present in all the nucleated cells of the body. Therefore, Kan *et al.* (*14*) were able to assay for the presence and relative number of α-globin genes, to distinguish the α-thalassemia syndromes by carefully quantitated hybridization of radioactive DNA complementary to α-globin genes with DNA from amniotic fluid cells. α-Thalassemia is rare in the United States, but common in south Asian countries.

Stage 2 Approach: Visualization of the Fetus

For the great majority of the many inherited diseases and birth defects there are no specific chromosomal or biochemical tests at present. A more direct approach to detection of significant abnormalities already manifest in the midtrimester of pregnancy is by visualization of the fetus. The three available methods are visual inspection with an instrument (fetoscope) inserted into the uterus, x-ray examination with or without injection of radiopaque dyes to outline the structures, and ultrasonographic scans.

Fetoscopy. Between 1968 and 1974, there was considerable enthusiasm for direct visualization of the fetus, in order to detect NTD's and other major anomalies associated with mental retardation or poor organ function (or both). There was progress in the instrumentation, with sophisticated fiberoptics, multiple lens systems, and video augmentation (*15*). However, relatively few applications of fetoscopy have been made to date because (i) noninvasive ultrasonographic methods developed much more rapidly, (ii) the AFP test provided a simple and safe alternative for most cases of open NTD's, and (iii) the narrow-diameter, rigid fetoscopes permitted only very limited views of fetal parts—often insufficient for orientation—and entailed significant risk of inducing hemorrhage or miscarriage (*15*). More recently, fine fetoscopes termed needlescopes (Hobbins) have been used as an aid to obtaining fetal blood samples (see below.)

Radiographic visualization. In order to provide early prenatal diagnosis in several conditions which are associated with an abnormal skeleton, but in which no biochemical or chromosomal abnormalities have been recognized, fetal radiography has been utilized. The syndrome of thrombocytopenia with absent radii (TAR) and severe dwarfing disorders, such as homozygous achondroplasia, are well-suited for this approach (*16*). Skeletal ossification is sufficiently well advanced by 16 weeks of pregnancy to permit identification and characterization of tubular bones, but parts of the pelvis, spine, and skull are still too poorly mineralized to be assessed.

Amniography, combining injection of radiopaque dyes with radiography, has been used late in pregnancy to outline fetal head, body surface, and intestinal configuration. In the second trimester, however, it has been unreliable in the major application, namely detection of NTD's. Even open defects have been missed entirely (*17*).

Ultrasound techniques. These approaches are based on the principle of transmitting high-frequency, low-intensity, pulsed ultrasonic waves through the body and detecting and displaying the reflected sound waves on a cathode-ray oscilloscope (Fig. 3), mapping tissue planes and discontinuities. Real-time ultrasonographic visualization of the fetus has emerged as a highly versatile, noninvasive, and reliable approach to visualization. It is readily combined with other methods, including amniocentesis, radiography, fetoscopy, and placental aspiration, to monitor the positioning of the placenta and fetal parts, to detect twins, and to estimate fetal age (*18*). Adaptations such as B-mode scanning permit visualization of internal structures of the fetus. The optimal time for ultrasound scanning is between 16 and 19 weeks of pregnancy. For seeking NTD's, scans are made first longitudinally to show the full length of the neural canal, and then transversely, in series, from the head to the pelvis. When the spine is normal, the neural canal is displayed on the transverse scan as a discrete circle, but when there is spina bifida, a U-shaped abnormality is noted. Thus, ultrasound provides an effective means of confirming cases suspected of NTD's from high values of the AFP. The dimensions of the head can be assessed quantitatively to estimate fetal age and to permit diagnosis or exclusion of autosomal recessive microcephaly (*19*), as well as anencephaly, hydrocephaly, and encephalocele (*18*). Ultrasound scans can be repeated over a period of weeks to assess the progression of findings and the rate of fetal growth.

In two pregnancies at risk for the autosomal recessive Ellis van Creveld syndrome of short-limbed dwarfism, congenital heart disease, and polydactyly, Mahoney and Hobbins used the needlescope to count the number of fingers and ultrasound to measure the length of the limb bones, and their resulting predictions in the two cases were correct (*20*). Ultrasound is likely to become useful in diagnosis of some kinds of congenital heart disease. It is already useful in polycystic kidney disease (*21*).

Stage 3 Approach: Sampling Fetal Blood and Serum

A number of important diseases that can be diagnosed very reliably with blood samples in children and adults cannot be diagnosed in utero with amniotic fluid samples because the relevant characteristics are simply not expressed in amniotic fluid cells. The most important examples are abnormalities of hemoglobin synthesis and hemoglobin structure in red blood cells, the β-thalassemias ("Cooley's anemia") and sickle cell anemia. These autosomal recessive disorders are very frequent in certain ethnic populations, carriers can be detected with a simple blood test, and many couples who are both carriers are fearful their children will inherit the disease (risk 1/4). β-Thalassemias are especially common among people of Mediterranean origin; the frequency of carriers in Sardinia is 12 percent, in Greek Cypriots 15 percent, and in many other groups 5 to 10 percent. Thus, the frequency of the homozygous disease state is one per 250 newborns in Sardinia and one per 200 newborns in the Greek Cypriots. Similarly, sickle cell anemia occurs primarily among blacks, with frequency of one per 600 newborns, reflecting an 8 percent frequency of carriers. These are not the only disorders that can be detected with

blood samples; enzyme deficiences in red and white blood cells, membrane abnormalities, platelet disorders, deficiencies of clotting factors and other plasma proteins, and elevations of plasma enzymes such as creatine phosphokinase (CPK) from muscle in muscular dystrophy may be detectable. However, the β-thalassemias and sickle cell anemia are the most common disorders being investigated in fetal blood samples.

In view of the worldwide distribution of the people at risk, especially for thalassemias, and the need for highly sophisticated laboratories to carry out the needed protein biosynthesis and hemoglobin molecular separation experiments, extensive international collaboration has developed (*22*). In February 1978, a group of investigators from all over the world met in Los Angeles under the sponsorship of the Sickle Cell Branch of the National Heart, Lung, and Blood Institute to assess the current status of prenatal diagnosis of hemoglobinopathies. About 300 different pregnancies have been studied, sometimes in multiple laboratories, after fetoscopy or placental aspiration (or both) to obtain fetal blood suitable for hemoglobin analyses. Thus, this field is now at about the same level of experience as amniocentesis for chromosomal and biochemical analyses (stage 1 technology) was in the beginning of 1971 when the National Amniocentesis Collaborative Study was undertaken to assess the safety and efficacy of the procedures and laboratory work. The laboratory demands for β-thalassemia diagnoses are much greater than for chromosomal work; an active laboratory can process only three samples per week at present. The blood samples are highly variable, ranging in volume from 1 to 400 microliters and ranging in content of fetal to maternal blood from less than 5 to 99.9 percent. In every group, there are cases in which insufficient fetal blood is obtained by either fetoscopy or aspiration; and fetal loss is about 5 to 10 percent in most major series, with fewer complications as experience has been gained. Several fetuses with sickle cell disease and more than 40 with homozygous β-thalassemias have been diagnosed with this approach.

Considerable progress has been made toward eliminating admixture of maternal blood (*22*). The percentage of blood that is fetal is readily determined with a Coulter counter on a sample before and after the adult cells are removed by lysis with alkali. Arithmetical corrections for the percentage of maternal blood have proved unsatisfactory, so that immunological or metabolic tricks are used to enrich the fetal cell proportion. In the first, antiserum to i factor is added to reversibly agglutinate fetal cells and separate them from the maternal; in the second, a carbonic anhydrase enzyme inhibitor (acetazolamide) is added to isotonic solutions containing ammonium chloride and ammonium bicarbonate, leading to highly preferential lysis of maternal cells because of their much greater carbonic anhydrase activity (Ørskov reaction) (*23*). These laboratory methods are replacing that formerly used, namely, transfusing the mother before the placental aspiration in order to suppress reticulocyte formation in the mother. Quantitative precision in determination of ratios of biosynthetic rates for β- and γ-chains is essential in attempting to distinguish homozygotes from heterozygotes for β^+-thalassemias (*22*).

Much continued progress in the techniques for sampling fetal blood and for analyzing globin or hemoglobin fractions can be expected. Improved safety in the sampling procedure and simplification of the laboratory methods may be essential before much larger numbers of pregnancies can be tested.

An even greater challenge is involved in the prenatal diagnosis of Duchenne muscular dystrophy via the fetal serum CPK activity. At least 26 male fetuses at risk for this fatal muscular dystrophy have been tested (*24*). Two positive cases have been diagnosed thus far; when pregnancy was terminated, histological changes suggestive of Duchenne muscular dystrophy were demonstrable in the fetal muscle. Requirements for the CPK assay are plasma, rather than blood cells; a larger volume than that sufficient for hemoglobin experiments; and plasma free of maternal contamination. If the technique can be perfected, the same sampling may be useful for analysis of factor VIII clotting activity to diagnose hemophilia. In both of these diseases, such capability would make it feasible to determine which male fetuses were actually affected, rather than only distinguishing male from female fetuses for these X-linked disorders.

Stage 4: Detection of Fetal Proteins or Cells in Maternal Blood

Small numbers of fetal blood cells and diffusible fetal metabolites and proteins cross into the circulation through the placenta. Efforts are now well under way to achieve initial screening for fetal abnormalities with samples of maternal plasma, rather than relying entirely on amniocentesis, visualization, or blood sampling of the fetus. The most progress has been made with the use of the AFP test to detect NTD's. More than 90 percent of such cases occur in families with no previous history. These pregnancies would not be subjected to amniocentesis for an AFP test. Brock noted in 1972 that maternal plasma from pregnancies which produced a child affected with NTD had elevated AFP. Measurements now have been made on thousands of pregnant women to establish normative curves of AFP values as a function of weeks of gestation.

In the U.K. experience, maternal serum AFP at 16 to 18 weeks exceeded by 2.5 times the normal median value in 90 percent of anencephalic, 80 percent of open spina bifida, and 3 percent of normal singleton pregnancies (*25*). If positive, the maternal test is followed by sonography to rule out multiple pregnancies, which often produce elevated maternal AFP, and then by determination of AFP on amniotic fluid to identify abnormal fetuses. Since the incidence of NTD in Britain (4.5 per 1000 births) is at least twice that in the United States, there is considerable interest in Britain to make such maternal plasma screening available to all women, according to an "all-party motion" signed by more than 200 members in the British Parliament (*26*). In a major U.S. prospective study on Long Island (*27*), 3800 pregnancies have been screened, of which 80 (2 percent) were above the 98th percentile threshold for further testing; after sonography and amniocentesis, seven cases with NTD were diagnosed correctly. Two other affected fetuses were missed, one because the mother was tested too early (week 13) and the other because the mother's AFP concentration was below the cutoff (96th percentile). Considerably more data will be required to assess the cost-effectiveness, reliability, public understanding, and acceptance of such voluntary screening programs.

Fetal metabolites appearing in maternal urine may be the basis for certain kinds of diagnosis, as in the case of methylmalonic aciduria (*28*). Other fetal proteins and fetal cells might be detected in the maternal circulation. Red blood cells do cross, producing feto-maternal transfusion, after such manipulations as amniocentesis, fetoscopy, and placental aspiration and may be a source of sensitization of the mother if she and the fetus are Rh-incompatible. Boyer *et al.* (*23*) used the Ørskov hemolytic reaction to

attempt to enrich the fraction of fetal cells in the maternal circulation. Others are working with laser-activated cell sorting equipment to detect fetal cells labeled with fluorescent antibody to histocompatibility antigens or other antigens that distinguish fetal cells from maternal cells in a particular pregnancy. Such maneuvers may eventually provide valuable new tests for routine prenatal care.

Stage 5 Approach: Treatment of Genetic Disorders In Utero

It is unsatisfactory that, for most genetic disorders diagnosed in midtrimester, the only active option is termination of the pregnancy to prevent the birth of a severely impaired child. Of course, there are all too many diseases diagnosed after birth for which there is no effective treatment. Efforts are being directed at devising means to prevent the initiation or progression of a disease process in utero. Only one notable success can be cited (*28*). Methylmalonic aciduria is an autosomal recessive disorder characterized clinically by recurrent vomiting, failure to grow, mental retardation, and life-threatening acidosis. At least four different biochemical defects result in accumulation of methylmalonic acid, two of which are blocks in the activation of vitamin B_{12}; the metabolic and clinical abnormalities can respond to high doses of vitamin. In a family with a previous affected child who died at 3 months of age, amniocentesis and biochemical studies at 19 weeks of pregnancy revealed that the next child also would be affected. It was already known that the methylmalonic acid accumulates during gestation and spills over into the mother's urine. Thus, it was feasible to administer large doses of oral cyanocobalamin (vitamin B_{12}) to the mother and monitor its effect on the fetus by measuring the methylmalonic acid in the mother's urine. Continued postnatal treatment has supported altogether normal development for this child.

Other prospects for effective treatment in utero are very limited at present. There are several other rare vitamin-responsive inborn errors of metabolism. Dietary manipulation may be indicated in cases of galactosemia; and prenatal administration of small doses of cortisol or thyroxine might be indicated in adrenogenital syndrome and hypothyroidism, respectively.

Conclusions

The technological capabilities for prenatal diagnosis of genetic disorders have grown remarkably in the past decade. That growth has been stepwise, with a logical progression of techniques and with early organized efforts to determine safety and validate effectiveness. Amniocentesis for mothers over age 35 and for couples with previous children with Down syndrome, NTD's, Tay-Sachs disease and other genetic disorders with known risks of recurrence now reaches only a very small fraction of all those who might elect to use this procedure were they aware of its usefulness. A great many families are fearful of having additional children or desperate to terminate an unplanned pregnancy because of these recurrence risks. Amniocentesis and appropriate tests can help these families to plan further children and to complete the vast majority of pregnancies because of the assurance that the baby will not be affected with the disease they fear most. In a small number of cases, less than 5 percent, the couple may have to choose between having an affected child and terminating the pregnancy, admittedly a wrenching decision for many couples.

The advances in prenatal diagnosis of genetic disorders have been built on a broad base of biomedical research into the mechanisms of birth defects and metabolic disorders, a host of collaborations with specialists in medical instrumentation, and a wide consultation with concerned laypersons and interested social scientists. It is clear that an important path bridging medicine, public health, and health education has been opened and that many technical innovations, demands for services, and concerns about the needs and rights of all parties will converge on that path.

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