The Prenatal Diagnosis of Metabolic Disorders

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The possibility of prenatal diagnosis of certain inherited metabolic disorders is based on the knowledge that many of them, particularly those showing simple recessive inheritance, are caused by deficiency of a specific enzyme which may be detectable in cultured cells, including those of amniotic fluid. In addition, the technique of amniocentesis, developed for the management of Rhesus haemolytic disease and adapted for the detection of chromosomal disorders, has now been applied to the diagnosis of inherited metabolic diseases in early pregnancy.

AMNIOCENTESIS

At present, making a prenatal diagnosis depends almost entirely on obtaining, culturing and analysing a sample of amniotic fluid, though other techniques such as diagnostic ultrasonics are developing rapidly and may in time prove to be safe and useful adjuncts (Campbell, 1972). Obtaining the fluid is now, in skilled hands, a relatively simple procedure; the transabdominal route is generally used, under local anaesthesia. In the largest reported series in early pregnancy (Nadler and Gerbie, 1970) 162 amniocenteses were done with no maternal or fetal complications and cultured cells were obtained in 97 per cent of the cases. It is important, however, to realise that problems (Table 1) can arise which may result in failure to obtain satisfactory results, or, more rarely, harm fetus or mother.

Failure to obtain fluid usually results from attempting amniocentesis too early in pregnancy, when the fluid volume is very small; the 14th to 16th weeks of gestation are probably the best time. A bloody tap may result from entering the placenta; not only may this result in a useless sample, but it may produce Rhesus isoimmunisation of the mother (Woo Wang et al., 1967). Localisation of the placenta by ultrasound will reduce the likelihood of this occurrence (Curtis et al., 1972), and may also demonstrate a twin pregnancy. The risk of precipitating abortion is less than 1 per cent when amniocentesis is done transabdominally; it was much higher when the vaginal route was used, as also was the risk of infection.

So far we have no evidence that removal of amniotic fluid causes any long-term effect on the fetus but it has been pointed out (Fuchs, 1971) that even

such a serious effect as a postulated drop of 25 points in IQ might not be recognisable until some years after birth.

The sample obtained (usually 10 to 20 ml) consists of the supernatant fluid and the cells within it. Studies of the composition of the fluid have shown considerable variation in concentrations of amino acids and other components (Emery *et al.*, 1970); some components such as blood group substances are fetal in origin (Harper *et al.*, 1971) while other proteins, such as group-specific component, appear to be maternal (Sutcliffe *et al.*, 1972). The cells in amniotic fluid are almost entirely fetal, and it is the existence of these viable cells reflecting fetal genotype that provides the basis for most of our present methods of intrauterine diagnosis.

INDICATIONS FOR PRENATAL DIAGNOSIS

There are basically two reasons for wanting to make a diagnosis *in utero*. In late pregnancy one may wish to diagnose a treatable condition in a fetus at risk so that therapy can be started before or immediately after birth. There are very few such indications at present; perhaps the best established is the adreno-genital syndrome, due to $21-\beta$-hydroxylase deficiency, which can be diagnosed in late (Jeffcoate *et al.*, 1965; Nichols, 1969) but not in early pregnancy (Merkatz *et al.*, 1969), by analysis of amniotic fluid steroids.

More commonly the aim is to diagnose an untreatable disorder sufficiently early in pregnancy to offer an abortion if an affected fetus is found. In these circumstances there must be certain criteria (Table 2) to judge whether a particular condition is really suitable for prenatal diagnosis.

If inherited metabolic disorders are both severe and untreatable there is no doubt about the desirability of prenatal diagnosis. The various lysosomal enzyme defects such as the mucopolysaccharidoses and the cerebral lipidoses (Table 3) fall into this category, but there is little justification for prenatal diagnosis in a disorder such as cystathioninuria, which appears to be an entirely harmless inborn error of amino acid metabolism (Perry, 1971). For severe disorders such as galactosaemia and phenylketonuria, which are treatable, the situation is much less clear, as the latter condition cannot yet be diagnosed *in utero*.

The practising physician or paediatrician faced with a genetic disorder will want to know whether prenatal diagnosis is established and available, or whether it is just a theoretical possibility.

At present, the diseases that can be diagnosed prenatally are mainly those in which the basic biochemical defect is an enzyme deficiency, which can be detected in cultured amniotic fluid cells.

Table 3 shows some of the biochemical disorders that can be prenatally

TABLE 1. Problems and Risks in
Diagnostic Amniocentesis

Failure to obtain fluid
Fluid contaminated by maternal blood
Twin pregnancy
Failure of cells to grow
Maternal cells cultured, not fetal
Risk of precipitating abortion
Damage to fetus
Rh isoimmunisation of mother

TABLE 2. Criteria for Prenatal
Diagnosis

1. Accurate prenatal diagnosis possible
2. High risk to pregnancy in question
3. Couple concerned agreeable to termin-
   ation of affected pregnancy
4. Severity of condition sufficient to
   warrant termination
5. Lack of effective treatment

TABLE 3. Metabolic Disorders Diagnosable *in utero*
using Cultured Amniotic Fluid Cells

	Diagnosis made and confirmed	*Diagnosis possible*
Lipidoses	Tay Sachs disease (O'Brien, 1971)	Juvenile G M (1) Gangliosidosis (O'Brien, 1971)
	Niemann Pick disease (Epstein *et al.*, 1971)	Generalised gangliosidosis (O'Brien, 1971)
	Metachromatic leuco-dystrophy (Nadler and Gerbie, 1970)	Refsum's disease (Steinberg, 1972)
	Krabbe's disease (Epstein *et al.*, 1972)	Sandhoff's Disease (O'Brien, 1971)
	Gaucher's disease (Epstein *et al.*, 1972)	
	Fabry's disease	
Mucopolysaccharidoses	Type I (Hurler's syndrome). (Fratantoni *et al.*, 1969)	Types II–VI
Amino acid disorders		Maple syrup urine disease (Nadler and Gerbie, 1970)
		Homocystinuria (Uhlendorf and Mudd, 1968)
		Cystathioninuria
		Argininosuccinic aciduria (Shih and Littlefield, 1970)
		Cystinosis (Schulman *et al.*, 1970)
Carbohydrate disorders	Glycogenosis type II (Nadler and Messina, 1969)	
	Glucose-6-phosphate dehydrogenase deficiency	
	Galactosaemia (Nadler, 1968)	
Other	Lesch Nyhan syndrome (Fujimoto *et al.*, 1968)	Congenital erythropoietic porphyria (Romeo *et al.*, 1970)
	Lysosomal acid phosphatase deficiency (Nadler and Egan, 1970)	Xeroderma pigmentosum (Regan *et al.*, 1971)

diagnosed. The list is not exhaustive; full lists of disorders diagnosable *in utero*, together with the underlying biochemical defects, are given in the excellent reviews of Milunsky *et al.* (1970) and Raine (1972)

The first column of the table lists disorders in which *in utero* diagnosis of an affected fetus was made and subsequently confirmed by examination of the terminated pregnancy or by the birth of an affected child. The second column lists disorders in which prenatal diagnosis appears to be feasible, as the defect can be demonstrated in cultured amniotic fluid cells, but the diagnosis of an affected fetus has not yet been made. In some cases pregnancies at risk have been monitored, a normal enzyme level found, and a normal child subsequently born.

The most important group is probably that of the lipidoses, progressive, untreatable and fatal cerebral degenerations of childhood, in which the accumulation of glycolipids in the cells is caused by the absence of the specific lysosomal enzymes normally responsible for their breakdown (O'Brien, 1971).

Most of the disorders shown in Table 3 are characterised by the complete or almost complete absence of activity of the particular enzyme; the exceptions are worth special mention.

In Hurler's syndrome, in which accumulation of mucopolysaccharide causes progressive mental deterioration and characteristic physical abnormalities, the affected fetus is identified by the abnormal accumulation of isotopically labelled sulphate in the cultured amniotic fluid cells (Neufeld and Fratantoni, 1970; Fratantoni *et al.*, 1969). The recent demonstration of α-L-iduronidase as the basic enzymatic defect in Hurler's syndrome (Weissmann and Santiago, 1972) is likely to lead to prenatal diagnosis by enzyme assay.

Cystinosis, although classed in Table 3 as an amino-acid disorder, is primarily a storage disease; blood levels of cystine are normal, but cystine is deposited intracellularly in the kidney and other organs. Although the precise enzyme defect is unknown, a prenatal diagnosis can be made from the greatly increased cystine content and sulphate uptake of amniotic fluid cells (Schulman *et al.*, 1970). Xeroderma pigmentosum can now be classed as a metabolic disease because the tendency to skin cancer has been shown to be caused by the absence of a specific enzyme involved in the normal repair processes that exist to correct chromosomal damage from ultraviolet and other irradiation (Cleaver, 1968). Cultured cells from affected individuals fail to incorporate labelled constituents of DNA when irradiated, indicating a failure of repair.

A considerable number of simply inherited inborn errors of metabolism remain undiagnosable *in utero*, including some of the more common and severe disorders. Among these are cystic fibrosis, phenylketonuria, haemophilia,

and the haemoglobinopathies. The reasons for our present inability to make a prenatal diagnosis vary in each instance.

In the case of cystic fibrosis, the commonest simple inherited metabolic disease of childhood in Europe, and one in which prenatal diagnosis would be of great value, the basic metabolic defect is unknown. Various histochemical abnormalities in cultured cells have been described (Danes and Bearn, 1969), but none is sufficiently reliable or specific to warrant its use in prenatal diagnosis.

The enzyme defect of phenylketonuria is known (phenylalanine hydroxylase in almost all cases), but this enzyme is normally confined to the liver, and is not normally present in cultured skin or amniotic fluid cells. For histidinaemia the enzyme concerned (histidase) occurs in skin, but is not found even in normal cultured skin fibroblasts or amniotic fluid cells. In haemophilia and the haemoglobinopathies the non-enzymic protein products are not found in cultured amniotic fluid cells. The cells must possess the genetic coding for these and other specialised products, but as yet no method has been devised of stimulating their synthesis.

At present it is impossible to diagnose prenatally the more complex metabolic disorders such as diabetes mellitus without much more specific information about the basis of their inheritance and their fundamental biochemical defects.

Culture of amniotic fluid cells is difficult; there is a considerable risk of failure due to infection and other technical problems. It also takes a number of weeks to provide a result. These factors have stimulated attempts to use the uncultured cells in the sample of amniotic fluid itself. Initial work suggested that the uncultured cells showed the same range of enzymes as cultured cells (Nadler and Gerbie, 1969; Schneck et al., 1970), but early enthusiasm was dampened by misdiagnosis caused partly by the presence of many dead or dying cells among the viable ones, which led to misinterpretation of the results of enzyme analysis (Sutcliffe and Brock, 1971).

The use of the supernatant fluid has been disappointing. It was hoped that the amino-acid levels (Emery et al., 1970) might be helpful in the diagnosis of such conditions as phenylketonuria and homocystinuria, but this is not so. The only disorder of this type that can be usefully diagnosed is methylmalonic acidaemia, a very rare inborn error of short-chain fatty acid breakdown (Morrow et al., 1970) but the use of cultured cells is likely to be more reliable in early pregnancy.

It was also thought that analysis of the mucopolysaccharides in amniotic fluid would make it possible to diagnose Hurler's syndrome and other mucopolysaccharidoses (Matalon et al., 1970); however, affected children have been

born after a normal amniotic fluid analysis (Brock *et al.*, 1971; Matalon *et al.*, 1972) so there is no alternative to the use of cultured cells.

The great majority of genetic disorders are undiagnosable *in utero* at present and many are likely to remain so. Nevertheless, it is sometimes possible to use indirect methods to predict whether or not a fetus is at risk, particularly in the group of disorders showing X-linked inheritance. At least 80 such conditions are known (McKusick, 1971) including such severe disorders as haemophilia A and B, Duchenne muscular dystrophy, and the Hunter (Type II) form of mucopolysaccharidosis.

Because these conditions are in general transmitted by healthy female carriers but affect only males, it is possible to test the pregnancy of a known or suspected carrier and to offer termination if the fetus is a male. Uncultured amniotic fluid cells are tested for the X chromatin or Barr body that represents the second condensed X chromosome, present only in the female; the Y chromosome of the male can be identified by the new fluorescent technique which shows it as a brightly fluorescing body (Pearson *et al.*, 1970). Fetal sexing does not differentiate between an affected and an unaffected male; such differentiation can be achieved only in those few X-linked disorders, such as the Lesch Nyhan and Hunter Syndrome, in which a direct method of prenatal diagnosis is feasible.

Comparable indirect prediction by the use of genetic markers may become possible for disorders that are not sex-linked. If a genetic marker such as a blood group is closely located on the same chromosome it may be possible to infer whether or not a harmful gene has been inherited even if the gene cannot be detected *in utero*. Thus, in the case of myotonic dystrophy close genetic linkage has been shown with the locus controlling ABH blood group secretion (Renwick *et al.*, 1971; Harper *et al.*, 1972). The secretor status of the fetus can be identified from analysis of amniotic fluid in early pregnancy (Harper *et al.*, 1971) providing the possibility of predicting with about 90 per cent of accuracy whether or not the fetus has inherited myotonic dystrophy, though unfortunately only a minority of couples have the correct combination of secretor types to allow such information to be used.

FUTURE DEVELOPMENTS

In considering future advances, first in potential importance is the technique of fetoscopy. A fibre-optic instrument has been developed, and is being used by obstetricians in Edinburgh and in Cardiff (McGarry, J. 1972, personal communication), which can be inserted percutaneously into the pregnant uterus as early as the 14th week of pregnancy, to allow direct inspection of the fetus. This has important applications for such structural conditions as spina

bifida and inherited limb defects, but it also offers the possibility of taking a fetal blood sample, either from the umbilical cord or from the fetus itself.

From such a sample it may become possible to diagnose a number of conditions prenatally, including haemophilia A and B, the red cell enzyme defects, acute intermittent porphyria, and the haemoglobinopathies, as well as the blood group antigens.

Secondly, the possibility of amniocentesis becoming a routine procedure in all pregnancies must be considered and that prenatal screening for metabolic diseases might be done in a way comparable to the present screening of newborn children for phenylketonuria and other disorders. This cannot be justified in our present state of knowledge of inherited metabolic diseases, but the situation may develop whether one wants it or not, because in some centres amniocentesis is performed on all pregnancies of older women to detect chromosomal defects. If it were to become a widespread practice it might be difficult to refuse to do biochemical tests at the same time.

A more fruitful approach is being adopted in some Jewish communities in the United States where Tay Sachs disease is a relatively common condition. The adult population is being screened to detect the heterozygous carriers of the gene, who show partial deficiency of the enzyme hexosaminidase A (Kaback, 1972). Prenatal diagnosis by amniocentesis can then be offered only to the couples who are both carriers and hence risk having an affected child. Such a programme makes potentially feasible the complete prevention of the birth of affected individuals in this population, but, even if the programme were to be completely successful, it would have no significant effect on the frequency in the population of the harmful gene since the great majority of such genes are carried and transmitted by healthy heterozygotes, not by affected homozygotes (Motulsky, 1971). The development of comparable methods of carrier detection for other inherited metabolic diseases, particularly for the commoner conditions such as cystic fibrosis in Europe and sickle cell disease in American negroes, might prove rewarding.

Prenatal diagnosis is becoming a powerful tool in the detection and prevention of inherited metabolic diseases. As yet its application in clinical practice is limited to a few relatively uncommon disorders, and it is important that enthusiasm should not run ahead of clinical judgement if there is to be a real benefit to families afflicted with these diseases. Properly used, however, there can be little doubt that within a few years, prenatal diagnosis will be playing an important role in an increasing number of these conditions.

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