Prenatal Diagnosis of Disease

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'Treatment' in fetal life is viewed quite differently from treatment in adult life, for the span of fetal life is divided by the two important milestones that mark the outer limit for abortion and the inner limit for viability. At present they stand at 20 and 28 weeks but are gradually being pushed towards each other by social opinion from one side and the efforts of obstetricians and paediatricians from the other. Indeed, an outer limit for abortion at 28 weeks is accepted. Fetal 'treatment' is in a way analogous to selective schooling where rejection of one group is followed by an intensive effort that will ensure that the selected group achieves success. But, unfortunately, selection is in both cases imperfect, and undetected flaws that appear later have to be dealt with before the goal is achieved. In pregnancy this selection leads to rejection and abortion of some, followed by great efforts to ensure the survival and normality of the remainder. This situation has partly arisen as young couples have become constrained to restrict their families, and it is they, in turn, who now demand that those they bear should be normal. The criteria used to achieve this end can be expressed more elaborately as follows—

1. The fetus with certain incurable diseases or serious malformations should be aborted, preferably before 20 weeks.
2. There is some advantage in recognising treatable malformations or disease during fetal life so that treatment can be given at the optimum time.
3. The fetus that is found in later pregnancy to be living in a hostile uterine environment and is ill as a result should be delivered as soon as survival seems possible.

Current diagnostic efforts are aimed at these goals.

There are set times for the detection and diagnosis of fetal disease and malformation represented by the boxes A, B and C in Fig. 1. Period 'A' represents a few intense weeks when the search is principally for spina bifida and mongolism. A number of rare and very rare but serious conditions can also be looked for.

The contents of periods 'B' and 'C' are of a somewhat different nature. First of all they represent groups of different and, on the whole, unexpected signs and complications of pregnancy that can signify fetal disease and it is these which have been used to separate 'B' from 'C'. The second reason for separating period 'C' from 'B' is to highlight a second period of active fetal testing to determine the state of fetal health and chance of survival after birth and the considerable efforts made by obstetricians to achieve this.

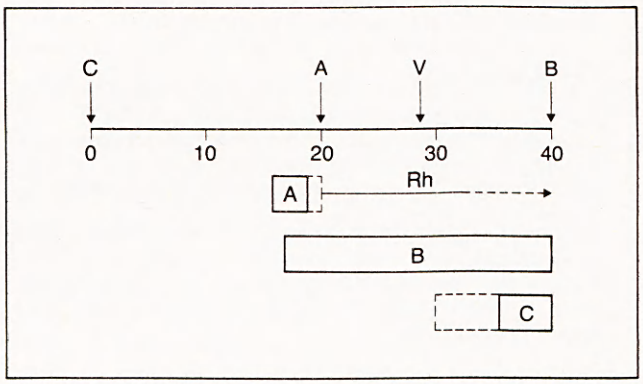


Fig. 1. *A diagrammatic representation of pregnancy. C = conception. B = birth. A = outer 'legal' limit for abortion. V = viability if fetus born prematurely. For contents of boxes 'A', 'B' and 'C' see text.*

During period 'B' two ominous signs appear. The first one is poor fetal growth and the second hydramnios. Poor fetal growth is more than a clinical impression. Fetal size can be assessed by ultrasound measurement of the fetus, principally by measuring the biparietal diameter, but the other measurements can be used and repeated frequently during pregnancy to determine the growth rate. The causes of smallness are heterogeneous: maternal, placental and fetal influences acting singly or in combination. Unfortunately, it is often difficult to determine which is the more important and, once poor fetal growth has been confirmed, the obstetrician simply has to follow the growth pattern and await the outcome and, when the 28 week milestone has been reached, repeatedly assess fetal health and viability. A drop-off in the fetal growth rate is usually ominous and birth may have to be induced if the baby is to survive.

The second sign is hydramnios, and clinical recognition is followed by an orderly sequence of ultrasound and X-ray investigation to try to find out what has disturbed the balance of fluid production and disposal. As disposal is affected by fetal swallowing and absorption from the gut, diagnostic efforts are focused on conditions that interfere with the reflex act of swallowing, as in anencephaly, and those which produce high-gut obstruction, usually oesophageal atresia, a condition that is finally confirmed by the impeded passage of a stomach tube after birth.

In period 'C' persistent unstable lie or, in the

primiparous woman, a failure of the fetal head to descend into the pelvis during the last weeks of pregnancy, suggest that there is disproportion between head shape or size and the pelvic cavity and, in the fetus, this may be the result of hydrocephalus or premature fusion of the skull bones. X-ray and ultrasound may again resolve the dilemma. If there is a suspicion that the head is too large, further careful ultrasound may successfully reveal the size of the lateral ventricles and this may well influence the strategic management of labour and delivery.

Period 'C' also envelops a time of intense activity and investigation for the obstetrician. Many of the tests carried out at this time are aimed at determining the state of the placenta 'at rest' and under stress to ensure adequate gas exchange for the fetus and so prevent the dire effects of hypoxia and metabolic acidosis. The number of tests is legion and it is not my purpose to describe them but to reserve special mention for an investigation that aims to determine whether a baby born prematurely will develop hyaline membrane disease. The test effectively measures pulmonary surfactant and predicts if the pulmonary alveoli will remain distended and stable after birth. The amniotic fluid test for surfactant measures the lecithin/sphingomyelin ratio[1]. The lecithin comes from alveolar surfactant that has become mixed into the lung fluid which, in turn, is mixed with amniotic fluid. Because so many variables are involved, absolute values are of limited value and so lecithin content is measured against the stable and unrelated sphingomyelin and expressed as a ratio.

Pulmonary surfactant has been recognised in the lungs of fetuses as immature as 24 weeks of gestation but at this early stage it is in an unstable form and production is easily switched off by hypoxia and metabolic acidosis. This tendency ebbs as pregnancy proceeds and is uncommon after the 36th week.

Period 'A' is the main topic of this article. It stretches from 12 to 20 weeks and in that time maternal blood and amniotic fluid may be obtained, the fetus scrutinised by ultrasound and occasionally viewed through the fetoscope, when blood samples may be taken. These tests tell us whether the fetus is at risk of attack by Rhesus antibodies, is protected from rubella, has a normal skull, if the skin is broken and body fluids are leaking out as in spina bifida, if the nuclei of amniotic cells are male or female and contain normal chromosomes and, in a very few highly-selected instances, whether certain enzymes and other proteins and polypeptides are normally formed. All these investigations have a physical component, each (except for simple maternal blood tests) with its hazards to the fetus, some of which are at present simply suspected, some suspected but awaiting further proof, and some certain. Which baby is to be submitted to these potentially traumatic procedures?

None of the tests, perhaps excepting blood-taking and ultrasound, is used as a routine. Amniocentesis and fetoscopy present well-recognised hazards and should not be embarked upon unless there is good and specific indication. They should certainly not be used as screening tests for unfounded maternal anxiety. These two procedures are carried out between 16 and 20 weeks, hedged into this short space of time by the limits imposed by the techniques themselves and the time taken for the laboratories to make an accurate diagnosis upon which termination may be recommended. In many cases the specimen has to be collected before the end of the 18th week. The risks inherent in amniocentesis above all else prevent its use for screening all pregnancies, though such screening is offered to women over 38 years because of the increasing risk of bearing a child with Down's syndrome. Of all the indications for amniocentesis, this is now the commonest, but there are two others which are as, if not more, important: the first is a history that the mother has already had a child with a neural tube defect (risk of recurrence one in 20) and the second is that she has already given birth to a mongol child (risk of recurrence of trisomy 21 one in 100 or greater). In all, there are three indications that should start a search for a mongol fetus and three indications to search for a child with spina bifida. Each indication bears its own recurrence risk (Table 1). Down's syndrome and spina bifida are, together, the commonest and, at the same time, the most serious handicapping conditions compatible with life and it is, therefore, not surprising that over 95 per cent of diagnostic amniocenteses are performed for their detection.

Table 1. Indications for mid-trimester amniocentesis (Rhesus disease excluded).

	Risk of Affected Fetus
A. Down's syndrome and other chromosomal disorders	
Previous child with Trisomy 21	1% +
Parent known translocation carrier, e.g. D/G translocation	5-10%
Mother 38 years old and over	1/180-1/20*
B. Neural tube defect (spina bifida and anencephaly)	
Previously affected child	1/20
Parent with visible spina bifida	1/20
High maternal AFP (twins excluded)	1/10
C. X-linked disorder	
Previous affected boy and family history	1/2 (for boys)
High risk determined on family history ± carrier testing	—
D. Certain inborn errors of metabolism	
Previously affected child	1/4
Both parents known carriers	1/4

*Some disagreement about exact risks, 1/250 approximate for 38 years, 1/20 at 45 years and over.

There is only one way to detect fetal mongolism and that is by chromosome culture of desquamated fetal and amniotic cells, but spina bifida can be recognised in one of three ways. Open lesions leak fetal proteins into the amniotic fluid and the estimation of one of these—alpha-fetoprotein—is now universally available. False positives are very rare, but small lesions may give equivocal results, and 'closed' defects or those with impervious coverings,

negative results. Some help in the differentiation of equivocal results may come from the examination of 'rapidly adhering cells' grown from the fluid[2]. These cells, of which there are many morphological types, include some which appear to be of neural origin and all grow with great speed over the first two or three days in the laboratory. This test is only used in a limited number of laboratories which find it a useful adjunct but errors of interpretation do occur[3]. The third technique is ultrasonography in which advances with the real-time equipment are likely to resolve difficulties in the diagnosis of spina bifida and may, in time, replace amniocentesis as the technique of choice for fetal diagnosis of this condition. Small encephaloceles and 'closed' or covered spinal lesions can already be recognised by a select few leaders in the field and it has become the practice of obstetricians to refer their more difficult problems to them.

A high maternal serum AFP is one of the indications for amniocentesis once twins have been excluded by ultrasound[4]. Unfortunately, serum cannot be usefully collected and AFP measured before the 16th week of pregnancy because abnormal and normal values do not diverge before then and 2-5 per cent of women tested at the appropriate time have to submit to amniocentesis to determine which fetus has an open neural tube defect. In practice, all those with anencephaly, and 70-80 per cent of those with spina bifida, are recognisable. A national screening programme aimed at the expulsion of all fetuses with spina bifida is at present under discussion and has been pressed upon the profession by the DHSS and various other groups. There are problems, some diagnostic, but mainly ethical and administrative, before this scheme can go ahead. It is, however, already well-established in Scotland and in various centres in the UK. However, like most well-laid plans, there is many a slip and perhaps the greatest obstacle is the failure of up to 45 per cent of women to book at antenatal clinics before 20 weeks of pregnancy.

It should not be forgotten that the procedures associated with the antenatal clinics are nearly all for screening out one problem or another and one of these is for the prevention and recognition of Rhesus incompatibility. The maternal blood group and presence or absence of antibodies are checked at the beginning of each pregnancy and Rhesus negative women are tested a number of times during pregnancy. Though the disease is now uncommon following the success of the anti-D programme (all at-risk Rhesus negative women receive an injection of 100 μg anti-D at birth, abortion and amniocentesis), it remains an important but eminently treatable disease. It is unique in this, for the only treatment that can be accorded to other serious genetic disease detected at this stage of pregnancy is abortion.

A few other genetic diseases can be discovered in the fetus at this early stage. All present great diagnostic challenges for the obstetrician and scientist. For the parents the stakes are high, for they have usually had one child handicapped or killed by the disease in question and the risk of recurrence is usually one in four. In practice such diseases are usually inherited in an autosomal recessive or X-linked fashion but, unfortunately, very few can at present be recognised. The simplest technique available is to determine sex by looking for chromatin bodies and fluorescent Y chromosomes in the nuclei of cells shed into the amniotic fluid. This is, of course, only relevant when dealing with X-linked disease in which males are at risk and if this is the only test that is available, positive diagnosis necessarily leads to the abortion of at least 50 per cent of normal boys. At best, sexing should only be considered a first step which in time will always be followed by others that confirm or disprove disease. The second step, using cell culture or, in some, ultrasonography and in others fetoscopy and fetal blood sampling, already exists or is proposed for certain conditions. The Lesch-Nyhan syndrome (hypoxanthine guanine phosphoribosyl transferase deficiency), an inborn error of purine metabolism, X-linked hydrocephalus, haemophilia A and Duchenne muscular dystrophy are examples. The Lesch-Nyhan disease of progressive infantile athetosis and later self-mutilation is at present one of the few X-linked disorders in which the diagnosis can be defined biochemically from cultured cells. With X-linked hydrocephalus the male fetus can be examined *in utero* by careful and expert ultrasonography so as to determine the size of the lateral cerebral ventricles. Finally, haemophilia A[5, 6] has already, and Duchenne muscular dystrophy will probably come within diagnostic reach because of advances in fetal blood sampling and measurement of factor VIII and creatine phosphokinase.

The diagnosis of autosomal recessive disease depends upon the direct or indirect measurement of enzyme activity and, for a very few diseases, is feasible. The mucopolysaccharidoses, Tay-Sachs disease (GM2) and cystinosis are examples. In the UK a number of laboratories specialise in the antenatal enzyme diagnosis of one or two conditions and some have become widely known for their expertise in this difficult field. Co-operation between individual laboratories has to be excellent and details of 'who does what' is known through the *Prenatal Diagnosis Group Newsletter*[7] while the *Inventory of Genetic Consultation Centres*[8] gives lists of European centres involved in this work. A supraregional unit funded to provide a comprehensive metabolic service has been set up in the UK but advice and help are often sought outside this country, principally in Rotterdam and in various centres in the USA.

These chemical analyses and the interpretation of results can be very difficult, for we must remember that the aim is to differentiate the affected and abort it from the carrier, whose life is to continue. This is particularly difficult when the genetic misprint leads to incomplete rather than complete enzymic block and the gap between the results obtained from the carrier and the affected fetus is small. Interpretation of results can be further complicated because the block for a given condition can vary from one family to another, the differences presumably reflecting a closely related mistake in the genetic code. In an ideal world these difficulties can be overcome by comparing fetal enzymic activity with that obtained from cells from a previously affected child. The

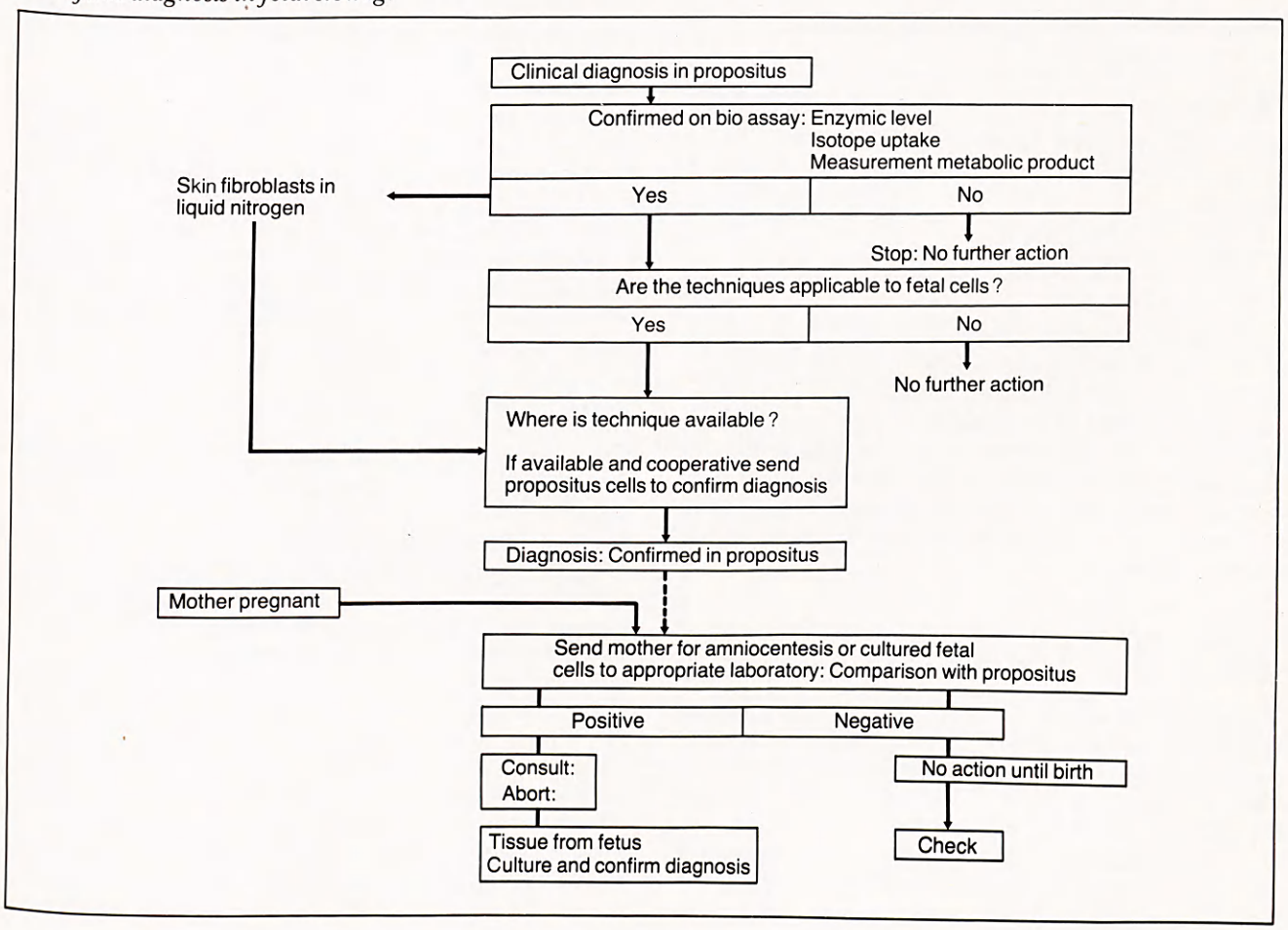
whole process that leads to the fetal diagnosis of one of these rare conditions can be summarised in a flow diagram (Fig. 2), the bottom of which includes the section where fetal cells are compared with those of the previously affected sib. Unfortunately, this comparison is not always possible because of the death of the first child before the conception of the second; this can only be guarded against by culturing skin from the first affected child at the time of its diagnosis and then storing the cells in liquid nitrogen, where they can provide an insurance against future possible antenatal interrogation. At present the number of banks that perform this service and the number of subscribers are small and usually restricted to trusted colleagues or where the diagnosis is beyond doubt, while the development of a single reference and diagnostic bank for the EEC is under discussion.

Until recently, fetal metabolic disease could only be assessed by examination of the amniotic fluid and its cultured cells but fetal blood sampling has now become a reality with the increasing use of the fetoscope.

The fetoscope has an outside diameter of 1.7 mm (for the operating model 2.7 mm) and is introduced through the maternal abdominal and uterine walls using a trocar. The standard models are not flexible and it is usually very difficult to see anything but the front of the baby, so it is of little use in the diagnosis of spina bifida. However, it can be used to examine limbs, face and genitalia and it allows the operator to take blood samples directly from vessels on the placental surface. This technique was principally developed in University College Hospital for the diagnosis of B-thalassaemia[9]; it has been wholly successful and is now used at King's College in an attempt to diagnose haemophilia and Duchenne muscular dystrophy. The technical advance is, however, being slowed for lack of normal blood values in the live fetus. A further complication as far as Duchenne muscular dystrophy is concerned is that fetal muscle at the time of abortion does not show recognisable change and confirmation of disease is consequently difficult to make.

Many of these advances are exciting but all produce their problems and casualties which have to be weighed against the benefits for each individual case. At present the disadvantages can be illustrated by answers to the following three questions.

Fig. 2. *Metabolic disease: steps in fetal diagnosis to confirm diagnosis in affected propositus and further steps to confirm diagnosis in fetal sibling.*



1. Does high fluid AFP always signify a neural tube defect?

Alpha-fetoprotein is the fetal predecessor of albumin, with a molecular weight of 65,000-70,000. It was originally selected by Brock and Sutcliffe[10] as a suitable fetal marker because it is present in the fetus but virtually absent from the amniotic fluid of the mother. (AFP is present in minute proportions in the maternal blood and can only be measured by sensitive radioimmunoassay.) Brock and Sutcliffe supposed that such a marker could be expected to leak out into the amniotic fluid if there was a break in the fetal covering, as in spina bifida and anencephaly. Their predictions were amply confirmed, first in anencephaly and then in spina bifida. However, non-specific tests of this kind must occasionally produce unexpected results.

It is not surprising that any cleft which allows the leakage of body fluid into the amniotic cavity will lead to a high AFP reading and, as amniotic fluid is mainly produced by the fetal kidneys, proteinuria will also be detected. This is fortunate, for it allows us to make the antenatal diagnosis of the congenital nephrotic syndrome for those couples who have already had one affected child. (The condition, which leads to death in the first few years of life, is inherited in an autosomal recessive fashion.) In addition, dead or nearly dead fetuses will also leak AFP. In summary (Table 2), it is clear that a high amniotic fluid AFP level is most likely to signify a neural tube defect and the only group in which there is fear of making a serious mistake is the exomphalos/gastroschisis group that paediatric surgeons consider to be eminently operable. However, as ultrasound improves and the use of cell morphology becomes more widely accepted in the diagnosis of neural tube defects, the differentiation from exomphalos will become routine.

Table 2. Possible causes of high amniotic fluid AFP in randomly obtained fluid.

	Population incidence
Neural tube defect	1/250
Exomphalos Gastroschisis	1/600?
Congenital nephrotic syndrome	1/100,000?
Fetus dead or moribund	

2. Is the interpretation of chromosomal results always straightforward?

Mongolism, trisomy 21, is not the only chromosomal disorder to be found (Table 3) though this is the usual condition searched for in the early antenatal period. The other aneuploids with 47 chromosomes are also age-related. Some are lethal within a short time of birth and, once they are recognised from cell culture, an earlier death does not give rise to misgiving. But what should be done with those conditions when the natural history is unknown and the IQ is often normal, as in the case of the sex chromosome defects, 47 XXX, 47 XYY and 47 XXY (Klinefelter's syndrome)[7].

Table 3. Population incidence of chromosomal disorders at birth.

Down's syndrome	1/600
Trisomy 18	1/3500
Trisomy 13	1/6000
47 XXX	1/800 of females
47 XYY	1/700 of males
47 XXY	1/700 of males

The unexpected *de novo* chromosomal rearrangements provide a great headache. Many are balanced in the sense that no chromosomal material has been lost or gained and the individual is effectively normal, though he or she may subsequently have abnormal children. On the other hand, there are some *de novo* 'balanced' translocations where, although the amount of chromosomal material appears to be normal under the microscope, the child is physically and mentally abnormal. In these cases we suspect that the chromosomal techniques are not giving us the resolution we would like and that there is indeed some loss or gain of chromosomal material. At present the appearance of the *de novo* balanced translocation in amniotic fluid cells simply produces anxiety and such pregnancies are allowed to go to term. The proportion of such rearrangements which will turn out to be abnormal and the children retarded is still unknown and the dilemma will not be resolved until better methods of chromosomal analysis become available.

The third problem with chromosomes is similar and concerns chromosomal polymorphism. Small alterations in the shape and structure of the chromosomes from normal individuals are being increasingly recognised and these may be quite unexpected in cells cultured for other reasons. Fortunately, comparison with chromosomes obtained from parents will usually resolve the difficulty but misinterpretations have occurred and these have led to the abortion of normal fetuses.

3. Is there any inherent danger in amniocentesis?

The short and not unexpected answer to this is 'Yes'. The most obvious is abortion or at least early fetal death, with abortion sometimes being delayed for some weeks. There has been a considerable amount of controversy as to whether a single attempt at amniocentesis is ever dangerous but it now seems generally accepted that each attempt is associated with a one per cent risk of abortion. The other complications to the fetus of amniocentesis are suggested by the MRC report published in 1978[11]. This study collated the outcome of just over 2,000 amniocenteses carried out in a number of centres before the 20th week of pregnancy. The results point to an increase in premature birth, an increase in neonatal respiratory disease, death from hyaline membrane disease and an association with fetal pressure deformities, especially talipes equinovarus. It seems that amniocentesis or the removal of fluid, or both, leads to a disturbance of fluid

dynamics which, in some cases, continues throughout pregnancy. The size of these individual risks is not clear and further studies are required.

As one might expect, the use of an even larger instrument, the fetoscope, should produce more complications and this was certainly the case in the early days when a 10 per cent abortion rate was recorded.

Summary

We are still in the early days as far as antenatal diagnosis of fetal disease and abnormality are concerned. All of the techniques show a high degree of accuracy if used in the correct circumstances but, apart from the use of maternal blood to assess the Rhesus status and screen for neural tube defects and amniocentesis to search for age-dependent mongolism, present techniques are too cumbersome, inaccurate and dangerous to be offered as routine antenatal tests. The rapid development in ultrasound equipment and expertise and the recent advances in fetal blood sampling and biochemistry suggest that there will be continued and rapid expansion within the field.

This article is based on a paper read at the College Regional Conference in Birmingham in September 1979.

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