Towards the prevention of fetal malformation / edited by J.B. Scrimgeour.

Towards the Prevention of Fetal Malformation

edited by
J.B.SCRIMGEOUR

J. B. Scrimgeour, editor

TOWARDS THE PREVENTION OF FETAL MALFORMATION

¶ Knowledge and skill in the early antenatal diagnosis and prevention of fetal malformation is growing fast. This book brings clinicians abreast of that knowledge. Important as an overall review, and specifically in its practical application to their particular speciality, its value doesn't stop there. It has a lot to offer those involved in laboratory diagnosis and research into malformations, and everyone responsible in any way for the forward planning of services, with special reference to cost-effectiveness.

¶ Comprehensive in scope, it emphasises screening by serum alpha-fetoprotein for neural tube defects, and its cost-effectiveness; the counselling and management of patients; drugs and toxins of various kinds, with reference to possible teratogenic effects; viral diseases during pregnancy, and the particular problem of vaccination during pregnancy; current indications, technique, and complications of amniocentesis; the present state of fetal blood sampling by fetoscopy and placental aspiration; the early diagnosis of sickle cell disease and β-thalassaemia; practical guidelines for minimising problems in amniotic fluid cell culture and metabolic diagnoses; and the techniques and hazards of termination of pregnancy.

¶ Finally, there is a consideration of the cost of these advances, from the medical, legal, and financial aspects. Administrators in Health authorities will want to inspect, to digest, and to respond to, these very exciting developments in the care of the world's population.

¶ J. B. Scrimgeour is Senior Lecturer in Obstetrics and Gynaecology, University of Edinburgh

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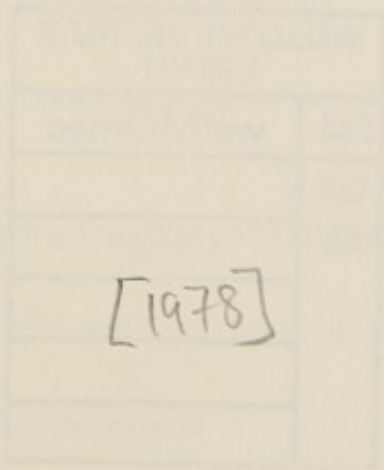

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Preface

The book was inspired by, and is based on, papers given at an International Symposium held in Edinburgh in March 1977. Contributors have included in the text not only material presented at the Symposium but also points made during discussion by delegates. In this way it is hoped that a balanced view of each aspect of this rapidly developing branch of medical practice has been given. The personal long-term prognostications of Professor Fairweather, Sir John Brotherston and Professor Polani, however, have been reproduced verbatim.

While the book is intended primarily for obstetricians it will be of interest to midwives, paediatricians, general practitioners and those involved in long-term planning for maternity services, as well as all those whose curiosity and research interests are aroused and fascinated by early human fetal development.

I am deeply indebted to my wife, my clinical colleagues, Mrs E. Aitken, Miss E. Stuart and Miss M. S. Watt, for the patience, understanding and invaluable help given during the Symposium. The skilled supervision and meticulous advice afforded me by Edinburgh University Press has resulted in the expeditious production of the completed book.

The entire enterprise would never have developed past the embryonic stage had it not been for the generous support and enthusiasm of Schering Chemicals Limited.

J. B. Scrimgeour
Western General Hospital
Edinburgh

THE CHALLENGE

Early Diagnosis and Prevention of Genetic Disease : Molecules and the Obstetrician

In the Western countries there is an increasing interest in early diagnosis, treatment and prevention of congenital disorders. This can be explained by several factors: congenital disorders have become the major cause of child mortality and morbidity in these countries; developments in biochemistry, cell biology, genetics and technology have led to new possibilities in diagnosis and prevention of an increasing number of congenital disorders; in modern Western societies most couples not only wish to determine the number of their children and the time of their birth, but they also want to do everything possible to prevent the birth of an affected child; the optimal medical and psychosocial care of the increasing number of mentally and/or physically handicapped means a heavy financial burden for society, which partially explains governmental interest in the prevention of congenital handicaps.

It is difficult to generalise about the role of the obstetrician in the various aspects of diagnosis and prevention of congenital disorders. First of all we should be aware that in the larger part of the world malnutrition and infections are still the major causes of infant mortality, and hence the interest of obstetricians for genetic diseases in these countries will be limited. Also, in Western countries the historical development of the obstetrician's interest has been rather technical and has been concentrated on the event of confinement. Although there have been quite some changes in the obstetrician's life during the last decades, generally there is still too little attention paid to the preventive aspects of their discipline. Another restriction in the contribution of obstetricians to prevention of congenital disorders is the limited time they have available for each patient and the fact that they have to deal with a large variety of different problems. In many countries one and the same obstetrician is expected to perform surgery and to deal with clinical, endocrinological, biochemical, pathological and psychosocial problems related to infertility, abortion, stillbirth, fetal development, birth and the perinatal period. It seems naive to suppose that the obstetrician can play an important role in the actual work that is involved in the early recognition of people at risk for affected offspring, the genetic counselling and the preventive measures. Yet, each obstetrician

must know about the risks (and recurrence risks) for the various categories of congenital disorders and the modern developments in early diagnosis, treatment and prevention.

In most instances the birth of a handicapped child is accompanied by an increased risk that the next pregnancy will end with another affected child (table 1). At present more than 2500 different congenital disorders have been recognised; together they are responsible for the failure of at least fifty per cent of all conceptions and for anomalies in four to ten per cent of the live-borns. The fact that most of the congenital diseases are relatively infrequent, and that many of them exhibit a large clinical heterogeneity, complicates their diagnosis.

Early diagnosis of people at risk for affected offspring implies the necess-

Table 1. Incidence and recurrence risk for some important categories of congenital disorders. (Data derived from Edwards 1974, Trimble & Doughty 1974, Carter 1976, Hamerton *et al.* 1975, Went 1975.)

Disorder	Incidence	Recurrence risk
Chromosomal aberrations	overall 0.5%	
Down's syndrome (non-familial)	1:10–3000 (depending on maternal age)	1–2%
translocation carriers	1:550	5–100% (depending on type)
sex chromosomal aberrations		
male	1:400	
female	1:700	
Monogenic disorders	overall 1%	
Autosomal recessive:	overall 0.2%	25%
cystic fibrosis	1:2600	
phenylketonuria	1:10000	
galactosaemia	1:50000	
X-linked recessive:	overall 0.05%	
Duchenne's muscular dystrophy	1:7000	50% of males
haemophilia	1:10000	
Dominant inheritance:	0.7–0.07%	
Huntington's chorea	1:5000	
blindness	1:10000	50%
deafness	1:10000	
Malformations due to genetic and/or environmental factors	overall 2.5–4%	
congenital heart defects	1:125–160	1–6%
spina bifida	1:250–1600	3–5%
anencephaly	1:500–1000	2–5%
club foot	1:250–1000	2–6%
cleft lip/palate	1:1000	3–4%

ity of early detection of abnormal development of a child, the execution of extensive clinical investigations, chromosome analyses, biochemical assays, investigation of patients and possible carriers in the family and sometimes extensive pedigree analysis. Most of this work requires the contribution of various experts and specialised laboratories, and often it is very time-consuming. Once an increased genetic risk has been established the people concerned have to be informed about their genetic risk and about the possibilities of preventing the birth of an affected child. Genetic counselling not only requires repeated talks with the counsellees, it also implies psychological care after the decision about future offspring has been made. These tasks should not be underestimated, since they involve the explanation of complicated issues like chromosome translocations, gene mutations, heterozygous carriers, X-linked disease or multifactorial inheritance. For the couple involved it means decisions about emotional subjects like no children (or no further children), accepting an increased risk of an affected child, adoption, artificial insemination, or prenatal monitoring, possibly followed by selective abortion.

It is important that the obstetrician is aware that optimal management of people at risk for affected offspring requires the collaboration of various experts, laboratories and centres. Decisions about the diagnosis, risk or recurrence risk, and various alternatives regarding future offspring, are best taken during one of the regular meetings of a multidisciplinary team for genetic counselling, of which the obstetrician is also a member. In the case of a pregnant woman the conclusions of this team can be discussed with the patient either by the geneticist or the obstetrician, and these should also initiate sufficient psychosocial care during the period following the decision about future offspring.

In discussions with patients the obstetrician, like most other clinicians, is used to a rather dominant position. Even today many doctors consider their counsellees as ignorant and dependent people who wait for the decisions to be taken for them by the expert. Such an attitude is unjustified for several reasons. In practice it appears that most doctors are rather ignorant themselves about the basic principles of genetics, the risks or recurrence risks of the various congenital disorders and about the modern methods of diagnosis and prevention (Passarge, this volume). Furthermore, many counsellees are rather well informed, especially when they are motivated because of sad experiences with handicapped family members. Finally, it should be realised that the consequences of a decision about future offspring primarily concern the couple at risk. They will have to live with the burden of a handicapped child (or another handicapped child) or with the disappointment of not having children at all. Therefore the decisions on the basis of the information given during genetic counselling should primarily be made by the counsellees themselves. Such decisions will be influenced by a variety of factors: the number of children in a family, whether these are affected, the severity of the handicap concerned, the age of the parents, their socioeconomic status, the attitude of

the family towards a handicapped person (and his care), and of course ethical and religious considerations.

The expert should help the counsellees to find out which decision would fit best in their specific situation. Unfortunately it is not uncommon for the expert to express quite strongly his own opinion about the severity of the handicap, the importance of the (recurrence) risk and about the feasibility of various alternatives regarding future offspring. As a result the counsellees do not feel free in making their own decisions, and they may also be blocked from choosing certain alternatives.

Generally speaking, the following alternatives exist for people at risk for affected offspring: decision of no (further) pregnancy in order to avoid the birth of an affected child; accepting an increased risk in the hope that a healthy child will be born; adoption; artificial insemination with donor sperm if the male partner is carrier for a chromosomal translocation, a dominant gene mutation or of the same recessive mutation as his wife; prenatal monitoring in early pregnancy followed by abortion if an affected fetus is detected (prenatal diagnosis is now possible for chromosomal aberrations, sixty different inherited metabolic diseases, and open neural tube defects like spina bifida aperta and anencephaly).

Several distinguished geneticists have calculated that reduction of the family size by people at risk is an effective means of decreasing the incidence of certain categories of genetic diseases. Such estimations seem, however, more of academic interest than of practical importance, since each individual couple will make decisions about their offspring according to their own wishes rather than on the basis of a possible benefit to society. For most people the decision of no (further) pregnancies is very difficult. Nevertheless this decision is made by quite a large number of couples who have had an affected child or close family member. In other instances, however, couples might decide to accept an increased risk either because they do not consider the risk as being very high or because they want to try everything to have a healthy child of their own. Again, generalisations about the acceptability of a genetic risk seem to me of very limited value. A risk of a few per cent might be considered as a 'small risk' by the expert, but people who really have experience with the handicap concerned may well find this a 'high risk'.

Chromosomal Aberrations

Incidence and Recurrence Risks

The role of the obstetrician in handling this category of abnormalities starts with families where patients with multiple malformations and/or recurrent abortions and stillbirths occur. It has been shown by various investigators that chromosomal aberrations are responsible for a large proportion of these cases, and each obstetrician should be aware of the fact. This aspect is discussed in detail by Boué and Boué (this volume), and they also point out in which instances it is useful to perform chromosome analysis in the female and/or male, to find out whether they are carriers of a balanced chromosome

translocation. Another important task of the obstetrician, together with other doctors involved in the care of the pregnant woman, is to avoid the prescription of any drug that is not absolutely necessary for the woman's health. Again, these aspects will be dealt with in detail in other papers.

Although there is a considerable natural selection against fetuses with a chromosomal aberration, they still occur in at least one in 200 liveborns (see, for a review, Hamerton *et al.* 1975). The obstetrician will be confronted with a relatively high proportion of these patients. Autosomal trisomies (overall incidence of one in 800 liveborns) usually result in multiple external and internal deformities and disturbed organ functions, which are apparent at birth or shortly thereafter. A number of other chromosomal aberrations, including sometimes Down's syndrome, may not be detected during the perinatal period, and these patients will thus be referred directly to the paediatrician. However, many of the patients with a sex chromosomal aberration (overall incidence one in 400 liveborns), where abnormal sexual development and sterility may be the first clinical manifestations, will also be seen by the obstetrician/gynaecologist.

Finally a number of obstetricians working in specialised centres will see all pregnant women at risk for congenital disorders that can be detected in early pregnancy by amniotic fluid (cell) analysis. It has been shown from large surveys on prenatal diagnosis that the vast majority of these cases (75%) are pregnancies at risk for a chromosomal aberration (Milunsky 1973, Galjaard 1976).

Early and precise diagnosis of a chromosomal aberration is of great importance for its prevention in subsequent pregnancies in the family. For the patient there is, unfortunately, no benefit in terms of therapy, but detection of a chromosomal aberration may prevent many unnecessary diagnostic investigations and will allow proper genetic counselling and a correct outline for future management.

As far as genetic counselling is concerned, it is absolutely necessary to establish the exact nature of a chromosomal aberration. There are still too many doctors who believe that in certain instances, like Down's syndrome and certain syndromes based on sex chromosomal aberrations, the clinical features are a sufficient basis for a diagnosis. This idea is wrong, because very similar clinical features may have a different aetiology and because the same chromosome abnormality may lead to different clinical and pathological manifestations (see, for reviews, Smith 1970, Hamerton 1971, Warkany 1971, Holmes 1974, McKusick 1975). In the case of Down's syndrome, for instance, it is mandatory for the determination of a recurrence risk to know whether the disorder is caused by a 'regular' trisomy 21 or by an unbalanced translocation that is or is not inherited from one of the parents. As shown in table 2, the recurrence risk derived from retrospective studies is supposed to be one to two per cent for younger mothers who gave birth to a patient with a 'regular' trisomy 21 (Mikkelsen and Stene 1970). A similar figure of 1.3% was found in prospective studies, based on the results of fetal karyotyping in preg-

Table 2. Incidence and recurrence risk for Down's syndrome based on a 'regular' trisomy 21. (Data derived from Collman & Stoller 1962, Stein *et al.* 1973, Lindsjö 1974, Galjaard 1976, Ferguson-Smith 1976, Sachs *et al.* 1977, and Mikkelsen & Stene 1970.)

	Incidence		
Maternal age	Retrospective studies from patients	Prospective data from pre-natal analysis	Recurrence risk
< 29 years	1:3000	—	
30–34 „	1:600	—	1–2%
35–39 „	1:270	1:80	
40–44 „	1:70	1:20	age dependent
> 45 „	1:16–40	1:8–10	

nancies from mothers who had already given birth to a child with a regular trisomy 21 (Galjaard 1976).

In a population with relatively young mothers about six per cent of the Down's syndrome cases were found to be caused by an unbalanced translocation (Mikkelsen *et al.* 1976). In about half of these cases one of the parents was a carrier of the balanced form of the translocation. In the latter case the recurrence risk depends on the type of translocation and on whether the father or the mother is the carrier. Table 3 shows some examples where the recurrence risk derived from retrospective studies varies from five to 100%. In a series of prenatal analyses in pregnancies where one of the partners was a carrier of a translocation the incidence of fetuses with an unbalanced karyotype was found to be seven per cent, and 25% were carriers (Galjaard 1976). More specific data are now being collected in order to obtain prospective risk figures for each of the most common translocations (Boué 1976).

Table 3. Recurrence risks for Down's syndrome based on a chromosomal translocation. (Data derived from Hamerton 1971, Mikkelsen & Stene 1970, Stene 1970 and Galjaard 1976.)

Retrospective data	
Father/mother carrier of 21/22 translocation	5%
Mother carrier 13/21, 14/21 or 15/21 translocation	10%
Father „ „ „ „ „	small
Father/mother carrier 21/21 translocation	100%
Prospective data	
Fetal karyotypes in case of parental translocation (unspecified)	7% unbalanced karyotype 25% balanced carrier

Obstetricians should be more aware of the recurrence risks mentioned above and about the preventive measures that can now be taken. In practice this implies that sufficient attention should be given to the exact diagnosis of

affected children or relatives who might have had a chromosomal aberration. In the case of an unbalanced translocation chromosome analyses should be performed in all those family members who might be carriers. The overall incidence of balanced carriers is one in 500 liveborns (Hamerton *et al.* 1975). Carriers at reproductive age should, of course, be informed about their risk and about the possibilities of prevention. These tasks of chromosome analyses, family studies and genetic counselling should of course be carried out in collaboration with other members of a multidisciplinary team for genetic counselling, where the obstetrician is only one of the members (Epstein *et al.* 1975).

An easier task for the obstetrician is to inform all pregnant women of advanced age about their risk of a child with a chromosomal aberration. The relation between maternal age and certain chromosomal abnormalities like Down's syndrome, trisomy 18 and Klinefelter syndrome has been known for a number of years from retrospective studies (Penrose and Smith 1966; Lenz, Pfeiffer and Tünte 1966; Collman and Stoller 1962; Stein, Susser and Guterman 1973; Lindsjö 1974). More recently, larger series of prenatal chromosome analyses in pregnant women of advanced age have resulted in prospective risk figures (Galjaard 1976, Ferguson-Smith 1976, Sachs *et al.* 1977). From these studies it appears that the risk for a child with a chromosomal aberration increases from one in eighty (1.2%) in women aged 35–38 to one in twenty for 38–40, up to more than one in ten (10%) in the highest age groups of 44 years and older (table 2). It is the task of each obstetrician to inform his patients about these risks and about the possibility of prenatal monitoring. In my view it is a mistake to think that such information causes 'unnecessary anxiety' or that 'risk figures should only be given if people ask for them'. The consequences of the birth of a seriously handicapped child are too large, both for the parents and relatives concerned and for society as a whole, to allow doctors to keep for themselves information that is so important for others.

Prevention of the Birth of Affected Children

Since we do not know how numerical and structural chromosome abnormalities originate during gametogenesis or early cleavage of the fertilised egg, we cannot prevent the cause of chromosomal aberrations. Yet, there are several methods of secondary prevention, e.g. adoption, artificial insemination, and prenatal monitoring.

Adoption can be considered in all instances where couples think that their risk is unacceptably high; in these cases contraception should of course be optimal and the possibility of sterilisation might be discussed. In practice this situation will occur, for instance, when one of the partners is the carrier of a 21/21 translocation.

Artificial insemination with donor sperm can be considered if the male partner is the carrier of a chromosome translocation. Our experience and that of other colleagues is that the number of requests for donor insemination by people at risk for an affected child is very limited.

Prenatal monitoring can in principle be offered to any couple at risk provided that sufficient personnel and financial means are available. In centres with sufficient experience fetal karyotyping can be performed between ten and sixteen days after amniocentesis in the fifteenth or sixteenth week of pregnancy (Stockholm Conference 1975, Boué 1976). As can be deduced from the results of a European survey of prenatal diagnoses (Galjaard 1976) half of the requests for fetal karyotyping are from pregnant women at advanced age. Some centres, as in Scandinavia, accept all women over 35, others take 40 as a limit and most groups, including our own, consider the age of 38 and older as an indication for amniocentesis. Every limit seems arbitrary, especially since transabdominal amniocentesis has now definitely been proven to be a safe procedure with a minimal risk for mother and fetus (Turnbull, this volume). In the long term each population and government will decide what funds they want to spend on this aspect of preventive health care. The obstetricians and cytogeneticists will decide whether the procedures of amniocentesis, amniotic fluid cell cultivation and fetal karyotyping are still worthwhile if the numbers of women to be monitored become too large. On the basis of these factors pregnant women of advanced age will, or will not, be encouraged to undergo amniocentesis.

The second important group of pregnant women to be considered for prenatal monitoring are those who have already given birth to a child with a chromosomal aberration. In the case of Down's syndrome (mongolism), the majority (about 95%) of the patients will be a 'regular' trisomy and a minority will have an unbalanced translocation. About half of the latter have arisen during gametogenesis in one of the parents, and in the other half one of the parents is a carrier of a balanced translocation. Although the recurrence risks are different, in these instances nearly all parents with a handicapped child or relative will ask for prenatal monitoring.

Genetic Metabolic Diseases

Incidence and Recurrence Risks

In McKusick's most recent catalogue (1975) more than 2300 different syndromes or biochemical abnormalities based on single gene mutations are described. The incidence of each of these genetic disorders varies from 1 : 1500 to 1 : 100000 (table 1) and it is assumed that, overall, 1 : 100 liveborns suffer from a monogenic disease. After the birth of an affected child the parents have a high recurrence risk (25% in recessive genetic diseases and 50% in dominant disorders) in each subsequent pregnancy. It is therefore very important that the diagnosis of a genetic metabolic disease is made as early as possible and that the diagnosis is followed by proper genetic counselling.

The large number of genetic disorders of Mendelian inheritance, their heterogeneity, and the relatively low incidence of each individual disease makes an early diagnosis difficult. During the last decades there have been considerable changes in the diagnostic approach of some genetic metabolic diseases. Detailed clinical observations supplemented with microscopical data

from tissue or cell alterations have always been the basis for the classification of the various syndromes. This is still true for the majority of genetic diseases, but for about 200 of them the responsible biochemical defect has now been elucidated. As a consequence the diagnosis can and should be based on the demonstration of this biochemical defect. This can be accomplished by biochemical analysis of blood cells, tissue biopsies or body fluids. The advantage of a known biochemical defect is that the diagnosis can be made with near absolute certainty and also before any clinical manifestations have occurred. For a few metabolic diseases like phenylketonuria, galactosaemia and congenital hypothyroidism this has led to mass screening of newborns (Scriver, Clow and Lamm 1973; Levy 1974, National Research Council Report on Genetic Screening 1975) in order to allow early treatment, which prevents the occurrence of severe physical and mental handicaps. The vast majority of genetic disorders, however, are not amenable to therapy and prevention should thus be accomplished by early recognition of parents at risk and by genetic counselling.

In about 100 genetic metabolic diseases the responsible biochemical defect can also be demonstrated in cultured skin fibroblasts of the patient (Stanbury, Wijngaarden and Frederickson 1972; Nitowsky 1972; McKusick 1975). This has led to the possibility of prenatal monitoring using biochemical analysis of cultured amniotic fluid cells (Nadler 1972; Milunsky 1973, 1976; Galjaard *et al.* 1975, 1976). The use of cultured human mutant cells also contributes to the solution of basic problems in biochemistry, cell biology and cell genetics. An example of the latter is the use of somatic cell hybridisation to investigate the question whether different gene mutations are responsible for the clinical heterogeneity that is frequently observed for 'the same' genetic enzyme deficiency (see, for review, Ringertz and Savage 1976). The future classification and diagnosis of some genetic disorders might well be based on such cell genetic studies.

Prevention of Genetic Metabolic Diseases

Again, the possibilities of prevention are very limited. The occurrence of gene mutations cannot be prevented, although known mutagenic factors (like ionising radiation, certain drugs and viral infections) should be avoided. The next step would be the early detection of heterozygous carriers and to offer genetic counselling to couples at risk before they have their first child.

In the case of dominant inheritance, heterozygous carriers will show clinical manifestations, and if these are detected before reproduction people at risk may be informed about their risk. If the male is the carrier (patient), artificial donor insemination might be considered. The only other approach would be either to accept a 50% risk of an affected child or to avoid pregnancy.

In the case of autosomal recessive disorders the first sign that both parents are carriers of the same gene mutation will be the birth of an affected child. Again, donor insemination or avoidance of a following pregnancy are two approaches to prevent the birth of a second handicapped child in the same family. Fortunately the possibility of prenatal monitoring offers a new pro-

spect to parents at risk for one of the genetic metabolic disorders that can be demonstrated by amniotic fluid cell analysis (table 4). At present practical experience with *in utero* diagnosis has been gained for thirty different genetic

Table 4. Prenatal diagnosis of genetic metabolic diseases. Data based on a survey in Canada and the U.S.A. by Milunsky (1973) and a survey of European experience with prenatal diagnosis by Galjaard (1976). Some data were collected by Svennerholm, Patrick and Galjaard for the Stockholm Conference (1975), and some data on Tay-Sachs disease are derived from Kaback *et al.* 1976.

Disease	Estimated total analyses	Analyses in Europe	Affected fetuses (Europe)
Acid phosphatase deficiency[1]	2	1	—
Argininosuccinic aciduria	few	2	—
Citrullinaemia	few	2	—
Cystathionuria	1	—	—
Cystinosis[1]	25	10	—
Fabry's disease[1]	few	1	1
Fucosidosis[1]	few	1	—
Galactosaemia	10	8	3
Gaucher's disease[1]	10	3	2
Glycogenosis II[1]	50	30	10
Glycogenosis III	1	—	—
Glycogenosis IV	1	—	—
GM_1-gangliosidosis[1]	20	8	—
GM_2-gangliosidosis (Tay-Sachs)[1]	370	42	—
GM_2-gangliosidosis (Sandhoff)[1]	10	8	3
Haemoglobinopathy	20	—	—
Homocystinuria	few	1	—
Hyperammonaemia II	1	—	—
Immune deficiency	few	—	—
Krabbe's disease	30	19	3
Lesch-Nyhan syndrome[1]	10	6	2
Maple syrup urine disease[1]	20	13	5
Metachrom. leucodystrophy[1]	40	24	2
Methylmalonic aciduria	20	10	5
Mucolipidosis II[1]	few	2	—
Menke's disease	1	1	—
Mucopolysaccharidosis I (Hurler)	50	34	9
Mucopolysaccharidosis II (Hunter)	50	28	?
Mucopolysaccharidosis III[1]	30	14	2
Mucopolysaccharidosis IV	1	1	1
Niemann-Pick disease	few	2	1
Ornithine carbamyl trans. def.	few	2	—
Propionic acidaemia	few	3	3
Wolman disease	few	1	1
Xeroderma pigmentosum	few	2	1

[1] For these genetic defects microchemical methods that allow rapid prenatal diagnosis are available.

metabolic diseases, and probably up to a thousand pregnancies at risk have been monitored in the U.S.A. and in Europe (Milunsky 1973; Kaback, O'Brien and Rimoin 1976; Galjaard 1976).

In order to facilitate prenatal diagnosis of a genetic metabolic disease each clinician should be aware of the necessity to take a skin biopsy from every patient with a disease that is amenable to prenatal monitoring. Cells from such a skin biopsy can be cultured and stored in a cell bank, and will then be available at any time when they are needed as control material for a prenatal diagnosis in another pregnancy of the mother. Because of the large variation in enzyme activities of cultured cells from different patients with the same genetic disorder, it is important to use cells from an affected sibling as control material and not cells from a non-related patient.

The demonstration of a genetic enzyme defect originally required biochemical analysis of $10^6 - 10^7$ cultured amniotic fluid cells; the cultivation period required to obtain this number ranged from four to eight weeks. Recently, microchemical techniques have been developed that enable biochemical assays in $10^2 - 10^4$ cells, and a prenatal diagnosis for about fifteen genetic metabolic diseases can now be established within two weeks of amniocentesis (Galjaard *et al.* 1973, 1974a,b, 1975, 1976; Wendel *et al.* 1973; Willcox and Patrick 1974).

The complexity of the analytical techniques, the biochemical and genetic heterogeneity, the relatively low incidence of each genetic disease and the variation of biochemical parameters with cell cultivation conditions are all factors that make centralisation of prenatal diagnosis of metabolic diseases mandatory (Galjaard 1976; Patrick, this volume).

Three X-linked recessive metabolic diseases (Fabry's disease, mucopolysaccharidosis type Hunter, and Lesch-Nyhan syndrome) can be detected in early pregnancy. For all other X-linked diseases, including the most common ones like Duchenne's muscular dystrophy and haemophilia, the biochemical defect is either not known or not expressed in cultured amniotic fluid cells. As an alternative, prenatal sex determination has been carried out in pregnancies at risk for such X-linked diseases. If a male fetus is found, there is a 50% risk that this will be affected and parents may then ask for abortion; when the fetus is female the parents can be reassured, since girls will not be affected. The disadvantage of this approach is that half of the aborted male fetuses will not be affected. On the other hand the possibility of prenatal sex determination offers a prospect of normal progeny to parents who should otherwise not have dared a pregnancy (or a new pregnancy) because of the high risk.

Studies are now in progress of fetal blood sampling, either by blind puncturing of placental vessels or under visual control using needle fetoscopy (Hobbins and Mahoney 1974; Golbus, Kan and Naglich-Craig 1976; Kaback and Valenti 1976; Valenti, this volume). In combination with microchemical assays (Galjaard *et al.* 1976) this may lead to a faster prenatal diagnosis of genetic metabolic diseases, and it may also become feasible

to detect the presence of Duchenne's muscular dystrophy by performing micro-assays of creatine phosphokinase in blood samples from a male fetus (Galjaard *et al.* 1976). A further widening of the scope of prenatal monitoring of genetic metabolic diseases may also be realised by experimental induction of enzymes that are normally not expressed in cultured amniotic fluid cells and skin fibroblasts (Galjaard *et al.* 1976).

The obstetrician's task in prevention of genetic metabolic diseases is to collaborate in early diagnosis of affected babies, to take a skin biopsy from such patients, to ask all pregnant women whether they have relatives with X-linked recessive disorders, to think of the (limited) possibilities of heterozygote testing (Kaback *et al.* 1974; Walton 1974; Brinkhous and Hemker 1975) and to be aware of the possibilities and limitations of prenatal monitoring.

Multifactorial Congenital Malformations

This category of malformations, which are (probably) caused by a combination of genetic and environmental factors, comprises the largest group of congenital disorders (Smith 1970, Warkany 1971, Myrianthopoulos and Chung 1974, Carter 1976). The overall incidence found in different studies varies greatly because of differences in geographic area, population sample, the criteria used and the diagnostic methods employed. Generally it is assumed that the overall incidence of congenital malformations is 2.5–4% of liveborns. The most common abnormalities are congenital heart defects, spina bifida, anencephaly, cleft lip (palate) and club foot; the incidence of each of these malformations is in the order of 0.05–0.8% (Carter 1976; table 1). When a child with one of these malformations has been born, the recurrence risk in a subsequent pregnancy is increased to the order of 2–6%. If there is a history of multiple abortions, stillbirths, or more than one affected relative, the recurrence risk may be considerably higher. Also, it is important to establish whether a congenital malformation is due to 'an inborn error of morphogenesis', in which case the recurrence risk may be as high as 25–50% (Holmes 1974).

The possibilities of prevention for this category of congenital disorders are very limited indeed. Except for abnormalities due to known harmful external factors (rubella, certain other bacterial and viral infections, ionising radiation, teratogenic drugs and chemicals, certain diseases of the mother, rhesus antagonism) nothing is known about the aetiology of congenital abnormalities that are not based on a chromosomal aberration or a gene mutation. Furthermore, the scope of prenatal monitoring is as yet limited to alpha-fetoprotein assays in amniotic fluid supernatant to exclude spina bifida aperta or anencephaly (Brock and Sutcliffe 1972, Brock 1976 and this volume). In the vast majority of cases prevention should be based on early diagnosis and genetic counselling.

Unfortunately too many clinicians are still of the opinion that it is unnecessary to inform parents with a handicapped child about their increased

recurrence risk. These doctors argue that 'one should not worry people with risk figures if one cannot provide help'. Also, there are still too many doctors who do not even know about the possibilities of prenatal monitoring for spina bifida aperta and anencephaly. According to most experts in genetic counselling it is the task of every doctor, including the obstetrician, to warn all those couples known to have an increased genetic risk. It should be the counsellee's own decision what they finally do with this information. A number of follow-up studies have shown that most couples adapted their reproductive behaviour to the information they had obtained during genetic counselling (Carter *et al.* 1971; Emery, Watt and Clack 1973). It has also been demonstrated that the information was better understood if the couple concerned had a better training in human biology (Leonard, Chase and Childs 1972). More attention to genetics in health care and in general education is required (Childs 1974).

Although the possibilities of preventing multifactorial congenital malformations are limited, there are a few developments that might lead to a broadening of the scope of prevention. The techniques of ultrasonography are improving each year, and it is to be expected that a number of external malformations and even some abnormalities of internal organs will be detectable at early pregnancy. The technique of fetoscopy as a means of visual inspection of the fetus has so far been discouraging (Scrimgeour 1973) but there seems to be improvement in this area as well (Kaback and Valenti 1976; Rauskolb, personal communication). Finally, there is a very exciting development in the area of basic studies of early embryonic development (Waelsch and Ericksson 1970, Bennett 1975, Ericksson 1975) and the differentiation of teratocarcinoma cells (Arzt, Bennet and Jacob 1974; Martin and Evans 1975; Illmensee and Mintz 1976). It is to be hoped that this basic research will finally lead to a better insight into the aetiology and pathogenesis of congenital malformations.

However, in addition to all these new developments, we should pay attention to the fact that only very limited use is made of the present possibilities of prevention. As a rough estimate only twenty per cent of the patients with chromosomal aberrations and genetic metabolic diseases are correctly diagnosed. Only ten per cent of the people who should obtain genetic counselling are referred for this purpose. Only seven to ten per cent of the pregnant women who should undergo amniocentesis are referred to the centres for prenatal monitoring. Even without any new developments there is very much to do to bring all the information and services to the people who are in need of them. In this respect there is also an important task for the obstetrician today: prevention of congenital disease means knowledge about genes, molecules, cells and organs, and it implies information and skill with physical, chemical and cytological techniques. It also requires understanding about what people know and do not know, what they feel and really want. The most difficult part of the task, however, is to realise one's own limitations, to acknowledge other colleagues' greater expertise and to accept the relative unimportance of one's own opinion in decisions about the future progeny of somebody else.

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Screening Populations for Genetic Disease

Patient care today encompasses not only the individual but also the community, the family and the fetus. Advances in the knowledge of human hereditary diseases mean that we are much better able to advise family members concerning their own health or the health of their offspring. As with the problem of nutrition and infection in the developing countries, an application gap exists between what is known and what is being done with respect to genetic disorders in developed countries. In this review I shall focus on the role of practising physicians, in particular obstetricians, when genetic knowledge could be applied to the care of pregnancies by screening for risk for genetic or congenital disorders.

Although on both theoretical and practical grounds fetal malformations cannot be prevented entirely, under certain circumstances the frequency of some types could be reduced substantially. Screening for such disorders will be an important means towards that end. Before reviewing the current state of genetic screening and considering future developments, it might be reasonable in the context of this presentation to ask: what is genetic disease?, what is screening?, and what is population?

What is Genetic Disease?

Broadly speaking, five categories of developmental disorders can be distinguished on the basis of genetic aetiology:

1. Unilocal genetic diseases, which are inherited according to the principles of Mendelian genetics.
2. Multilocal (or polygenic) genetic disorders, which show familial aggregation, but the effect of genes at the different gene loci involved cannot be individually determined.
3. Aneuploid or chromosomal disorders, where the subject may have part or a whole chromosome missing or too much.

The other two categories are not strictly of a genetic nature, but by tradition they fall into the realm of the medical geneticist:

4. Prenatal disorders caused by exogenous influences such as rubella virus or chemicals.

5. Developmental defects of undetermined aetiology, some of which may be either genetic or non-genetic, and yet others that may be caused by interaction of genetic and non-genetic factors.

Unilocal Genetic Disorders

These are diseases caused by the effect of a mutant gene at a single gene locus. Depending whether a mutant gene causes a noticeable effect on the phenotype when present on one gene locus but not the other (heterozygous state), or when present at both homologous gene loci (homozygous state), one can distinguish a dominant or a recessive pattern of inheritance, respectively. Most gene loci are located on one of the twenty-two pairs of autosomes (autosomal inheritance), but about 100 are known to be located on the X-chromosome (X-linked inheritance). A mutant gene on the X-chromosome is always apparent in males with XY sex chromosomes regardless of whether its effect is dominant or recessive (hemizygous state). Genes with recessive effect can generally not be recognised in the heterozygous state ('carriers' of gene). For further reading some excellent brief texts well suited for the general physician are available (Carter 1969, Fraser Roberts 1973, McKusick and Clairborne 1973, Emery 1975).

A total of about 1500 well-defined single gene disorders (or characters, for some are not diseases in the usual sense) have been recognised in man (McKusick 1975) and an additional 1400 are known but await definitive confirmation. Some examples will follow later.

Each person carries about six mutant genes in the heterozygous state (the mutant allele on one locus, the normal 'wild type' on the other) that would cause a severe genetic disease in the homozygous state (a mutant allele on both loci). With some exceptions, it cannot be determined for which genes one is a carrier. Single mutant gene disorders occur, with some exceptions, at individual frequencies of less than 1 : 10000. However, owing to their number, one of them is present in about 0.25% of newborn infants.

Multilocal Genetic Disorders

This group is presumably based on the presence of mutant genes at several different gene loci. Their individual effect on the phenotype and their inheritance, although following Mendelian principles, cannot be recognised individually. The result of their accumulative effects is a genetic predisposition for a certain defect that follows a continuous distribution in the population and cannot be accurately determined on an individual basis. It is only beyond a certain threshold that a defect actually occurs, thus betraying the genetic predisposition.

In contrast to the qualitative nature of the single gene disorders (affected or not affected) and their clear-cut segregation pattern among offspring, the genetic predisposition of multilocal or polygenic disorders is of quantitative nature (degrees of predisposition). An estimate of the frequency of recurrence in a given family or population is based on empiric data obtained by observation. Since such risk figures are averages from populations with, presumably, not identical variance, any given individual or family may deviate from the

population mean without the possibility of this being recognised in advance. Thus, by necessity, individual genetic counselling is usually somewhat imprecise with multilocal disorders.

Examples from this group are congenital heart defects, cleft lip and/or palate, congenital hip dysplasia, pyloric stenosis, open neural tube defects (myelomeningocele/anencephaly), congenital intestinal aganglionosis and some others (for review see Carter 1974, Fraser 1976b).

This group of disorders is important because they have an average individual incidence of about 1 : 1200 to 1 : 800 in the population, and about 1 : 50 to 1 : 10 in the families of patients affected with one of these disorders. As a group they occur in at least 0.5% of newborns. Since possibilities for prenatal screening for genetic disorders of this group are limited at present to detection of defects of the closure of the neural tube, which are discussed by Dr Brock (below, this volume), this group will not be further considered here.

Aneuploid (Chromosomal) Disorders

About 1 : 180 newborn infants has an aberration of the normal chromosome number or structure recognisable by light microscopy, and in about 1 : 420 this is the cause of a severe disease with multiple developmental defects. About twenty clinically defined chromosomal diseases are known. Anomalies of the two sex chromosomes X and Y or the twenty-two pairs of autosomes are involved in about equal proportions. Although most of these occur sporadically with a low risk of recurrence in the same family, some are transmitted by carriers of balanced chromosomal rearrangements with a high risk of recurrence. In addition, parental age has been identified as a risk factor, as described below. Only one chromosomal disorder, trisomy 21 (Down's syndrome, mongolism), is relevant to genetic screening, as discussed later.

The two other categories of congenital disorders need not be considered here, although they account for a significant proportion of congenital defects. In all, defined disorders of genetic aetiology as described above, have to be anticipated in about one per cent of newborns. The occurrence of a genetic disorder, therefore, should be more readily anticipated by physicians than at present. A state of awareness of genetic disorders is an important aspect of good prenatal care, as will be seen later.

What is Screening?

Genetic screening sets out to identify persons at risk for a genetic disease affecting either themselves or their offspring before this risk is apparent or incurred. Three principal forms of genetic screening can be distinguished: (a) screening for medical intervention to provide early diagnosis and therapy, (b) screening to provide reproductive information, and (c) screening for surveillance to establish or monitor the frequency of congenital or genetic disorders.

Screening is not diagnosis, but merely the presumptive identification of unrecognised disease or risk by a screening test, examination or some other procedure, which can be applied to large numbers of people. It must be confirmed by full diagnostic evaluation, counselling, and treatment if available.

The subject of screening has been reviewed extensively in recent years (Levy 1973, Bergsma *et al.* 1974, Whitby 1974, Chard 1974, Laurence 1974, Raine 1974, Genetic Screening Report 1975, Kaback 1975, Erbe 1975, Motulsky and Boman 1975, Reilly 1975, Childs *et al.* 1976a and b).

Screening for genetic disease can be directed towards the entire population (mass screening), or towards certain parts of the population identified with an increased risk for a certain disease or group of diseases (selective screening).

What is Population?

Population studies have revealed a high degree of genetic diversity in human populations. At many gene loci more than one allele (a gene at the same gene locus with a different effect) may occur. If the frequency of a certain allele rises over one per cent, it is called genetic polymorphism. It has been estimated that at least thirty, and probably more than fifty, per cent of human gene loci are polymorphic. Each person may be heterozygous for at least seven per cent of them, and perhaps as many as twenty per cent.

Although most single gene disorders occur at a frequency of less than 1 : 10000, the frequency of unaffected carriers of a recessive gene in the heterozygous state is of the order of 1 : 400 to 1 : 50 for most of the conditions known. This means that, on the average, each person has a small but definite probability for mating with another carrier of the same gene. Such a mating will have a twenty-five per cent risk of an affected child from each pregnancy. We may conclude again that the occurrence of a genetic disorder is not a unique event, befalling just a few unfortunate families or individuals, but an ever-present possibility.

The following sections will summarise the current state of genetic screening, outline the potential and requirements for developments relevant to antenatal care, and finish with some recommendations pertinent to these problems.

Genetic Screening : Current State

Since screening for genetic hyperphenylalaninaemia (phenylketonuria or PKU) began in 1962, mass screening for this disorder has been carried out in many countries and has resulted in early therapy for numerous infants. This success was not achieved without problems, and many questions had not been answered at the inception of mass screening (Levy 1973, Genetic Screening Report 1975). Since then at least twelve disorders of the amino acid metabolism, two carbohydrate disorders (galactosaemia, galactokinase deficiency), some red blood cell disorders (glucose-6-phosphate dehydrogenase deficiency, haemoglobin diseases), and some individual disorders such as Tay-Sachs' disease, hereditary angioneurotic oedema, cystic fibrosis, alpha-1-antitrypsin deficiency, Duchenne's muscular dystrophy, hyperlipidaemias, chromosomal disorders and others have been subject to screening (tables 1–4). Most of these require small amounts of blood obtained in the newborn nursery. Often more than one disorder is screened for from one blood sample (multiphasic

screening). While they have provided useful incidence data, often relevant to genetic counselling, they have on occasion failed to contribute directly to the welfare of patients and, in some instances, even caused unwarranted anxiety and problems.

Table 1. Inborn errors of metabolism detected by mass screening of newborn population. (Adapted from Levy 1973, Shih 1975 and Sveger 1976.)

Disorder	Number screened	Number detected	Incidence
Amino acid disorders			
Hyperphenylalaninaemias	13 665 664		
'classic' phenylketonuria		1190	1: 11 500
other hyperphenylalaninaemias		133	1: 30 000
Maple syrup urine disease	3 372 363	17	1:200 000
Hypermethioninaemia (homocystinuria)	2 781 042	12	1:230 000
Hereditary tyrosinaemia	1 401 777	0	n.d.
Histidinaemia	1 098 215	47	1: 23 000
Hyperprolinaemia	631 025	4	1:150 000
Hartnup disorder	651 323	26	1: 25 000
Iminoglycinuria	651 323	53	1: 12 000
Cystinuria	651 323	83	1: 8 000
Argininosuccinic aciduria	364 025	4	1: 90 000
Cystathioninuria	364 025	5	1: 73 000
Propionic acidaemia	364 025	1	1:300 000
Hyperglycinaemia (non-ketotic)	364 025	2	1:180 000
Hyperlysinaemia	364 025	1	1:300 000
Hyperornithaemia	350 176	1	1:300 000
Fanconi tubulus dysfunction	350 176	1	1:300 000
Vit. D dependent rickets	350 176	1	1:300 000
Carbohydrate disorders			
Galactosaemia	3 099 738	41	1: 75 000
Galactokinase deficiency	3 099 738	2	1:750 000
Other disorders			
Hereditary angioneurotic oedema	180 000	0	n.d.
α_1-antitrypsin deficiency	200 000	190 (120PiZ, 48SZ)	1:300 for Z allele
Overall incidence of all inborn errors of metabolism ~ 1:2400			

The only forms of mass screening currently relevant to antenatal care are screening for PKU (and the problem of maternal PKU, see below) and, possibly, screening for open neural tube defects (see Brock, below, this volume). In contrast, selective screening of defined segments of the population at risk has great potential, only part of which is now being realised.

Screening for Reproductive Information

Selective screening for genetic disease is possible, and is in fact being practised for three segments of human populations that have been shown to be at in-

creased risk: certain haemoglobin diseases in African and Asian populations, Tay-Sachs' disease in Ashkenazi Jewish populations, and Down's syndrome in older mothers. In these groups it is possible to identify an unaffected carrier or an affected fetus, or both, during mid-pregnancy, and thus potentially prevent these disorders in the populations at risk.

Table 2. Other genetic disorders detected by selective screening of certain populations. (Data from Levy 1973, Stephan *et al.* 1975, and Beckmann & Scheuerbrandt 1976.)

Disorder	Number screened	Number detected	Incidence
G6PD[1]	> 500 000	~ 9000	~ 1:26
Sickle cell disease	> 500 000	?	1:500
Sickle trait	> 500 000	?	~ 1:11
Duchenne muscle dystrophy	16 520		
male newborns	8 590	5	1:1718
female newborns	7 930	0	
Cystic fibrosis	34 300	19	1:1800

[1] Glucose-6-phosphate dehydrogenase deficiency (Desforges 1976).

Screening for Haemoglobin Diseases

Sickle cell anaemia and thalassaemias (Cooley's anaemia), both occurring in a number of clinically and genetically different degrees and types, are prevalent among populations of African and Asian origin. For example, eight to ten per cent of the American black population is heterozygous for haemoglobin S, which in the homozygous state or in combination with some other haemoglobin mutant alleles such as haemoglobin C, D, β-thalassaemia, or persistent fetal haemoglobin, causes sickle cell anaemia. About two to three per cent are heterozygous for haemoglobin C and one to two per cent for β-thalassaemia. Screening for heterozygotes has been beset with social, legal and educational problems related to misconceptions about the purpose of a screening programme within a minority group. In addition, some programmes had been started in a haphazard, poorly planned way, which did not adequately regard the consumer's views (Genetic Screening Report 1975, Erbe 1975).

With the prospect of antenatal diagnosis of some haemoglobinopathies (Alter *et al.* 1976; Kan, Golbus and Dozy 1976a and b), genetic screening for these disorders is now being developed under appropriate auspices.

Screening for Tay-Sachs' Disease

Tay-Sachs' disease screening was initiated by Kaback in the Baltimore-Washington area in 1971 and has proved to be an example of well-planned successful genetic screening that has been, on the whole, well received by those screened (Genetic Screening Report 1975, Kaback 1975, Childs *et al.* 1976a and b). Similar programmes have been started in other parts of the United States, in Canada, England, and South Africa. Some aspects of the

programme, in particular its social and psychological impact as well as the motives for participation, are of interest for any screening programme.

Tay-Sachs' disease is a lysosomal lipid storage disorder caused by a deficiency of the enzyme N-acetyl hexosaminidase A (Hex A), which normally removes the terminal β-N-acetyl galactose from GM_2 ganglioside, a ceramide hexosamine primarily present in neurones. As a consequence, GM_2 ganglioside accumulates there and causes a progressive neurologic disease

Table 3. Screening human newborn infants for chromosomal aberrations. (Data from six newborn studies in Århus, Boston, Edinburgh, London (Ontario), New Haven and Winnipeg, adapted from Hamerton *et al.* 1975.)

Total number screened	46 150	
males	29 930	
females	16 220	
Total abnormalities	258 (0.56% or 1:178)	
Autosomal trisomies	57 (0.12%)	(22% of affected)
trisomy 21	47 (0.10)	
trisomy 18	6 (0.01)	
trisomy 13	3 (0.007)	
triploid	1 (0.002)	
Sex chromosome anomalies (males)	75 (0.16)	(29% of affected)
47,XXY	27 (0.09)	
47,XYY	30 (0.10)	
other	18 (0.06)	
Sex chromosome anomalies (females)	23 (0.05)	(9% of affected)
45,X	2 (0.01)	
47,XXX	16 (0.10)	
other	5 (0.03)	
Structural rearrangements—balanced	84 (0.18)	(32.6% of affected)
Robertsonian translocations		
t(DqDq)	33 (0.07)	
t(DqGq)	9 (0.02)	
reciprocal & insertional translocations	36 (0.08)	
inversions	6 (0.01)	
Structural rearrangements—unbalanced	19 (0.04)	(7.3% of affected)
Robertsonian translocations	2 (0.004)	
reciprocal & insertional translocations	1 (0.002)	
inversions	1 (0.002)	
deletions	2 (0.004)	
supernumerary	7 (0.015)	
other	6 (0.013)	
Chromosomal polymorphism (marker chromosomes)	230 (1.65)	(230/14 069)[1]
single autosomal markers	148 (1.05)	
single Y-chromosomal markers	79 (0.56)	
double markers	6 (0.043)	
other	4 (0.028)	

[1] Winnipeg study only (Hamerton *et al.* 1975).

beginning three to six months after birth and resulting in death by the age of three to six years. This dreadful disease is caused by an autosomal recessive gene in the homozygous state. Among Ashkenazi Jews it occurs with a frequency of about 1 : 3600 births, and 1 : 30 (3.3%) are heterozygous carriers. Thus 1 : 900 matings in this population have a twenty-five per cent risk per pregnancy for an affected child.

Tay-Sachs' disease is well suited for screening, because the heterozygous carrier can be identified by a simple, reliable, automated, and relatively inexpensive test (Genetic Screening Report 1975, Kaback 1975) and because it can be diagnosed *in utero* following amniocentesis in mid-pregnancy. Therefore, primary prevention is possible, because the genetic risk is signalled before the birth of an affected child. Up to now, several thousand people have been screened, several dozen matings at risk identified, and a similar number of affected children prevented, which would not have been possible without screening. Some social and psychological aspects are discussed below.

Childs *et al.* (1976a) reported non-involvement of the medical profession (only 6.5% of the approximately 7000 persons screened had heard about the disease from medical sources) and poor quantitative and qualitative knowledge of the genetics of the disease among the population screened (only fifteen to twenty per cent could say what a Tay-Sachs' carrier is and only four per cent had a good or fair qualitative and quantitative knowledge of the genetics of the disease), although they had asked well-educated young people. Still the programme was well received and there was no evidence of adverse impact on reproduction plans or interpersonal relations, although about half the carriers expressed discomfort when they learned that they were heterozygotes (Childs *et al.* 1976b). The authors concluded that counselling would be helpful in this respect, but still some residual unease had to be anticipated. They suggested that screening would best be carried out under the ordinary medical system, rather than outside it.

Childs *et al.* (1976a) also noted that people differ in the way they employ numerical odds in decisions of daily life. A tendency to minimise high risks and to maximise low risks may be relevant to some counselling situations. This study shows the need for the physician to learn how genetics relates to preventive medicine, and for the public to be more informed about human biology.

Screening for Down's Syndrome by Amniocentesis

Since the studies by Shuttleworth in 1909 it has been known that advanced maternal age is associated with increased risk of Down's syndrome, long before the chromosomal aetiology was established by Lejeune in 1959 (Lejeune, Gautier and Turpin 1959) and chromosomal studies in the fetus by cultured amniotic fluid cells were first done by Klinger in 1965. Although the basis for the effect of maternal age is still not known, it defines a population at risk and permits selective screening by amniocentesis. Since the procedure is reviewed elsewhere in this volume, the following remarks are restricted to the epidemiology of Down's syndrome as relevant to antenatal screening.

Down's syndrome is caused by an additional chromosome 21 in all or most somatic cells after it has entered an ovum, and perhaps sometimes a sperm cell, by non-disjunction during meiosis. Thus the zygote has three instead of two chromosomes 21 (trisomy 21). This event accounts for about 95% of cases of Down's syndrome. The remainder is the result of a chromosome translocation involving chromosome 21 or non-disjunction in somatic cells in the early stages of embryonic development. The latter two cases need not be considered for the purpose of screening.

Table 4. Frequency of trisomy 21 in relation to maternal age. (Figures adapted from Hook 1976, Ferguson-Smith *et al.* 1976, Polani *et al.* 1976 and Mikkelsen *et al.* 1976.)

Maternal age (yr)	Frequency of trisomy 21 in newborns (approx.)	Risk factor relative to population (approx.)
< 30	1:1500	0.3
31–35	1: 650	1
35–36	1: 400	1.5
36–37	1: 300	2
37–38	1: 250	3
38–39	1: 180	4
39–40	1: 130	5.5
> 40	1: 50	13
all ages	1: 650	—

Numerous studies have shown a rather consistent incidence of trisomy 21, as shown in table 4 and figure 1. These figures indicate the striking increase in risk beyond a maternal age of thirty-five, in particular beyond thirty-eight. In principle it would thus be possible to detect an affected fetus *in utero* early enough to terminate the pregnancy. The question is whether the public and their physicians will accept such an approach, whether the capacities for

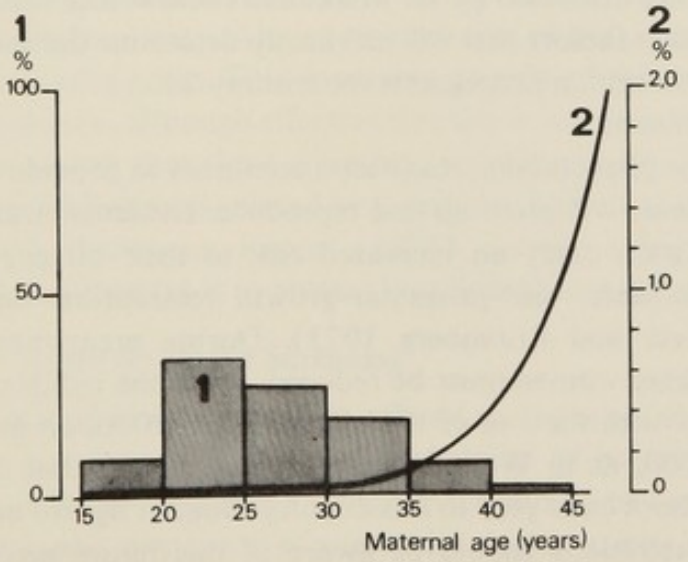

Figure 1. Maternal age groups and proportion of total birth rate (1) in relation to the incidence of Down's syndrome (2).

counselling and diagnosis are sufficient, and whether economic considerations would induce public health authorities to support such a programme.

Table 5 shows the proportion of infants with trisomy 21 in relation to maternal age and the overall birth rate. The data in this table indicate that if antenatal screening for trisomy 21 were carried out on all mothers older than 40, 23% of patients with trisomy 21 would be detected *in utero*; beyond the age of 39 it would be 27%, beyond 38 years 31%, beyond 37 years 34.5%, beyond 36 years 38.8%, beyond 35 years 42.5%. Mikkelsen *et al.* (1976) recently reported slightly smaller proportions of trisomy 21 born to older mothers from the Copenhagen area, but the principle holds.

Table 5. Proportion of infants with trisomy 21 in relation to the overall birth rate. (Data for the Federal Republic of Germany, based on a total birth rate of 635633 in 1973.)

Maternal age (yr)	No. of births with trisomy 21 expected	% of births with trisomy 21	Total no. of births	% of all births
35–36	34.95	3.58	13978	2.39
36–37	39.43	4.03	11829	2.06
37–38	37.78	3.86	9445	1.04
38–39	42.89	4.38	7720	0.95
39–40	39.45	4.04	5140	0.70
40+	221.58	22.65	11079	1.78
35+	977.90	42.54	59191	8.92

However, a prohibitive expansion of existing diagnostic facilities and expenditure would be required for screening below the age of 39–40, as suggested by Stein, Susser and Guterman (1973). Before we briefly consider the economic aspects, which are discussed by Dr Mikkelsen (below, this volume), it is pertinent to review other factors that will ultimately determine the success or failure of an antenatal screening programme for trisomy 21.

Maternal Phenylketonuria

Genetic screening for phenylketonuria (PKU) continues to provide early therapy. Affected individuals will grow up and reproduce. Evidence accumulates that mothers with PKU carry an increased risk to their offspring of congenital malformations, ante- and postnatal growth retardation, and of mental retardation (Scriver and Rosenberg 1973). During pregnancy the phenylalanine intake of these women must be reduced. Since the incidence of newborn females affected with PKU is of the order of 1 : 20000, an annual birth rate of about 600000, as in West Germany today, means that about thirty girls with PKU are born each year in this country. Similar figures can be expected in Britain. Obstetricians should be aware of this future problem, and the future has already begun: the first girls treated successfully are now reaching puberty.

Screening for Rhesus Incompatibility

With fifteen per cent of the population having rhesus-negative blood group, screening for Rh-negative mothers developing antibodies against a Rh-positive fetus during pregnancy has been an extremely successful form of preventive medicine for a basically genetic problem. With the possibility of anti-D (Rhesus) prophylaxis by immunisation, screening for Rh-negative mothers will remain a useful form of genetic screening (Clarke 1973). Since fetal blood may enter the maternal circulation during amniocentesis, it is important to immunise Rh-negative mothers at that time.

Screening for Cystic Fibrosis

About one person in twenty-two of Western and Central European origin is heterozygous for a mutant gene *cf* (cystic fibrosis), which in the homozygous state causes a progressive generalised dysfunction of the bronchial cilia and the exocrine pancreas. This disorder is the most common recessive trait in European populations (disease frequency 1 : 2000). Although causal therapy is not available, early and persistent treatment appears to be beneficial. Heterozygotes cannot be identified with certainty and no antenatal diagnosis is possible yet. Screening, therefore, is aimed at early diagnosis to provide therapy and genetic counselling. A test-strip has been developed for screening by stool examination (Stephan *et al.* 1975).

Cystic fibrosis will be a candidate for premarital and antenatal screening in European populations when the heterozygotes can be identified and antenatal diagnosis can be achieved. This will probably take another few years, but is likely to come eventually.

Screening for Genetic Hyperlipidaemias

Three distinct forms of genetic hyperlipidaemia, hypercholesterinaemia, hypertriglyceridaemia, and combined hyperlipidaemia, are each determined by a mutant gene in the heterozygous state (autosomal dominant inheritance). These genes are present in about twenty per cent of hyperlipidaemic survivors of myocardial infarction under the age of sixty, and in about seven per cent of patients over sixty. Almost one per cent of the population carries one of these lipid-raising genes. This poses a great public health problem and calls for early recognition, although effective therapy is not yet available. Screening attempts have shown that these disorders are difficult to identify, and genetic hypertriglyceridaemia is missed by screening. Motulksy and Boman (1975) and the Genetic Screening Report (1975), therefore, suggested that screening for hyperlipidaemias is at present best limited to small, well-designed studies.

Aims of Genetic Screening

Genetic screening should be offered and viewed as a service to the individual who may have an increased probability of producing offspring with a congenital or genetic defect. This service should be coupled with adequate counselling, and discussion of alternative decisions about pregnancy and offspring. No coercion must be used. In particular, it should be made clear that political or eugenic motives are excluded, and that screening does not strive for illusion-

ary betterment of mankind. Any success of a screening programme is thus closely linked to the degree of acceptance it has gained with the physicians and the public.

Acceptance of Genetic Screening and Awareness of Genetic Disorders

In most areas of the western hemisphere physicians are not sufficiently aware of genetic diseases. This is not to say that they should know all the different genetic disorders, which is impossible, but that many physicians do not seem to understand the basic principles underlying the occurrence of genetic disorders. An interesting survey has recently been carried out by the Committee for the Study of Inborn Errors of Metabolism, under Drs Barton H. Childs and Artemis P. Sinopoulos, for the National Academy of Sciences of the USA (Genetic Screening Report 1975) concerning knowledge of and attitude towards genetic screening among general practitioners, obstetricians, and paediatricians. Although the data are based on a sample of only 1092 doctors, the results are of sufficient interest to warrant a brief summary here.

The following areas were explored by questionnaire: exposure to genetics in formal education, experience with potential or actual genetic disorders, perceived frequency of genetic defects, genetic disorders encountered in practice, opinion about some specific defects, beliefs about genetic screening, perceived impact of genetic disease on affected families, perceived importance of detecting genetic disease, and others. The response rate was 57% for paediatricians, 41.5% for obstetricians, and 38.4% for family practitioners.

About three-quarters reported that no courses in genetics had been available during their medical training. Genetic training had been available to only half those who had graduated in the previous six years. Almost 30% of obstetricians had 'never or rarely' seen a patient with a potential genetic disorder (same proportion of paediatricians and 49% of practitioners), and 63% of obstetricians (17% paediatricians, 55% practitioners) 'never or extremely infrequently' reported actual experience with genetic disorders in children. When asked about the perceived frequency of genetic defects in live births, about 50% of obstetricians assumed a figure of under one per cent (too low) or did not respond. The obstetricians' subjective opinion of the risk for Down's syndrome, given a stated probability of occurrence of 1 : 100 (six times higher than the population average), was perceived as 'low' by 38% (paediatricians 40%, practitioners 42%), as 'medium' by 39% (45% and 40%, respectively), and as 'high' by 22% (14% and 18%, respectively).

Nevertheless, when asked their opinion of the effectiveness of their own genetic counselling, 82% considered it highly or partially effective. On the other hand, 96% of obstetricians and paediatricians, and 94.5% of practitioners, found screening for genetic disorders desirable, in spite of the belief of 28% of obstetricians that screening for phenylketonuria over the past few years has not been beneficial (paediatricians 35%, family practitioners 33%).

The committee (Genetic Screening Report 1975) concluded that the

medical profession as a whole is not ready to accept the importance of genetic disease and of screening for it at the present time. But this survey also indicated that a greater knowledge among physicians of genetics and of the impact of genetic disease would increase their readiness.

Legal Considerations

The legal aspects of genetic screening have been reviewed extensively in recent years (Bergsma *et al.* 1974, Reilly 1975, Genetic Screening Report 1975) and only a few comments are included here. Since the aim of a genetic screening programme is to provide information not available otherwise, it should be clear that any such programme must be able to render the information of the screening test to the person screened in proper medical counselling.

Difficulties may arise when a disorder is found that has not been screened for: for example, XXY or XYY karyotype, which in most cases does not cause undesirable intellectual or social attributes, or when evidence for non-paternity of the putative father is obtained (adventitious findings). The results of screening may be relevant to other family members but the person screened may wish that the results are not conveyed to other members of the family or third parties. In general, confidentiality of the results and the protection of individual privacy are important features in any legal system dealing with genetic screening. The experience of screening for phenylketonuria indicates that laws are generally not needed for genetic screening.

Ethical Aspects

The Genetic Screening Report (1975) states that most of the troubling problems in genetic screening are not scientific but ethical. Since screening should be offered as a service, conscious efforts must be made to protect the consumer's interest. Genetics is viewed by many people as a science that can be subject to perversion by genetic engineering or reproduction manipulation in pursuit of eugenic goals. In particular, antenatal screening *in utero* could be viewed as discrimination unless guide lines for proper use and an understanding of its goals have been instilled in the public and the medical professions. The decisions not to be screened should rest on knowledge of the potential benefits on one hand, but remain free from social pressures and coercions on the other.

The Genetic Screening Report (1975) quotes an 'ideal ethical observer' with the following characteristics to be emulated by the decision-making process: to be omniscient of the relevant facts, to be omnipercipient of the feelings and influences on all parties involved, to be free of self-interest, to be dispassionate and free from emotional involvement, and to be consistent in all stages of planning and application.

Economic Considerations

These are discussed by Dr Mikkelsen (below), but a few comments are included here. Any screening programme must have a sound economic basis

and it would be futile to screen regardless of cost. The average cost of an antenatal diagnosis with chromosomal analysis from cultured amniotic fluid cells has been estimated to be of the order of US $275 (£160) in the USA (Conley and Milunsky 1975), DKr1300 (£128.5) in Denmark (Mikkelsen and Nielsen 1976), DM565 (£134) in West Germany (Passarge 1976), and £78 in Britain (Hagard and Carter 1976).

Basically, cost-benefit analysis and cost-effectiveness analysis can be used to develop the economic basis of a screening programme. Cost-benefit analysis establishes the relationship between the cost required for screening and the benefits obtained, while cost-effectiveness analysis compares the costs incurred to reach a certain goal by different approaches. The identification of benefits is thus an important part of the economic strategy of a screening programme. The Genetic Screening Report (1975) has rightly emphasised that there are difficulties inherent in a simple valuation scheme that identifies benefits simply as costs expected without screening *versus* costs incurred by screening. Since the cost of screening a large number of people must be matched against the benefits to a few, the incidence and the natural history of the disorder screened for are important. Therefore, the cost of identifying one affected person per number screened (case finding cost) should be determined.

Table 6. Costs of case finding in antenatal screening by amniocentesis in West Germany. (Data from Passarge 1976, based on a total birth rate of 635 633 in 1973 and an estimated cost of DM565 (approx. £134) per antenatal test.)

Maternal age group	Number of tests required per year	Expected number of trisomy 21 detected *in utero*	Total expenditure (million DM)	Cost per case detected (DM)
35–36	14 000	35	9.70	225 714
36–37	12 000	39	6.78	173 846
37–38	9 500	37	5.36	144 865
38–39	7 700	43	4.35	101 162
39–40	5 140	40	2.90	72 500
over 40	11 000	222	6.22	28 000

I have recently done a cost-benefit analysis for the Federal Republic of Germany (Passarge 1976), which is summarised in table 6. The figures would probably be similar in other countries in Western Europe. These data indicate a cost-benefit ratio for mothers over 40 years of age of 0.14, for mothers over 39 years 0.25, over 38 years 0.33, over 37 years 0.43, and over 36 years 0.51. I have estimated the minimal average total costs for the care of one patient with trisomy 21 to be of the order of DM200 000 (£47 619), taking morbidity and mortality of this disorder into account (Passarge 1976). However, it should be emphasised that economic considerations alone are not a sufficient basis to start a genetic screening programme.

Prospects for Genetic Screening

The Genetic Screening Report (1975) set forth guide lines for establishing a genetic screening programme, which should be consulted if such a programme is initiated. The following is a brief summary. Public interest and acceptance is considered to be important for an initial assessment. This includes such factors as frequency and severity of the disease to be screened and the family and public burden, professional knowledge and attitudes towards the disorder.

The next step would be to review the feasibility of screening for the disorder by considering existing facilities, laboratory readiness, method to employ, educational possibilities, and benefits. If these are judged to be able to support a screening programme, a pilot study or field trial would follow. This should ideally be under the same auspices as the actual screening to follow. Factors important in this respect are laboratory (quality, false positive, false negative tests), setting where the test and the follow-up is to be done, how and where consent to be obtained, economic and legal considerations, and evaluation of benefits derived from the programme.

A particular problem in antenatal screening may be the concurrent consent of the father. The Genetic Screening Report (1975) recommends that screeners would be best advised to obtain the mother's consent before amniocentesis that the information will be shared with the father. A policy on the disclosure of adventitious findings would be needed, such as the detection of an XXY or XYY or other karyotype anomaly that was not screened for but that is, nevertheless, considered a deviation from the normal. It is necessary that the person screened agrees explicitly what information the screener is obliged to reveal as well as what will not be revealed.

We are planning an antenatal screening programme in the city of Essen, and amniocentesis will be offered to all women over 38 years. In a city of about 700000 inhabitants with an annual birthrate of about 5000 this will require about 185 antenatal diagnoses, almost four per cent of all pregnancies. This should discover two to three fetuses with trisomy 21 and reduce the eight live birth trisomies expected per year to about five or six, corresponding to a case finding rate of about thirty per cent.

All obstetricians (about fifty) and eleven maternity units in the city are asked to inform their patients about this service. Amniocentesis will be done at just a few units where the expertise exists and the amniotic fluid transferred to our laboratory. Amniotic fluid cells will be cultured to obtain chromosomal preparations and the content of alpha-fetoprotein will be determined. We expect to gain insights into the feasibility, acceptance, benefits, and possible pitfalls of such a programme, which might eventually be extended to the whole country.

Summary

Screening for genetic disease is a service that extends the application of genetic knowledge to identify persons at risk for a genetic disease, affecting either

themselves or their offspring, before the risk is apparent or incurred. Genetic screening may be for early diagnosis and therapy, to provide reproductive information not available otherwise, or for surveillance of disease frequency. Genetic defects constitute a substantial proportion of congenital disorders, and screening may be a rational approach towards early diagnosis or prevention of some of them. Physicians should be more aware that genetic disorders can occur in any pregnancy.

Genetic disorders due to a single mutant gene in the homozygous state (recessive inheritance) or the heterozygous state (dominant inheritance) occur in about 0.25% of newborns, disorders due to mutant genes at several different gene loci occur in about 0.5% of newborns, and disorders due to chromosomal aberrations occur in about 0.5% of newborns.

Genetic screening has been successfully carried out to provide early therapy in phenylketonuria (PKU), galactosaemia, and some other inborn errors of metabolism.

Genetic screening for reproductive information appears to be useful in certain segments of the population known to be at a defined risk for a certain disorder (selective screening). Four examples are:

1. Screening for haemoglobin diseases among populations of African or Asian origin.
2. Screening for Tay-Sachs' disease among Jewish populations of Ashkenazi origin.
3. Antenatal screening by amniocentesis in mothers over 38–40 years, who have 4–13 times the population average risk of offspring with trisomy 21 (Down's syndrome, mongolism).
4. Screening for open neural tube defects from maternal blood is being developed (see Brock, below).

Successful genetic screening depends on the feasibility and reliability of the test, benefits derived by screening and costs incurred, frequency and severity of the disease that is screened for, the family and the public burden imposed by the disease and by screening, professional knowledge and public attitudes, and finally ethical and legal considerations.

On the whole, physicians are not sufficiently involved in dealing with genetic disorders, and the public is not well enough informed about human biology to allow genetic screening without conscious efforts to make it a service to the community and to be perceived as such.

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Screening for Neural Tube Defects

The idea of screening for fetal neural tube defects early in pregnancy by the measurement of alpha-fetoprotein (AFP) in the mother's blood arose from the discovery of very high concentrations of AFP in the amniotic fluid surrounding fetuses with spina bifida (Brock and Sutcliffe 1972) and with anencephaly (Brock and Scrimgeour 1972). It seemed possible that AFP might traverse the fetal membranes and be absorbed into the maternal circulation, so that a sensitive assay technique could detect these abnormalities in a readily accessible tissue. But even since the introduction of the idea it has been clear that maternal serum AFP measurement is a comparatively crude screening procedure, which will identify some but not all of the abnormalities (Brock, Bolton and Monaghan 1973; Seller *et al.* 1974; Harris *et al.* 1974; Brock, Bolton and Scrimgeour 1974; Wald, Brock and Bonnar 1974). Furthermore between one and five per cent of all pregnancies would appear as 'false positives'. The screening technique had therefore to be seen as operating at all times in close conjunction with amniocentesis and determination of amniotic fluid AFP—a diagnostic method of high precision (Brock, Scrimgeour and Nelson 1975).

In the past four years most research on AFP screening has been directed at two questions: (a) what proportion of fetal neural tube defects can be identified by serum AFP determinations, and (b) at what cost to the disturbance of normal pregnancies by requiring mothers to undergo an amniocentesis? There is no doubt that the most definitive answers to these two questions come from the U.K. collaborative study on AFP in relation to neural tube defects (1977). In this paper, a description is given of the experiences of my own laboratory in attempting to answer these questions.

Methodology

Of the various methods for determining the ng/ml quantities of AFP found in maternal serum, namely enzyme-linked immunoassay, haemagglutination, radioimmunoautoradiography and radioimmunoassay, the latter was selected as being most appropriate to the large numbers of samples that may have to be routinely measured in a population screen. Three procedures are now available to most laboratories: the second-antibody method uses a precipita-

ting antibody directed against anti-AFP to bring down the primary antigen-antibody complex; the polyethylene-glycol method uses chemical precipitation of the antigen-antibody complex; and the solid-phase method uses a single first antibody (anti-AFP) bound in insolubilised form to sepharose beads. In our laboratory the second-antibody technique has turned out to be slow and expensive. Polyethylene glycol and solid phase are about equally cheap and rapid; the former has the disadvantage of comparative non-specificity while the latter requires special agitation equipment during the incubation stage. For laboratories commencing screening the polyethylene-glycol assay is probably the method of choice.

When the detection efficiency of the three different methods was evaluated, using a panel of maternal sera taken from pregnancies in which the fetal outcome was anencephaly (33) or spina bifida (20), each assay showed about comparable effectiveness (table 1). None of the differences was statistically significant. Since the same percentiles of the normal range were used to define the upper limit of normal in each case, the 'cost' in terms of false positive normal pregnancies was approximately the same. Because of early worries about possible non-specificity of the polyethylene glycol assay, the studies that follow were conducted using either second-antibody or solid-phase methodology.

Table 1. Comparison of radioimmunoassay methods in detecting neural tube defects. (All samples at 14–21 weeks.)

	Number and Percentage above:	
	95th percentile	98th percentile
Anencephaly (33 cases)		
Second antibody	32 (97%)	30 (91%)
Solid phase	32 (97%)	31 (94%)
Polyethylene glycol	33 (100%)	31 (94%)
Open Spina Bifida (20 cases)		
Second antibody	14 (70%)	10 (50%)
Solid phase	15 (75%)	9 (45%)
Polyethylene glycol	15 (75%)	11 (55%)

Retrospective Studies

In collaboration with Dr N.J.Wald of the Regius Department of Medicine, Radcliffe Infirmary, Oxford, a large-scale trial of maternal serum AFP measurement was undertaken using samples that were collected and stored frozen until the outcome of pregnancy was known. The samples from Oxford were serum and were assayed blind, while those from Edinburgh were plasma and were assayed with knowledge of the outcome of pregnancy (Brock *et al.* 1975). Figure 1 shows in graphical form the outcome of this study. The line designated 2 × median coincides roughly with the 95th percentile of the nor-

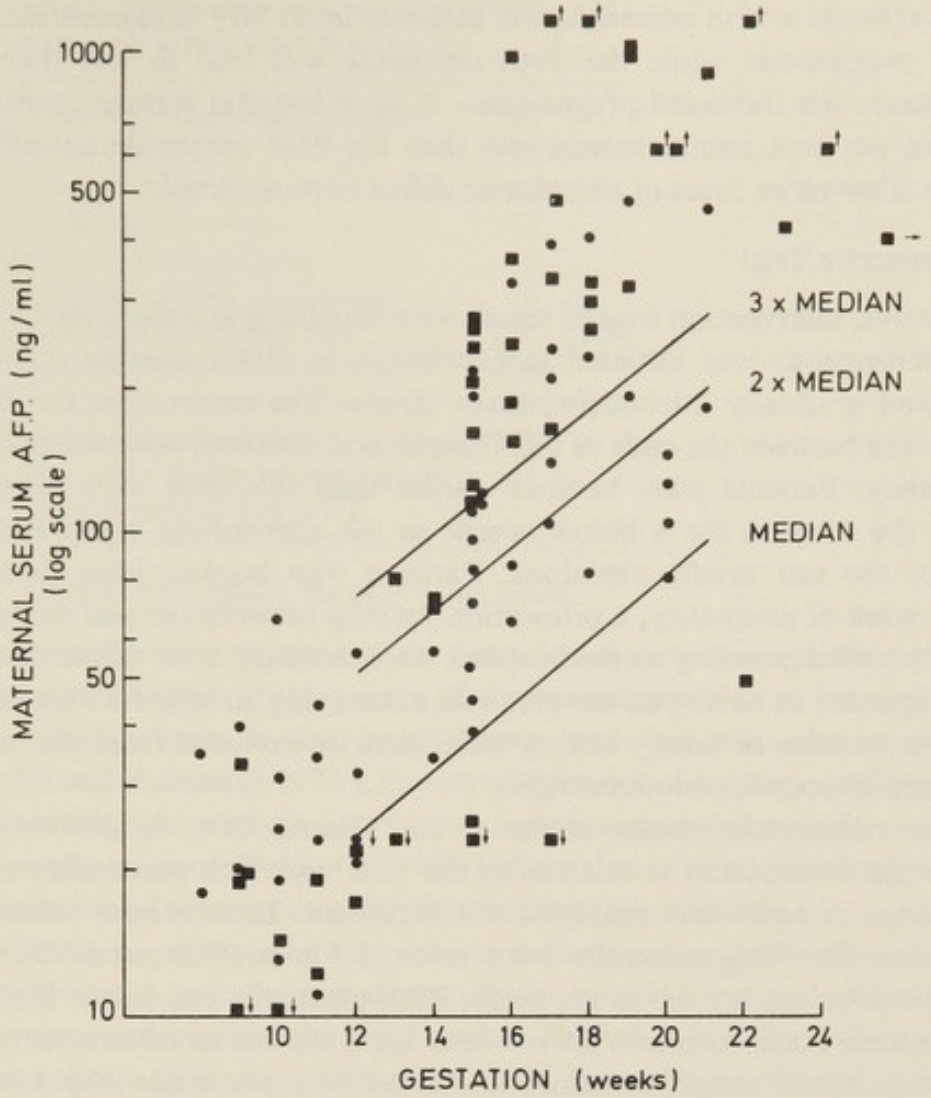

Figure 1. Maternal serum alpha-fetoprotein concentrations in cases of anencephaly (squares) and open spina bifida (circles). Solid lines represent multiples of the median value for the gestational week.

mal range, while that designated 3 × median corresponds to the 98th percentile. Multiples of the median were chosen in place of percentiles to define the cut-off point because they are easier to derive and more stable in the face of the inevitable assay fluctuations.

It is clear that the method is completely ineffective before the end of the thirteenth week of pregnancy, and thereafter increases in efficiency with advancing gestation. The U.K. collaborative study (1977) has shown that the optimal gestation is 16–18 weeks. Detection efficiencies are higher for anencephaly than for open spina bifida, and, as expected, negligible in the case of closed spina bifida. It is also clear that detection efficiency decreases as the chosen cut-off point is moved from the 90th to the 98th percentile. Since the object of maternal serum AFP screening is to identify high-risk pregnancies that will be followed up by amniocentesis and amniotic fluid AFP determination, the cut-off point has to be chosen rather carefully in the light of local facilities and resources for coping with increased numbers of amniocenteses.

In practical terms a 95th percentile will generate up to fifty amniocenteses per thousand pregnancies while the 99th percentile will lead to less than ten amniocenteses per thousand pregnancies. If local hospital services can cope with a five per cent amniocentesis rate then the 95th percentile cut-off will obviously allow more cases of neural tube defect to be detected.

Prospective Trial

A prospective, intervention trial of serum AFP screening in collaboration with Dr J.B.Scrimgeour was initiated in Edinburgh in 1975, starting with one hospital and gradually introducing three others. The target date for blood sampling was between the ends of the fifteenth and sixteenth completed weeks of pregnancy. Patients who 'booked' earlier than this time were asked to return to the hospital for a blood sample at the appropriate week, and the reason for the test briefly explained. Patients who booked later than the twentieth week of pregnancy, a proportion varying between ten and thirty per cent of the total depending on the hospital, were excluded from the screen. All patients regarded as having an elevated risk of carrying an infant with a neural tube defect because of family history were likewise excluded from the screen and referred directly for amniocentesis.

After radioimmunoassay of the serum/plasma AFP, results were reported to the obstetrician in relation to the 95th and 98th percentiles of the normal range or equivalent multiples of the median. Those whose values lay on or above the 98th percentile were referred for possible amniocentesis. Those whose values lay on or above the 95th percentile but below the 98th percentile were asked to return a week later for a second blood sample. If the value of the second sample remained above the 95th percentile they were referred for amniocentesis.

The outcome of the prospective trial to the end of January 1977, for those pregnancies where it has been possible to follow the outcome, is shown in tables 2 and 3. Raised AFP values after the first blood sample were obtained on 5.5% of the total, indicating that the cut-off point was slightly below the 95th percentile. The total of raised values after a second sample dropped to 3.2%.

Before amniocentesis the 194 patients with raised values were investigated more closely. Clinical and ultrasound examination excluded some whose gestational dates had been underestimated and whose serum AFP be-

Table 2. Prospective maternal serum alpha-fetoprotein screen (January 1977).

Samples assayed (15–21 weeks)	6034
Raised AFP values (1st sample)	329 (5.5%)
Raised AFP values (2nd sample)	195 (3.2%)
Outcome known	4059
Lost to follow-up	127
Yet to deliver	1848

Table 3. Prospective maternal serum alpha-fetoprotein screen (January 1977).

Raised AFP values (2nd sample)	195
Wrong dates	42
Twins on sonar scan	11
Threatened abortion	13
Declined amniocentesis	1
No amniocentesis carried out	3
Amniocentesis carried out	125 (2.1%)
Normal amniotic fluid AFP	106
Abnormal amniotic fluid AFP	19
Fetal neural tube defects	18[1]
Other abnormality (exomphalos)	1
∴ Amniocentesis Ratio 7:1	

[1] Twelve anencephaly, six open spina bifida.

came normal with a revised dating. Amniocentesis was not performed on any patient who showed clinical signs of threatened abortion at the time of AFP determination, since it has been shown that this may elevate the value (Seppälä and Ruoslahti 1973). However, episodes of painless vaginal bleeding up to a week before serum AFP testing were not regarded as contra-indicating amniocentesis, since Wald *et al.* (1977) have demonstrated that such bleeding and elevation of serum AFP concentrations usually coincide.

Ultrasonography was also used to indicate the presence of twins (eleven patients) and to exclude intrauterine death (missed abortion). Amniocentesis was not carried out in twin pregnancies. Though it is technically possible to aspirate the individual sacs in such cases the potential ethical problems of such a procedure are considerable. Concordance for a neural tube defect in a twin pregnancy is rarely encountered, and if one fetus is shown to be affected and the other normal, the parents are presented with a problem which it is hardly fair to expect them to resolve. Only one patient has so far definitely declined amniocentesis, although three others who failed to present themselves should probably be included in this category.

Of the 125 amniocenteses carried out, normal amniotic fluid AFP values were found in 106 cases, about two thirds of whom have now come to term and delivered normal infants. About half the fluids had visible bloodstaining. It is our practice to set aside an aliquot of such fluids until after the amniotic fluid AFP value has been determined. If the value is raised the cell button in the reserved aliquot is then examined by the Kleihauer technique for the presence and number of fetal red cells. With moderately increased AFP concentrations (between three and ten standard deviations above the mean for the gestational week), and fetal erythrocytes exceeding 10^6 per ml amniotic fluid, a repeat amniocentesis is requested. If a second amniocentesis cannot be performed an attempt may be made to calculate the expected contribution of fetal serum to the amniotic fluid AFP concentration (Brock 1977). This is, however, a somewhat imprecise process. Recently Gosden and Brock (1977)

have shown that a more reliable procedure is to examine the morphology of the rapidly adhering cells in such fluids. Characteristic cells are seen when the fetus has a neural tube defect, even in the presence of fetal blood contamination (Brock and Gosden 1977).

In the remaining nineteen amniotic fluids raised AFP concentrations were encountered. Termination of pregnancy led to twelve cases of anencephaly, six cases of open spina bifida and one case of exomphalos. The ratio of amniocenteses performed to fetal abnormalities discovered was thus 7 : 1.

One of the disconcerting features of the screening programme has been the relative dearth of neural tube defects identified. It is possible that calculations of expected numbers on the basis of a local incidence rate of 5.5 per thousand is an overestimate (table 4). It is likely that we have missed some cases of both spina bifida and anencephaly, which will be revealed when all the pregnancies come to term. However, as shown in table 4, amongst the 4059 completed follow-ups only one case of open spina bifida has been missed. Three other cases of open spina bifida have been born in the hospitals covered by the screen but these were to mothers who booked too late to have serum AFP measured. There have also been two cases of closed spina bifida that showed normal serum AFP values.

Table 4. Prospective maternal serum alpha-fetoprotein screen (January 1977).

	Neural Tube defects:		
Samples	expected	found	missed
6034 measured	33	19	?
4059 outcome known	22	9	1

Problems of Maternal Serum AFP Screening

Some of the problems encountered in the prospective screening trial are shown in table 5. One of the most difficult of these to assess is the cost of screening, which is dealt with in detail by Dr Glass (below, this volume), and only two comments on this point will be made. The first is that if wide-scale screening is contemplated it is important that laboratories use their own reagents and do not rely on commercial kits. The cost of consumables in kits is approximately fifty times that of home-prepared laboratory reagents, and, furthermore, commercial companies have a disconcerting habit of withdrawing or modifying their kits with little or no warning. The second is the question of whether amniotic fluid obtained because of a high serum AFP should also be screened for the normality of fetal karyotype. Since our trial has been research-orientated chromosomes have been studied in all amniotic fluids taken, but it is unlikely that this can be continued if serum screening is extended to all pregnancies. It would rapidly undo the cost-effectiveness of serum screening.

One of our biggest problems in screening has been obtaining blood

Table 5. Problems of maternal serum alpha-fetoprotein screening.

1. Laboratory load	Use own reagents
	Karyotyping of amniotic fluids
2. Gestation	Early booking
	Late booking
3. Follow-up of high values	Second sample
4. Obstetrical load	Correct sampling time
	Ultrasonar scan
	Amniocentesis
	Termination of pregnancy
5. Maternal anxiety	Amniocentesis declined
	Amniocentesis contra-indicated
6. Maternal expectations	
7. Costs	Karyotyping of amniotic fluids

samples at the optimal point in gestation. When pressure on maternity services is high patients tend to 'book' early, and in a recent survey of four Edinburgh hospitals we found that the peak booking period was at the tenth week of pregnancy. Obviously such patients have to be brought back some weeks later for their AFP blood sample. In three of the four hospitals within our trial this has been carried out by arranging an additional antenatal clinic appointment, and the reporting rate has been greater than ninety per cent. In the fourth hospital, general practitioners have been asked by letter to provide a sample as close to the sixteenth week of pregnancy as possible. The return rate in this hospital has been around seventy per cent.

A much greater problem for screening comes from the 'late bookers', those patients who first register with a hospital after the twentieth week of pregnancy. In our experience this group represents between twenty and thirty per cent of total pregnancies, and we have not considered it worthwhile measuring serum AFP in these patients. At present there seems little prospect of reducing this proportion of patients, except by the time-consuming process of making the public aware that early booking in pregnancy confers an advantage that is lost to those who delay until after the twentieth week.

In this trial we have used the 98th percentile as the primary cut-off point and the 95th percentile as the secondary cut-off point, for reasons that have been more subjective than objective. There is no doubt that if antenatal clinics and laboratories can cope, sequential blood samples will give a higher detection efficiency and lower false positive rate than a single sample. The fact that a 95th percentile cut-off has in practice led to only just over two per cent of amniocenteses, after various exclusions are made, suggests that an even lower cut-off point might be practical in some areas. We have not so far felt impelled to test this idea because of the high detection efficiency experienced during the trial.

One of the major consequences of screening that has to be assessed is the load that it throws on local obstetrical resources. Arranging special antenatal appointments for blood sampling, or persuading general practitioners to

perform this task, are labour-intensive processes and require a degree of commitment and a knowledge of the benefits of blood sampling among the personnel involved. Ultrasonography and amniocentesis are extremely skilled procedures, and become more effective when the operators have constant practice. One must also not forget that termination of pregnancy is regarded with distaste in many maternity hospitals, even when the outcome is virtually certain to be a fetus with a neural tube defect.

Perhaps the biggest unknown factor in screening at the present time is the effect on mothers undergoing it. In our experience patients who have high serum AFP values followed by amniocentesis and reassurance have few ill-effects, and may benefit from the feeling that their antenatal care has been exceptionally well-conducted. We are less certain about those where serum AFP indicates a need for amniocentesis but other factors contra-indicate, and clearly this is a group that needs especially skilled counselling and management. Since, to date, only one patient has declined amniocentesis when all other factors indicated it, this category would appear to be of minor importance in this part of the world.

As screening becomes more widespread and knowledge of its potential permeates the public consciousness, we must expect some degree of back-lash among mothers who still give birth to children with neural tube defects. However much one may state that screening by serum AFP is a comparatively crude and partial process, mothers will begin to anticipate protection from the birth of a child with spina bifida or anencephaly, which all studies to date indicate cannot be completely satisfied. It is therefore important that whenever screening is discussed in public fora its limitations should be made clear, and claims that it will eventually abolish neural tube defects carefully avoided.

Implications of Maternal Serum AFP Screening

The disadvantages of screening encountered in the prospective trial have been outlined in the previous section. Nonetheless our personal experience suggests that it is very worthwhile. Obviously we are strongly influenced by the fact that we have achieved a detection efficiency amongst the mothers in our screen of 95% over a period of two years. It is probable that this high rate cannot be maintained, though we doubt that it will fall much below 90%. However, as indicated in table 6, the twenty per cent of mothers who, through late booking, avoid the screen will lower the effectiveness of screening in reducing the overall detection of neural tube defects to a figure of about 75%. Even if this were to fall as low as 50% (and we doubt that with efficient laboratory procedures and energetic persuasion of hospitals to embark on screening it can go much lower) we still feel that it would be worthwhile. Indeed, in parts of the country where serum screening is more widespread the incidence of infants born with neural tube defects is already beginning to fall (M.A. Ferguson-Smith, personal communication).

Few medical screening procedures can have such an impressive benefit-cost ratio, and yet we are dealing with a group of disorders that have such

Table 6. Implications of maternal serum alpha-fetoprotein screening.

Local incidence of neural tube defect	5 per 1000
Screening percentile	95%
Raised maternal serum AFP	3.2%
Amniocentesis rate	2.1%
Detection efficiency	95%
Mothers missing screen	20%
∴ Reduction in neural tube defect incidence	75%

appalling consequences for the affected children, their families, the community and the attending medical personnel that in many ways a straightforward economic approach is quite inappropriate.

Acknowledgements

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THE AETIOLOGY

Chromosome Anomalies associated with Fetal Malformations

At the beginning of the century, it was realised that certain developmental anomalies lead principally to fetal wastage (Mall and Meyer 1921). Later the high frequency of abnormal embryonic developments was demonstrated by Hertig and Sheldon (1943), who reported the results of an analysis of 1000 consecutive spontaneous abortions (table 1). Following their work, it was suspected that intrinsic zygote defects were among the most important causes of fetal malformations. The discovery in 1959 that congenital syndromes may result from chromosome anomalies shed new light on the studies seeking the aetiology of fetal malformations. The first observation of a chromosome anomaly in a macerated embryo was reported by Penrose and Delhanty in 1961. Since then, several studies have demonstrated the importance of chromosome anomalies in humans and have shown their relation to the different stages of reproductive failure: subfertility, spontaneous abortions, stillbirths and congenital malformations.

Table 1. Simple morphologic results of an analysis of 1000 consecutive spontaneous abortions (Hertig & Sheldon 1943).

Pathologic ova with absent or defective embryos	489
Embryos with localised anomalies	32
Placental abnormalities	96
Anatomically normal ova with macerated embryos	146
Anatomically normal ova with non-macerated embryos	74
Uterine abnormalities	64
Others	99

Anomalies in the number of chromosomes are the most frequent. These numerical errors result first from abnormal events during meiotic divisions of paternal or maternal gametes: malsegregation of one chromosome leading to conceptuses with trisomies or monosomies, failure of segregation of the complete set of chromosomes leading to triploid conceptuses by either digyny or

diandry. Triploid conceptuses may also result from dispermy at the time of fertilisation. Abnormalities during the first mitotic divisions of the zygote may lead to tetraploidy or to mosaicisms. All these numerical errors are observed in parents with a normal chromosome constitution.

Structural anomalies of the chromosome, which are considerably less frequent, may appear *de novo* or may be transmitted by one of the parents who is a balanced carrier.

Frequency and Types of Chromosome Anomalies

Incidence in liveborn children. The incidence of chromosome abnormalities in newborn infants has been established by six studies on a total of 54 746 consecutively liveborn children (Hamerton *et al.* 1975, Nielsen and Sillesen 1975). The incidence of major chromosome abnormalities is between 1 : 150 and 1 : 200 liveborn infants. Some details of these results are given in table 2.

Table 2. Incidence of chromosome abnormalities in newborn babies (from Hamerton *et al.* 1975).

Type	%	Incidence
Sex chromosome anomalies	0.21	1:500
Males	0.25	1:400
47,XYY	0.10	
47,XXY	0.09	
Females	0.14	1:700
45,X	0.01	
45,XXX	0.10	
Autosomal trisomies	0.12	1:800
47,21+	0.10	
47,18+	0.01	
47,13+	0.007	
Structural anomalies		
balanced	0.18	1:500
unbalanced	0.04	1:2500
Total	0.56	1:178

In stillbirths and neonatal deaths. Cytogenetic studies done in the investigation of stillbirths and neonatal deaths showed a frequency of 6–7% of abnormal karyotypes (Bauld, Sutherland and Bain 1974; Machin and Crolla 1974; Kuleshov 1976). The frequency of abnormal karyotypes is higher if macerated stillbirths are compared to non-macerated stillbirths, for example, 7 : 54 (13%) compared to 5 : 151 (3.3%) in the cases investigated by Bauld and Bain (1977). Among the lethally malformed infants the frequency is also higher: 13% to 36% (Machin and Crolla 1974, Waller and Waller 1973). The types of chromosome abnormalities are in general the same as those observed in liveborn infants but their relative frequency is different, as exemplified by

the greater frequency of trisomies 13 and 18 and unbalanced translocations (table 3).

Table 3. Incidence of chromosome abnormalities among stillbirths and neonatal deaths. (Data from 828 observations: Bauld *et al.* 1974, Machin & Crolla 1974, Kuleshov 1976.)

Type	%
Sex chromosome anomalies	1.2
Autosomal trisomies	
trisomy 21	0.7
trisomy 18	1.8
trisomy 13	0.5
Structural anomalies	
balanced	0.35
unbalanced	0.5
Others	0.7
Triploidy	0.35

In spontaneous abortions. Investigations of human abortions have provided the most important data related to the importance of chromosome anomalies in fetal malformations. Methodologic difficulties, due to the way of collecting specimens (at home or in hospitals without knowing the population from which they are derived) or to the different percentages of specimens which did not yield chromosome results, are the main reasons for the variations in the reported frequency of chromosome anomalies in spontaneous abortions.

From the largest series collected in Europe (Boué and Boué 1973; Kajii *et al.* 1973; Therkelsen *et al.* 1973; Creasy, Crolla and Alberman 1976) it can be estimated that fifty to sixty per cent of early spontaneous abortions are due to a chromosome anomaly. Early spontaneous abortions represent most of the recognised abortions occurring during pregnancy. The prospective studies done after amniocentesis for prenatal diagnosis have shown that the incidence of spontaneous abortion after the sixteenth week is less than two per cent of the pregnancies (Turnbull, this volume). The relative frequency of the different types of chromosome anomalies in abortuses is similar in the different surveys published. Figure 1 shows the results of four series collected in Canada (Carr 1967), Denmark (Therkelsen *et al.* 1973), France (Boué and Boué 1973) and Switzerland (Kajii *et al.* 1973).

Monosomies and trisomies are the most frequent. Nearly all monosomic zygotes are 45,X and autosomal monosomies are extremely rare. In contrast, the extra chromosome in trisomic zygotes is nearly always an autosome. In late abortion specimens, in stillbirths and in neonatal deaths the autosomal trisomies that have been found are similar to the autosomal trisomies ob-

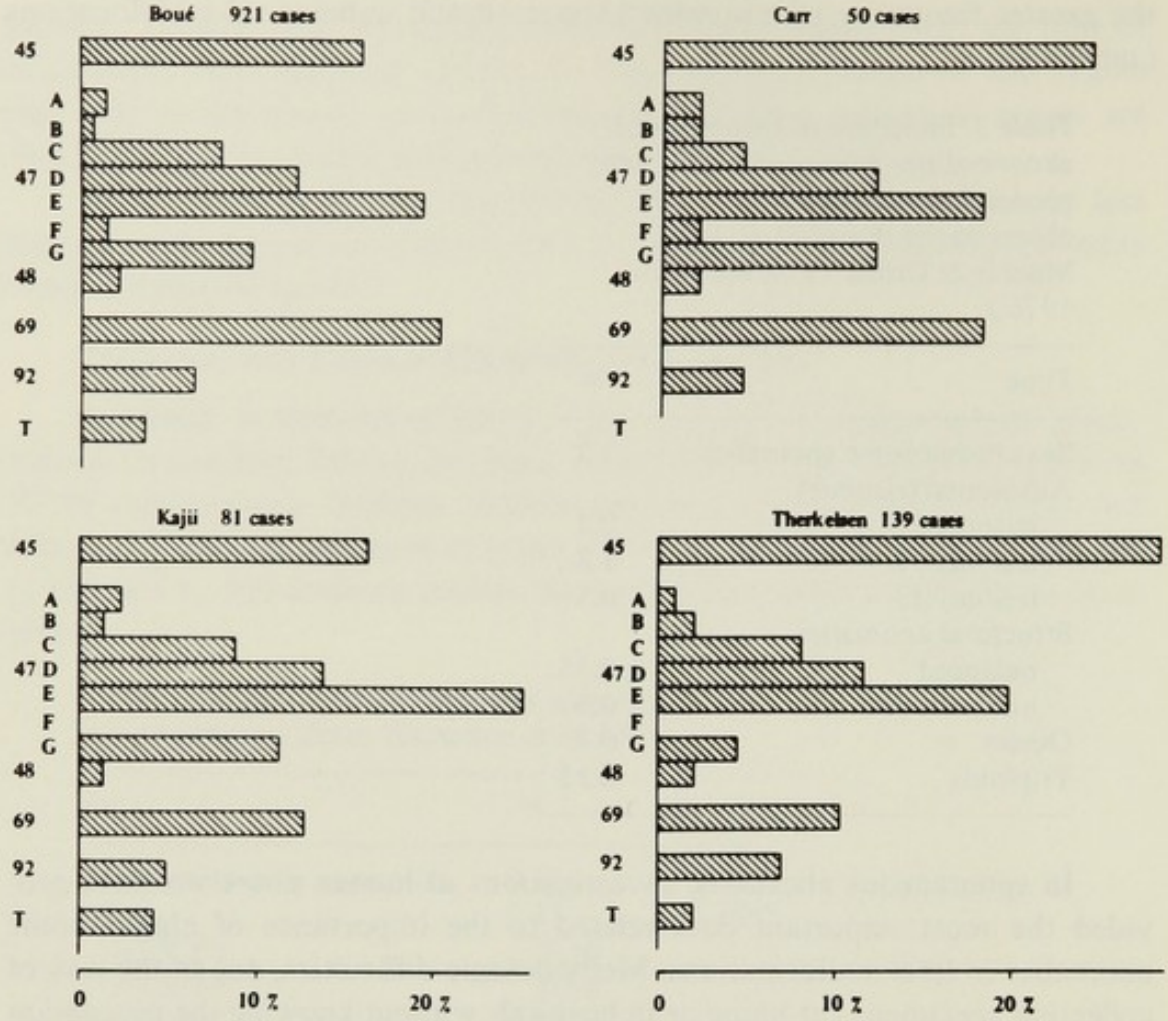

Figure 1. Relative frequency of different chromosome anomalies in spontaneous abortuses (Results from four surveys: Boué & Boué 1973, Carr 1967, Kajii *et al.* 1973 and Therkelsen *et al.* 1973).

served in liveborn infants (mainly trisomies 13, 18 and 21). In early abortuses trisomies of all chromosome groups are found. Recent studies with banding techniques have shown that nearly all chromosome pairs are involved in lethal autosomal trisomies. Table 4 summarises the results of five recent studies in which precise identifications of the extra chromosome have been done with banding techniques (Boué *et al.* 1976b). To explain the absence of autosomal monosomies and the low frequency of some trisomies it is hypothesised that these anomalies must lead to very early developmental arrests, most of the zygotes being eliminated before the pregnancy is recognised. This explanation is supported by morphologic examination of specimens with rare autosomal trisomies that showed very precocious developmental arrests (usually three weeks or less).

This hypothesis of precocious elimination of zygotes with autosomal monosomies and certain autosomal trisomies has been confirmed experimentally in a translocated mouse, the tobacco mouse, *Mus poschiavinus* (Gropp 1973; Ford and Evans 1973; Gropp, Putz and Zimmerman 1976). Due to the presence of seven balanced translocations in this experimental system, a high rate of meiotic malsegregation occurs and monosomic and

Table 4. Identification of the extra chromosome in lethal trisomies. Results from five studies: Boué (France), Carr (Canada), Creasy (UK), Kajii (Switzerland) and Lauritsen (Denmark).

Extra chromosome		No.	Extra chromosome		No.
A	1		D	13	10
	2	12		14	32
	3	2		15	32
B	4	4	E	16	104
	5			17	
				18	20
C	6	1			
	7	14	F	19	1
	8	15		20	5
	9	12			
	10	10	G	21	34
	11	1		22	37
	12	3			
				Total	349

trisomic zygotes are frequent. Chromosome analysis shows that in the preimplantation period monosomic and trisomic zygotes are observed with symmetrical distribution around the peak value of diploid zygotes. After implantation monosomic zygotes disappear; trisomic zygotes survive longer but are progressively eliminated before the end of gestation (table 5). Gropp has shown that the sequential elimination of the trisomic embryos depends on the chromosome involved. In man similar observations have been made: for example, in the trisomies of the G group, trisomies 22 have an early developmental arrest (four weeks) and in lethal trisomies 21 the peak of the developmental arrest is at six to seven weeks (42–50 days) (Boué *et al.* 1976c).

Table 5. Survival pattern of euploid and unbalanced embryos: F1 (tobacco mouse × lab. mouse) back-crossed with lab. mice (Gropp *et al.* 1976).

Stage of development	Number studied	Percentage of embryos: monosomic	diploid	trisomic
Preimplantation				
day 4	95	31	46	23
day 8	128	2	63	35
day 10–15	223	1	78	21
day 20	58		97	3
liveborn	36		100	

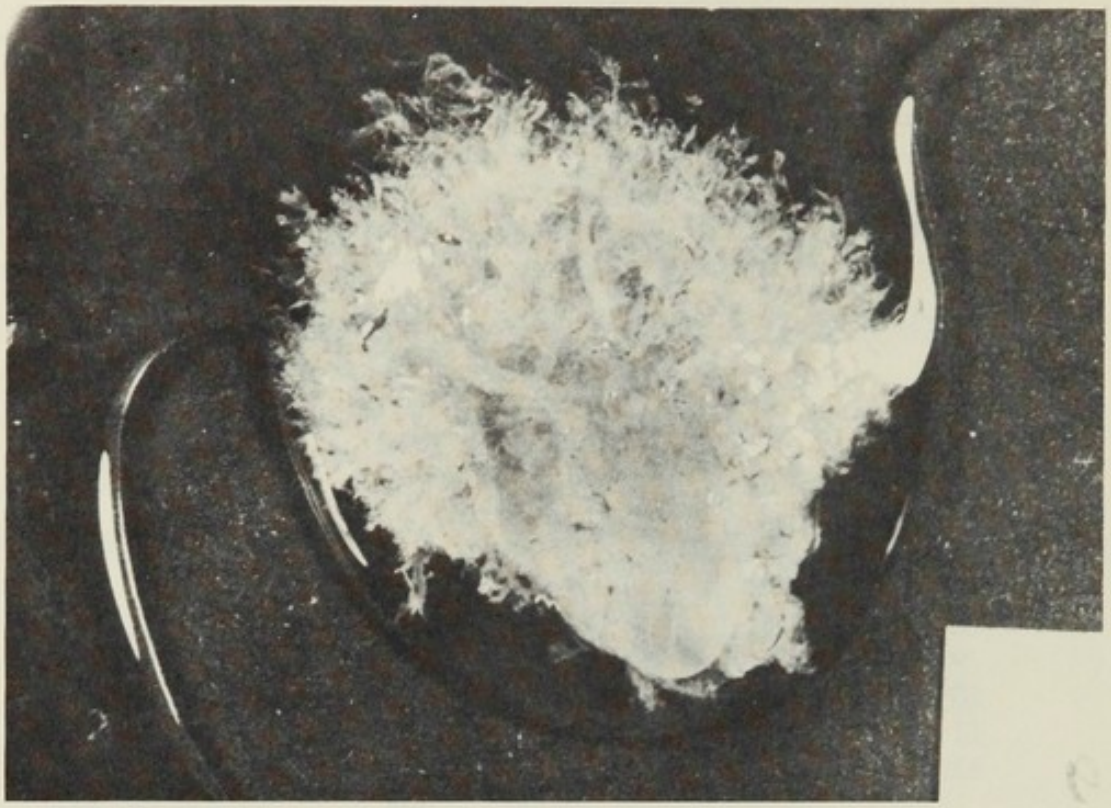

Figure 2a. Blighted ovum. This type of specimen is frequently associated with tetraploidy or with autosomal trisomies (e.g. trisomies A, B and F).

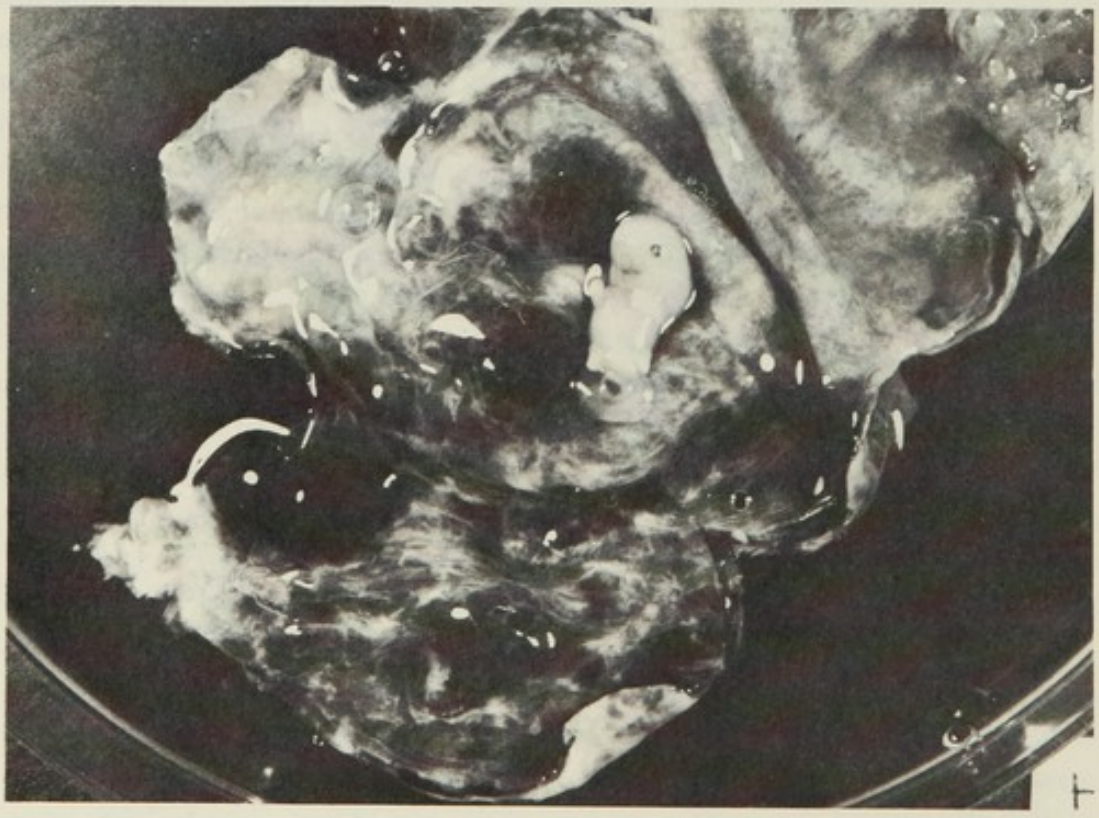

Figure 2b. Specimen with trisomy 14. Intact sac of 60 mm, macerated embryo of 40 days with under-developed nasal processes. Hypoplasia of placenta, which has developmental age of less than 35 days. Gestational age 104 days.

Figure 2c. Specimen with monosomy X. Intact sac (60 mm) of six weeks' development with well-defined cord. At end of cord, small amorphous mass of embryonic tissues. Marked subchorionic thrombosis. Gestational age 92 days.

Figure 2d. Specimen with triploidy. Intact sac of 50 mm with pseudomolar degeneration of the villi. Gestational age 184 days.

It would be important to estimate the frequency of early losses of zygotes and the proportion of chromosome anomalies in these zygotes. There was the fundamental work of Hertig *et al.* (1959), with a systematic search for early fertilized ova in the first two weeks of development, which was carried on from 1938 to 1954. Summarising their data these authors concluded that 'in any one month, when conditions are optimal only 42 per cent of fertilized ovocytes are of such viability as to cause the patient to miss her expected menstrual period'. Using a different approach Roberts and Lowe (1975) have estimated that women aged 20–29 may abort more than seventy per cent of their conceptions.

Actually it is possible to detect a pregnancy at day 7–8 post conception by measurement of chorionic gonadotrophin (HCG) by radioimmunoassay. Some preliminary data have confirmed the frequency of early losses producing no interruption in menstrual rhythm or only a delayed period (Fuchs 1977, Chartier 1977).

It seems that chromosome anomalies may explain a large number of these early losses. If one accepts the hypothesis that meiotic non-disjunctions (in male and in female) occur with equal probability for each chromosome pair, an identical number of zygotes should be conceived with monosomy and trisomy for each chromosome pair, most of them being eliminated very early. From the data collected in spontaneous abortions it has been calculated that the order of magnitude of these chromosome errors is about one in every two conceptions (Boué and Boué 1973).

What are the practical consequences of these findings?

It is unlikely that chromosome analysis of abortuses will ever become routine laboratory procedure. The simple and careful morphologic examination of fresh specimens of abortions will very often yield enough information to tentatively diagnose a chromosomally abnormal zygote (figure 2), and later histologic examination of fixed material will provide complementary information that will confirm the diagnosis. The description of phenotypic expression of a chromosomally abnormal abortus is based on different parameters:

a) *The evaluation of developmental age of the abortus and the comparison with gestational age at expulsion.* In spontaneous abortions developmental arrests occur mainly before the eighth week (post fertilisation), and about two-thirds of these specimens have a chromosome anomaly (figure 3). Embryos with a developmental age of eight to twelve weeks are less frequently collected and the incidence of chromosome anomalies is lower (around one quarter); after twelve weeks few chromosome anomalies are detected.

In chromosomally abnormal abortuses there is a lengthy *in utero* retention and there is a wide discrepancy between gestational age and the developmental age of the abortus (Boué and Boué 1973, Miller and Poland 1973). Figure 4 shows the distribution of developmental ages of autosomal trisomies, and clearly illustrates the long retention *in utero* (six to seven weeks on the average). At that stage of pregnancy, maternal hormone secretions are still active and maintain the pregnancy. The abortion occurs when the maternal

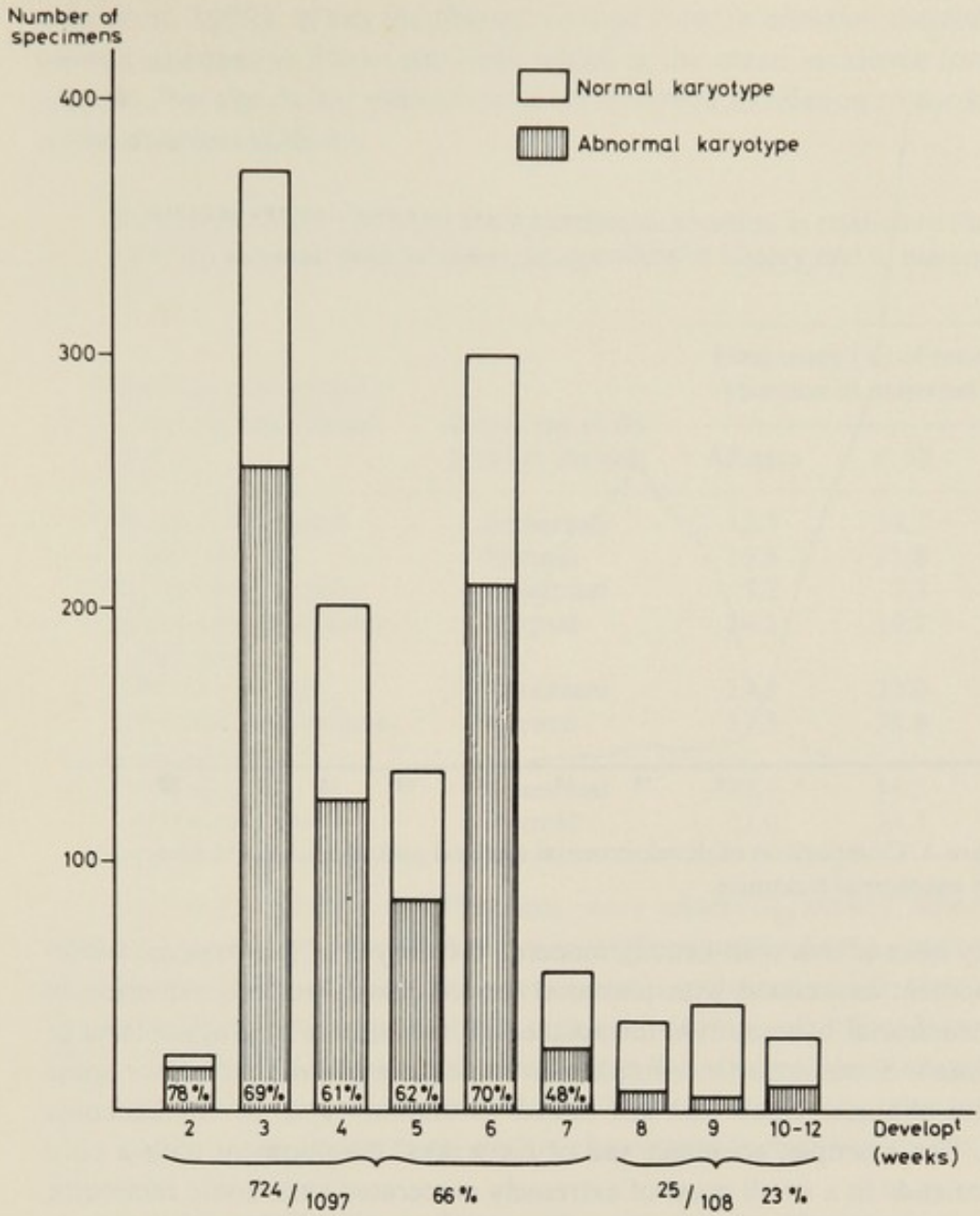

Figure 3. Frequency of chromosome anomalies in abortuses in relation to developmental arrest (Boué & Boué 1975).

secretions stop, e.g. around the twelfth week. Generally, the more advanced the developmental age the less the abortion is likely to be due to a chromosome anomaly.

b) *The simple morphologic examination of the fresh specimen.* This examination, which, firstly, evaluates the developmental age of the zygote, consists of a macroscopic description of the whole sac, the embryo and the placenta. In association with other anomalies, a wide range of central nervous system malformations is observed (Creasy and Alberman 1976).

A high incidence of chromosome anomalies was observed (Boué *et al.* 1976c; Creasy, Crolla and Alberman 1976) in blighted ova or intact empty sacs, in specimens with disorganised embryos (three to four weeks of development), with malformed embryos (five to six weeks of development) and in

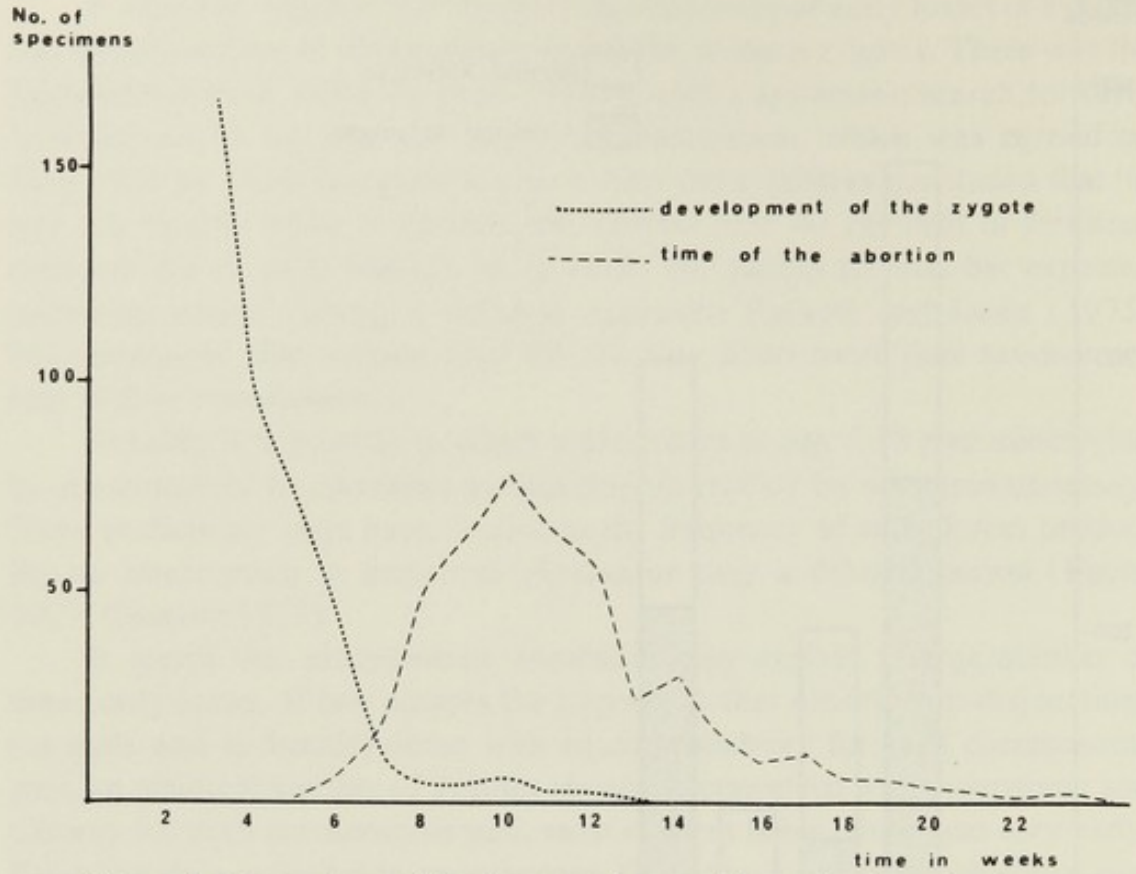

Figure 4. Comparison of developmental age and gestational age in abortuses with autosomal trisomies.

apparently normal ova with heavily macerated embryos of five to seven weeks of development associated with placental lesions related to long retention *in utero* (intrachorial haematoma, intraplacental haematoma and hypoplasia of the placenta). Sometimes this simple morphologic examination shows some phenotypic characteristics that are linked to certain types of chromosome anomaly. For example, an intact sac of six weeks' development with a cord stump that ends in a small mass of extremely macerated embryonic remnants, and with a chorion invaded by haematoma, is generally associated with a monosomy X.

c) *Histologic examination of the placenta* completes the description of the phenotypic expression, and may provide clues for a precise diagnosis (Boué *et al.* 1976c). For example, a triploidy can be suspected by morphologic examination of a hydropic molar placental degeneration with a large amniotic sac containing an especially small embryo. Histologic examination of the swollen villi shows a hypotrophic trophoblast and trophoblastic invaginations leading to formation of microcysts (Philippe 1974; Honoré, Dill and Poland 1976).

This information can be used practically in counselling for a subsequent pregnancy when there is a suspicion of spontaneous abortions with chromosome anomaly in the obstetrical history. Studies of the reproductive histories of couples before and after a karyotyped abortion have provided information concerning risks for future pregnancies (Boué, Boué and Lazar 1975; Alber-

man *et al.* 1975). When *the first obstetrical event is abortion* the risk of a recurrent abortion is fifteen per cent, which is the mean incidence for all pregnancies. No significant differences were observed in relation to the karyotype of the abortus (table 6).

Table 6. Frequency of recurring spontaneous abortion in relation to the karotype of the previous abortus, to reproductive history and to maternal age.

Reproductive history before the karyotyped abortus	Karyotype of the abortus studied	Frequency (%) of recurring abortion at maternal age:		
		All ages	⩽ 30	> 30
No obstetric events (144 cases)	Abnormal	13.3	13.3	13.3
	Normal	15.8	15.6	16.7
Delivery(ies) without spontaneous abortions (95 cases)	Abnormal	7.2	7.3	7.7
	Normal	24.3	19.2	33.3
Delivery(ies) with spontaneous abortions (78 cases)	Abnormal	24.5	23.0	24.0
	Normal	37.5	38.9	33.3
Abortions only (103 cases)	Abnormal	22.1	14.3	41.5
	Normal	21.0	23.3	12.5

However, significant differences were observed among those women who had *previously given birth to normal offspring and had a single abortion.* The prognosis for subsequent pregnancies is good when the abortus had a chromosome anomaly (seven per cent of recurrent abortions), in contrast with a high risk of recurring abortion when the abortus had a normal karyotype (table 6). It may be postulated that, in these latter cases, maternal causes such as incompetent cervix or uterine abnormalities are responsible for these abortions.

Among women who had *more than one abortion* the frequency of recurring abortion is generally high, especially in the older maternal age group after abortions with abnormal karyotype (table 6).

In patients with recurring abortions it is important to try to differentiate abortions resulting from zygotic causes (mainly chromosomal abortions) from abortions due to maternal causes, using the criteria developed for the description of phenotypic expressions. In patients having abortions occurring after the twelfth week with non-malformed, non-macerated embryos, with a high correlation between developmental and gestational ages, the frequency of fetal chromosome anomalies is low. These abortions are mainly due to maternal causes, so there will be an increased frequency of immature and premature births, which are not chromosomally abnormal, in following pregnancies. Prenatal diagnoses do not need to be undertaken in these instances.

It is important to give genetic counselling to those patients who have produced first trimester abortuses that were blighted ova, or macerated em-

bryos, with growth retardation and lengthy *in utero* retention. There is always a discrepancy between the small size of the conceptus arrested in the early stages of embryogenesis and the growth which should have been expected, based on the gestational age. This group has an increased risk of having subsequent early abortions with a high frequency of chromosome aberrations. After three abortions karyotypes of both parents should be done in order to detect structural anomalies.

When each parent has a normal karyotype, prenatal diagnosis may be indicated in some cases. Other factors influence the decision: advancing maternal age and the clinical evolution of the pregnancy. All signs signalling abnormal development of the conceptus must be considered, e.g. uterine bleeding and contractions, inadequate uterine growth, low urinary hormonal assays (Boué and Boué 1977).

Table 7. Prenatal diagnosis in structural chromosomal anomalies in either parent.

Type	No.	Affected
Robertsonian translocation	39	2
Reciprocal translocation	38	3
Pericentric inversion	17	1
	—	–
	94	6

When a structural chromosome anomaly is diagnosed a prenatal diagnosis will encourage this couple to plan a pregnancy. From the analysis of the results of 94 prenatal diagnoses made in couples with a parent carrying a structural anomaly only six fetuses with unbalanced karyotypes were found (table 7). This incidence is much lower than the expected risk. During the same period other couples with a parent carrier of a balanced structural chromosomal aberration were referred for genetic counselling. Some pregnancies aborted spontaneously before the day scheduled for amniocentesis. In 29 pregnancies with a parent carrying a balanced Robertsonian translocation

Table 8. Outcome of pregnancies in families with 14q–21q balanced translocations.

		Outcome			
Carriers[1]	Number of pregnancies	spontaneous abortion	unbalanced (trisomy 21)	balanced carrier	normal
Mother (16)	23	5	2	8	8
Father (3)	6	4		1	1
	—	–	–	–	–
Total	29	9	2	9	9

[1] Some couples had several pregnancies monitored.

14q,21q which were followed prospectively (table 8), nine spontaneous abortions occurred before amniocentesis, four were karyotyped (three trisomies 21, one trisomy 14). Twenty amniocenteses were performed and only two cases of trisomy 21 were detected. This study indicates that the risk of conceiving unbalanced conceptuses is high, and in agreement with the expected risk, but that the risk of giving birth to a Down's syndrome infant is much lower, a high selection occurring spontaneously during the early pregnancy (Boué *et al.* 1976a).

Future Developments in This Field

Studies to determine the causes of chromosome errors and the predisposing factors or external causes that may increase their prevalence have, thus far, not been entirely successful. In spontaneous abortions, stillbirths, and living newborns chromosome anomalies resulting from malsegregation during meiosis are those most frequently found. One of the major difficulties in epidemiologic studies of trisomies is that the exact nature of the non-disjunctional event is unknown. Trisomy may result from four possible events occurring in paternal or in maternal meiosis, and in either at the first or at the second meiotic division. Recently, with chromosome markers (mainly fluorescent markers), it has been possible to identify the exact non-disjunctional event in some cases of trisomy 21. In the observations collected by Langenbeck *et al.* (1976) from fifteen different published works the four possibilities were documented (table 9). It is impossible from these few selected observations to calculate the relative incidence of each of these possibilities.

Table 9. Tracing of non-disjunctional events in standard trisomy 21. Data from fifteen studies compiled by Langenbeck *et al.* (1976).

Number of families studied	243
Number of informative cases	62
1st meiotic division	
maternal	21
paternal	6
2nd meiotic division	
maternal	17
paternal	11
1st or 2nd meiotic division	
maternal	5
paternal	2

Thus, it appears that, using epidemiologic studies in which the exact non-disjunctional events are unknown, it will be difficult to elucidate those factors that increase risk. This may explain, for instance, why the results of the studies on the effect of X-irradiation on the incidence of chromosome anomalies (and mainly on trisomies) were either of borderline significance (Alberman *et al.* 1972; Boué, Boué and Lazar 1975; Patil *et al.* 1977) or con-

flicting. Also, data on triploid specimens show that different mechanisms are involved in the formation of triploid conceptuses in humans but that it is still impossible to try to estimate the relative frequency of each.

Hence even when dealing with a chromosome anomaly as precisely defined as triploidy, epidemiologic studies are difficult. Here again it is essential to improve techniques. Animal experiments indicate that the events leading to triploid conceptuses are sensitive to external factors. In humans an increased frequency of abortions, mainly those resulting from triploidy, has been noted after the use of ovulation inducing drugs (Boué, Boué and Lazar 1975; Alberman, this volume). In mice it has already been shown that the administration of human chorionic gonadotrophin results in a significant increase of triploid embryos (Takagi and Oshimura 1973, Fraser *et al.* 1976).

Another important question is: can we understand why the same type of chromosome anomaly may lead to either an early arrest of development or a liveborn infant with a life expectancy of many years? This problem is illustrated by the curve in figure 5, which shows the development attained by trisomies 21. From our data it was estimated that at least 85% of zygotes with trisomy 21 are aborted, results which are in good agreement with those of Creasy and Crolla (1974).

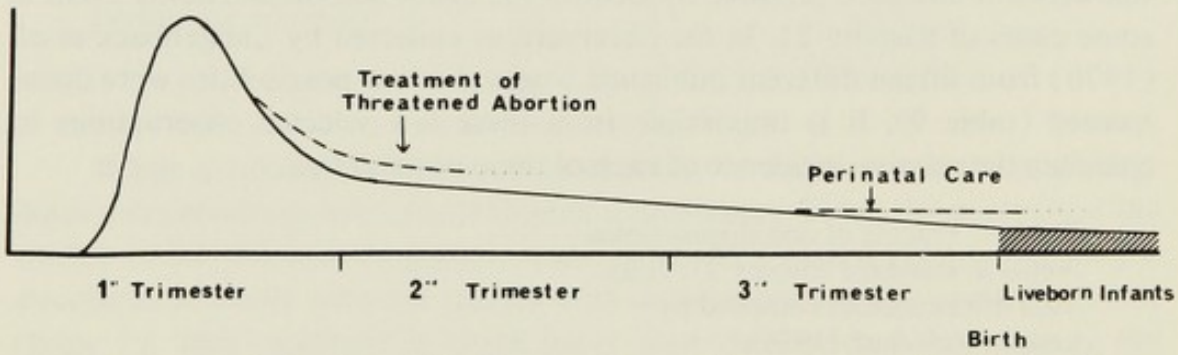

Figure 5. Distribution of developmental ages of conceptuses with trisomy 21.

It is unlikely that external factors may act on the survival of the zygotes that are arrested at five to seven weeks of development, but a small number of those that abort after eight weeks are on the borderline of possible survival. Macroscopic and microscopic examinations of such embryos did not reveal lethal malformations, and it was suspected that placental insufficiency might be responsible for these abortions. Hormonal treatment can, in some of these cases, improve placental function and ensure continuation of the pregnancy to term. The long-term hazards of high-dosage hormone therapy may yet be unforeseen: for example, female fetuses may subsequently be found in teenage to have a predisposition to vaginal carcinoma. Clinical observations of Down's syndrome pregnancies show many cases of threatened abortions that had been treated by hormone therapy (Boué, Boué and Spira 1974).

During the last decade there have been great improvements in the prevention of premature births and of perinatal deaths. In France, with a static annual number of births (880000) the perinatal deaths (stillbirths and neo-

natal deaths) decreased from 26000 (2.95%) in 1964, to 18000 (2.15%) in 1972. Trisomies 21, which were detected in about one per cent of stillbirths and neonatal deaths, also benefited by these improvements. Such variation, due to better prenatal care, may increase the number of newborns with Down's syndrome.

This illustrates a general problem in malformations in which the miscarriage rate is high and, as Leck (1976) pointed out, it is important to distinguish the 'incidence' of an anomaly in the zygote and its 'prevalence' at birth.

In conclusion, the implications of current knowledge of prenatal fetal wastage is important in the day-to-day work of the general practitioner, the gynaecologist and the obstetrician. However, it will often be difficult to arrive at a perfect decision:

First, before conception, especially when counselling couples who are subfertile, and those who have obstetrical histories of abortions. These couples desire children and exert pressure for special investigations and treatments. Certain of these investigations, such as radiologic studies or treatments such as ovulation-inducing medication, may be factors increasing the risk of chromosome anomalies. Even when useful, they must only be recommended with caution.

Likewise, it is not easy for a pregnant woman with an early threatened abortion to accept the fact that it may be better to avoid treatment that is intended to carry her to term.

It is important that physicians and their patients understand that prenatal fetal wastage represents the sacrifice that the human species must pay for its evolution—'Le prix de la vie' (de Grouchy 1976)—and that from these innumerable mutations and chromosome anomalies remain a few that have been incorporated into our hereditary patterns and have resulted in the great diversity of human beings.

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Environmental Hazards

In discussing environmental hazards and aetiological factors that are important in relation to the development of fetal anomalies, we are concerned with a wide variety of possible causal factors. In the context of genetics, however, we are particularly interested in those factors that may result in mutation. If a mutational change occurs in a germ cell, and this cell is involved in the formation of an embryo, then all the cells in the developing fetus will have the mutation and we can refer to this as a *constitutional mutation*. On the other hand, mutations may arise in somatic cells, either during the development of the fetus, or later in life, and we may refer to these as *acquired mutations*. In general, an agent that induces acquired abnormalities in somatic cells will produce similar abnormalities in germ cells, so that if these are transmitted they will appear as constitutional changes in the fetus.

In this paper, we shall consider particularly mutations that affect chromosomes or parts of chromosomes ('chromosomal mutations') and that can be seen under the microscope in dividing cells from peripheral blood, or from fetal cells in amniotic liquor. These mutations can be broadly classified into two groups: those that involve changes in chromosome number, and those that involve changes in chromosome structure (figure 1). The mechanisms giving rise to these two sorts of change are different, but both result in genetic imbalance and they may share at least some common consequences in terms of the development of fetal anomalies. Spontaneously arising or mutagen-induced acquired changes in chromosome structure can be readily observed in the peripheral blood lymphocyte chromosomes of individuals, particularly in

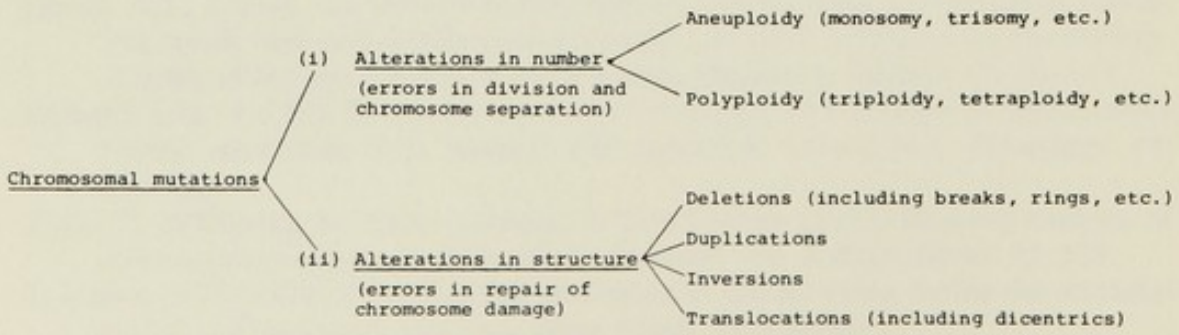

Figure 1. Categories of chromosomal mutations.

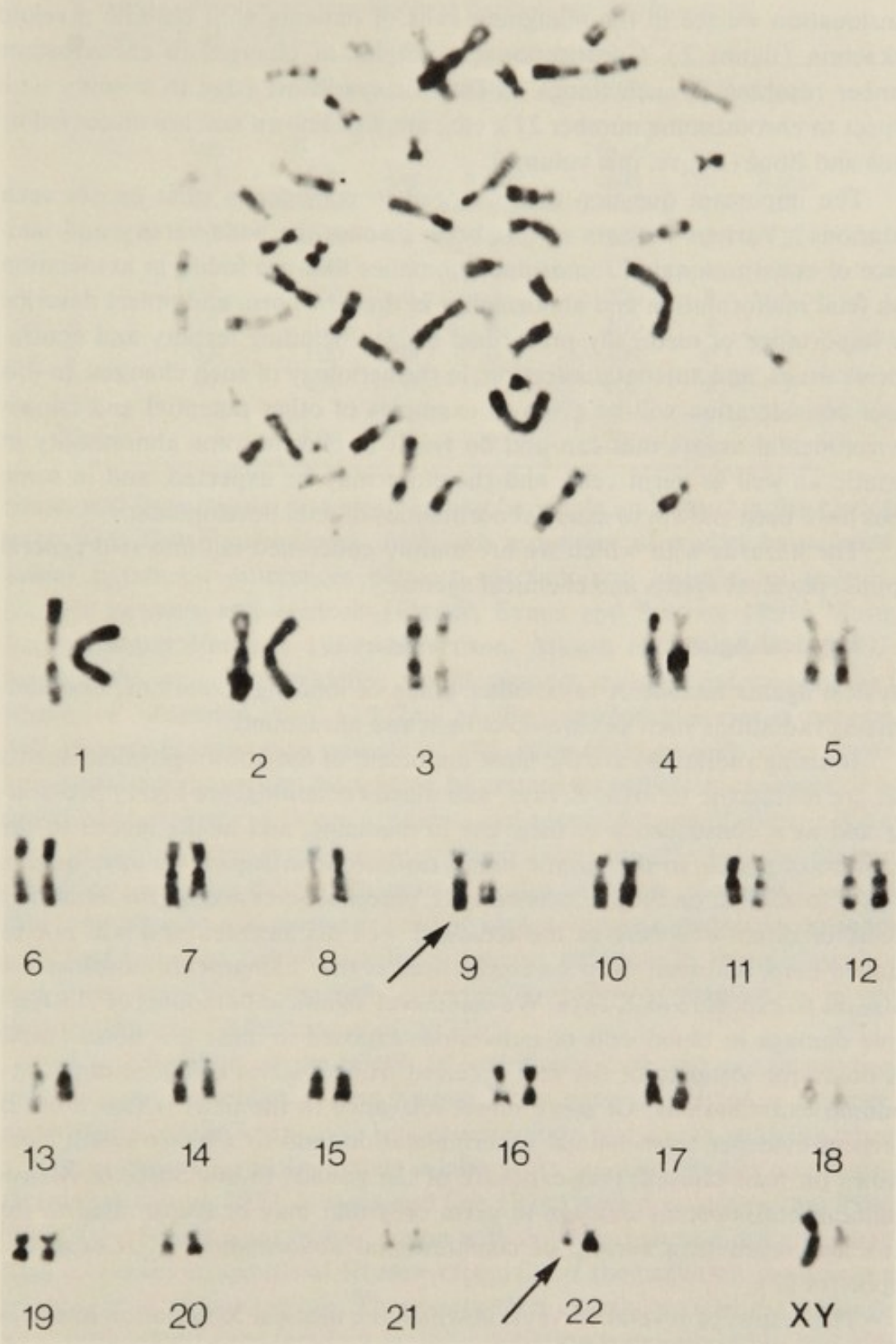

Figure 2. Metaphase cell, plus karyotype, from an R-banded preparation of bone marrow from a patient with chronic myeloid leukaemia. The leukaemic cells are characterised by the presence of a chromosome structural change (a translocation) involving an exchange between a chromosome 9 and a chromosome 22; the rearranged 22 is smaller than its partner and is referred to as the Ph^1 chromosome.

those exposed to mutagens, and a good example of an acquired change is the translocation we see in the malignant cells of patients with chronic myeloid leukaemia (figure 2). Constitutional examples of changes in chromosome number resulting in such things as Down's syndrome (due to trisomy with respect to chromosome number 21), etc., are well known and are discussed by Boué and Boué (above, this volume).

The important question that we need to consider is what causes such mutations? Various authors in this book discuss the wide variety and incidence of constitutional chromosome anomalies that are found in association with fetal malformation and abnormality in the new born, and others describe the importance of medically prescribed drugs, including fertility and contraceptive drugs, and antenatal infection, in the aetiology of such changes. In this paper consideration will be given to examples of other potential and known environmental agents that can and do result in chromosome abnormality in somatic as well as germ cells, and therefore may be expected, and in some cases have been shown, to cause abnormalities of fetal development.

The hazards with which we are mainly concerned fall into two general groups: physical agents and chemical agents.

Physical Agents

Physical agents include X-rays, other kinds of ionising radiations, and non-ionising radiations such as ultraviolet light and ultrasound.

Ionising radiations are the most important of the known physical agents that are mutagenic to man. X-rays, and allied radiations, are highly penetrating and as a consequence of their use in medicine, and of the results of the exposure of people to the atomic bomb explosions in Japan, we have quite a lot of information on their effects on cells, chromosomes and on the fetus. The effects of direct exposure of the fetus are well documented and will not be detailed here, but what must be emphasised is that human chromosomes are sensitive to exposure to X-rays. We can detect significant amounts of chromosome damage in blood cells of individuals exposed to quite low doses (table 1), doses for instance of the sort received from a series of fluoroscopic abdominal examinations. Of more direct relevance to the fetus is that there is excellent evidence from animal experimentation, and to a lesser extent from studies on man himself, that exposure of the gonads to low doses of X-rays results in chromosome damage in germ cells that may be transmitted to the fetus and result in a variety of constitutional abnormalities (U.N.S.C.E.A.R. Report 1972).

The results of several surveys, in which the medical X-radiation histories of parents of affected children and controls have been analysed, have suggested that exposure of the human ovary to low dose levels of radiation increases the risk of chromosome anomalies in future pregnancies (Uchida and Curtis 1961; Sigler *et al.* 1965; Uchida, Holunga and Lawler 1968; Alberman *et al.* 1972a and b). A variety of different chromosome anomalies may be induced, many of which are lethal and lead to 'spontaneous' abortion, but

Table 1. Effect of low dose (25 R) whole body exposure to X-rays (2 MeV) on peripheral blood lymphocyte chromosomes (Buckton *et al.* 1971).

		Before irradiation			After irradiation		
Case no.	Age (yrs)	No. of cells	Dic.	Rings	No. of cells	Dic.	Rings
1	50	100	1	0	200	3	2
2	61	100	0	0	200	1	1
3	79	100	0	0	200	8	1
4	60	100	0	0	200	5	1
5	53	100	0	0	200	8	0
6	40	100	0	0	200	5	1
Totals		600	1	0	1200	30	6

some, and in particular trisomy 21, may be viable and result in the birth of a baby with Down's syndrome. Although a number of studies have failed to reveal significant differences between the radiation histories of parents of Down's patients and controls (Carter, Evans and Stewart 1961; Marmol, Scriggins and Vollman 1969; Stevenson, Mason and Edwards 1970), on balance the weight of evidence would support such an association. In the studies of Alberman *et al.* (1972a), on the radiation histories of parents of 465 Down's patients and parents of 465 other children with other types of congenital handicap not thought to be related to radiation exposure, it was shown that mothers of Down's patients had received more diagnostic X-rays, both in number and in total dose, than controls; the effects being greater in the older age group mother. In strict quantitative terms, the results indicated that mothers who had received 2 rad or so, ten years or more prior to conception, had twice the overall risk for producing babies with Down's syndrome and some three times the risk of having pregnancies terminating in spontaneous abortion (Alberman *et al.* 1972b).

These findings on the effects of low X-ray dose and age in producing chromosome anomalies in the human fetus have stimulated a number of experiments on the interaction between radiation and age in inducing trisomy and other chromosome anomalies in laboratory animals. Studies on the mouse (Yamamoto *et al.* 1973, Uchida and Lee 1974) have now shown that irradiation of the female prior to conception with doses as low as 5 rad gives significant increases in aneuploid fetuses, especially if the radiation is given many months prior to conception. These data then exactly parallel the findings in man and underline the fact that ionising radiation is an important, and indeed powerful, teratogen in so far as man is concerned, and that we should be on our guard against its *excessive* use as a diagnostic aid.

The other important radiation that is used in diagnosis is the non-ionising ultrasonic radiation now in routine use, particularly by the obstetricians, and a brief comment is relevant.

Some early experiments on human lymphocytes exposed *in vitro* to ultrasound at diagnostic intensities, suggested that ultrasound exposure resulted in significant amounts of chromosome damage (Macintosh and Davey 1970, 1972). This immediately raised concern at the possibility that its use in obstetrics could result in chromosome damage in the developing fetus and lead to serious consequences. As a result, at least half a dozen different laboratories in this country made intensive studies of fetal chromosomes exposed to ultrasound prior to hysterectomy and examination, as well as studies on lymphocyte chromosomes exposed *in vitro* to a range of ultrasound intensities. Thankfully, all these studies proved negative (Evans 1975). The original findings can be discounted as an error of observation, so that it would seem that, at least in so far as genetic damage is concerned, ultrasound would appear to have a clean bill of health.

Chemical Agents

At the present time there can be no question that our main concern lies with the influence of a vast variety of chemical agents to which man is either knowingly or unknowingly exposed, and which are either known or suspected to be a cause of fetal abnormalities. Even excluding compounds used in medicine, there are thousands of chemicals to which we are exposed and which have been introduced into our environment without adequate testing for their possible effects in inducing fetal abnormalities, mutation and carcinogenesis.

Early concern was expressed at the exposure of man to heavy metals such as zinc, tin, lead, cadmium and mercury, all of which have been implicated in the development of fetal abnormalities. Some are known carcinogens, and all have some suspicion attached to them with regard to their properties for producing mutations (Clegg 1971, O'Riordan and Evans 1974, Skerving *et al.* 1974, Sirover and Loeb 1976). Excesses of such metals may, for example, be present in water supplies, from industrial effluent and even from cooking utensils. Significant numbers of individuals are being chronically exposed to fairly high levels of some of these metals and, unfortunately, sporadic outbreaks of poisoning due, for example, to methyl mercury, as in the Minimata incident in Japan (Irukayama 1969), or to cadmium sulphide, are all too well known and may result in deaths, much sickness, induced abortion and fetal abnormalities. It is perhaps worth noting that some of the fungicides that we use in our gardens are frequently based on heavy metals, and there is some evidence that seed-dressings based on zinc produce chromosome damage in man (Pilinskaya 1970).

The possible effects of heavy metals are well known, as indeed are the mutagenic and carcinogenic properties of a variety of insecticides such as captan and dieldrin (Ahmed, Hart and Lewis 1977) and processing chemicals such as methyl methane sulphonate. More insidious are the possible hazards attached to such things as flame-retardant chemicals (Blum and Ames 1977), certain food preservatives, herbicides, etc. Our chemical industries have produced vast quantities, measured in billions and billions of tons, of organic

bromides and chlorides that are used for an infinite variety of purposes and are not natural components of our environment. It turns out that many, and probably the majority, of these compounds are carcinogenic and teratogenic, and may provide us with a world-wide health problem. It is difficult to put these hazards, or potential hazards, into proper perspective, but discussion of a few relatively recent incidents will be made, with examples illustrating the general problem of exposure to chemical agents in relation to the development of fetal malformations.

Dioxin and the Seveso Affair

In July of 1976 an explosion in a chemical plant making trichlorophenol (TCP), at Seveso in North Italy, resulted in the release of a cloud of TCP, which also contained the highly toxic by-product chlorinated dioxin or TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin). The chlorinated dioxins are found as contaminants in the herbicides 2,4-D and 2,4,5-T—herbicides in use in gardens—and were used as the active ingredients in defoliants in the Viet-Nam war. Studies in Viet-Nam suggested that individuals exposed to dioxin showed a higher than normal incidence of miscarriage and of fetal abnormalities (Tung *et al.* 1971), although the evidence is somewhat unclear. However, experimental studies on laboratory animals (e.g. Neubert *et al.* 1973) unambiguously show that dioxin, and particularly 2,3,7,8-tetrachlorodibenzo-dioxin, is a very powerful teratogen, and that quantities of around 1–10 μg/kg are sufficient to produce cleft palate, hydronephrosis, etc., in fifty per cent of the offspring of exposed rats and mice (table 2). In relative terms, its potency as a teratogen in the mouse can be gauged by the fact (figure 3) that it is a thousand times more effective, weight for weight, than cyclophosphamide, an alkylating cytotoxic drug that is itself a powerful mutagen and teratogen.

Table 2. Embryotoxic effects induced by dioxin (TCDD) given on days 6–15 of pregnancy (Neubert *et al.* 1973).

Species	Abnormality	Dose producing ~50% effect (μg/kg)
Rat	Intestinal haemorrhage	0.5?
	Kidney abnormalities	1–2?
	Lethal	1–2?
Mouse	Cleft palate	6
	Kidney abnormalities	1–3
	Lethal	7

In addition to its obvious effects on the fetus, dioxin is also extraordinarily toxic to adult animals, producing all manner of deleterious effects in a variety of tissues in the body, and doses of 10 μg/kg, given orally, have been reported to be lethal for the rabbit (Kimbrough 1974).

There is also some evidence, as yet unsubstantiated, that dioxin can produce chromosome aberrations, and it turns out that it is one of the most effective known stimulators of the enzyme aryl-hydrocarbon-hydroxylase

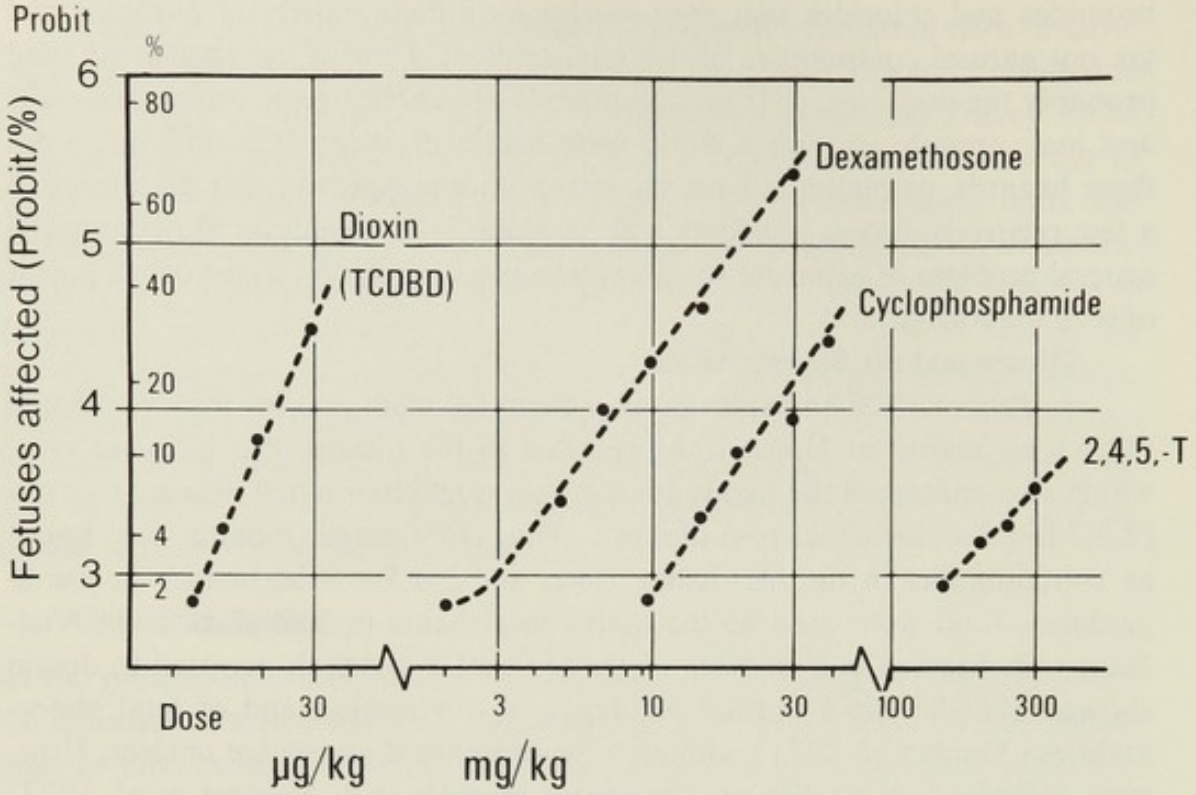

Figure 3. Dose response for cleft palate induction in the mouse. The compounds were administered on day 13 of pregnancy, and fetuses from 12–30 litters were scored for each point (after Neubert *et al.* 1973).

(Poland and Glover 1973). Its enzyme-inducing activities can be detected in both maternal and fetal livers of exposed pregnant rats (Berry *et al.* 1976), so that dioxin may be an important enhancer for the activity of other carcinogens that may be present in the environment.

In the Seveso incident, an area of some 700 acres in and around the town was contaminated with dioxin, and large numbers of people were exposed and numerous animals died as a consequence of exposure (Hay 1976a and b). Although the explosion occurred some eight months ago (10 July 1976), it was recently reported that some 340 school children are still suffering from chloracne, a condition of the skin that is a direct consequence of exposure to dioxin. The substance, in fact, is rather indestructible and is still present in high concentrations around the area of the accident. One consequence of the explosion was the exposure to dioxin of 100–150 women who were in their first trimester of pregnancy (Hay 1976c). Some thirty of these obtained abortions, but the results of a study on these abortuses have not yet been published. The first live births involving women who were in the very early stages of pregnancy at the time of exposure are now taking place and the full consequences of the incident will not be evident for some time yet.

Anaesthetics and Theatre Staff

In 1967 Vaisman published results of a survey carried out in the U.S.S.R. on working conditions in operating theatres, and produced the shattering finding that 18 of 31 pregnancies among 303 female practising anaesthetists exposed to nitrous oxide and/or diethyl ether had terminated in

spontaneous abortion, an incidence far in excess of that expected in a normal population (Vaisman 1967). This report caused something of a stir and was followed by a publication from Denmark (Askrog and Harvald 1970) showing that the rate of spontaneous abortion amongst Danish anaesthetists was twenty per cent, compared to a rate of ten per cent in the same group prior to employment in operating theatres.

Table 3. The populations sampled in the U.S.A. national study on occupational disease among operating room personnel. (From Cohen *et al.* 1974.)

Exposed populations	
American Society of Anesthesiologists (A.S.A.)	
American Association of Nurse Anesthetists (A.A.N.A.)	
Associations of Operating Room Nurses/Technicians (A.O.R.N./T.)	
Control populations	
American Academy of Pediatrics (A.A.P.)	
American Nursing Association (A.N.A.)	
Circularised	
Operating room personnel	49585 individuals
Unexposed groups	23911 individuals
Initial response 55% of sample (40044 out of 73496)	
Final response from A.A.P. 72.1% and A.S.A. 75.7% No significant difference between initial responders and non-responders	
Matching Samples matched for age and smoking habits	

The Russian and Danish studies were open to various criticisms but, by and large, their findings have generally been upheld in more carefully controlled and larger surveys such as those carried out in this country (Knill-Jones *et al.* 1972, 1975; Pharoah, Alberman and Doyle 1977) and in the U.S.A. (Cohen *et al.* 1974). The U.S.A. study was sponsored jointly by the American Society of Anesthesiologists and the National Institute for Occupational Safety and Health, and involved questionnaires being sent to doctors, nurses and technicians in various professional organisations, including those whose members worked in operating theatres and also non-theatre staff in comparable organisations. The organisations involved are listed in table 3, from which it may be seen that there were some 40044 initial responders, and these were divided into 'exposed' (those who had worked in operating rooms during the first trimester of pregnancy and/or during the previous year) and 'control' groups. Data from initial responders and non-responders, who were then pressurised into agreeing to respond, showed no important differences and all were adjusted to take account of age and smoking habits. The essential results on spontaneous abortion and congenital malformation rates in these populations is summarised in tables 4, 5, 6 and 7.

The results on spontaneous abortions (tables 4 and 5) show that there was an increased risk in female physician anaesthetists, nurse anaesthetists

Table 4. Spontaneous abortion rates for female respondents (Cohen *et al.* 1974).

Exposed[1]		Controls		
Organisation	Rate[2]	Organisation	Rate[2]	P
A.S.A.	17.1 ± 2.0 (468)	A.A.P.	8.9 ± 1.8 (308)	< 0.01
A.A.N.A.	17.0 ± 0.9 (1826)	A.N.A.	15.1 ± 0.9 (1948)	0.07
A.O.R.N./T.	19.5 ± 0.9 (2781)	A.N.A.	15.1 ± 0.9 (1948)	< 0.01

[1] 'Exposed' = exposure during first trimester and worked in operating room over previous one year.
[2] Rates/10^2 ± SE standardised for age and smoking habit at time of pregnancy.

and operating room nurses and technicians, with those exposed to the highest levels of anaesthetics having the highest risk, the range of risk extending from 1.3 to 2.0 times that of unexposed personnel. Other data, which are not presented here, showed no increased risk of spontaneous abortion for the wives of exposed male respondents compared with the wives of unexposed male respondents.

Table 5. Spontaneous abortion rates for exposed and unexposed members of the different organisations (Cohen *et al.* 1974).

	A.S.A.	A.A.N.A.	A.O.R.N./T.
Unexposed mother	15.7 ± 3.3 (138)	14.4 ± 1.4 (676)	15.1 ± 1.2 (1533)
Exposed mother	17.1 ± 2.0 (468)	17.0 ± 0.9 (1826)	19.5 ± 0.9 (2781)
P	0.35	0.06	< 0.01

In the case of congenital abnormalities, significant increases were observed amongst the newborn babies of exposed women, the increases amounting to more than sixty per cent compared with the controls (tables 6 and 7). The class exposed to the highest concentrations of anaesthetic gases, the female physician anaesthetists, showed a two-fold increase compared with two sets of controls, the unexposed female physician anaesthetists and the female paediatricians. Moreover, there was a significant increase, amounting to around twenty-five per cent, in the incidence of congenital abnormalities for the wives of exposed male physician anaesthetists.

The British study carried out by Knill-Jones and his colleagues from Glasgow (Knill-Jones *et al.* 1972, 1975) showed essentially similar findings on exposed females. In this study, matching of exposed and non-exposed preg-

Table 6. Congenital abnormality rates for children of female respondents (Cohen *et al.* 1974).

Exposed		Controls		
Organisation	Rate[2]	Organisation	Rate[2]	P
A.S.A.	5.9 ± 1.4 (384)	A.A.P.	3.0 ± 1.1 (276)	0.07
A.A.N.A.	9.6 ± 0.8 (1480)	A.N.A.	7.6 ± 0.7 (1629)	0.03
A.O.R.N./T.	7.7 ± 0.6 (2210)	A.N.A.	7.6 ± 0.7 (1629)	0.47

[1] 'Exposed' = exposure during the first trimester and worked in operating room over previous year.
[2] Rates/10^2 ± SE standardised for age and smoking habits.

nancies with respect to birth order, maternal age, and smoking habits was carried out, and the risk of abortion was shown to increase with maternal exposure to the environment in operating theatres by from 1.6 to 2.7. Moreover, there was some evidence that children born to female anaesthetists working during pregnancy had a higher frequency of congenital abnormalities than those not at work, a finding also noted in the U.K. survey by Pharoah, Alberman and Doyle (1977). On the other hand, studies on exposed males showed that paternal exposure did not appear to influence the over-all abortion rate, or the frequencies of major congenital abnormalities.

Table 7. Congenital abnormality rates for children of exposed and unexposed women within the various organisations (Cohen *et al.* 1974).

	A.S.A.	A.A.N.A.	A.O.R.N./T.
Unexposed mother	3.4 ± 1.2 (116)	5.9 ± 1.0 (566)	7.0 ± 0.9 (1275)
Exposed mother	5.9 ± 1.4 (384)	9.6 ± 0.8 (1480)	7.7 ± 0.6 (2210)
P	0.13	< 0.01	0.23

Although there would appear to be clear increases in the instances of spontaneous abortion and in congenital abnormalities in offspring of females working in operating theatre environments, there is no *direct* evidence that these anomalies are a consequence of exposure to anaesthetic vapours. Indeed, it has been suggested by various people (e.g. Mehta and Burton 1976) that individuals working in such an environment may be subject to undue stress and that this might be an important factor in inducing miscarriages, etc. On the other hand, there is plenty of evidence to show that inhalation anaesthetics administered to laboratory animals in concentrations similar to those found in operating theatre environments, and well below the concentrations that are

anaesthetically effective, result in teratogenic effects on embryos of the rat and also the chicken (Corbett *et al.* 1974). Furthermore, we should note that metabolites of halothane can be found in the urine of patients many weeks after exposure, and that traces of halothane and nitrous oxide can be detected in the expired air of anaesthetists many hours or days after a routine session in the operating theatre (Cohen *et al.* 1974).

Now, despite the fact that it is still not proven that the increased spontaneous abortion and congenital malformation associated with the operating theatre environment is a consequence of exposure of females to anaesthetic gases, the findings are of particular interest to the geneticist, since it has been known for over thirty years (Östergren 1944) that nitrous oxide can behave like the drug colchicine in preventing chromosome separation at mitosis, resulting in the formation of polyploid cells (Brinkley and Rao 1973). Moreover, N_2O appears to be efficient in inducing trisomies and other aneuploidies in plants (Dvorak, Harvey and Coulman 1973) and indeed is used by plant breeders as a standard and well-established treatment to give rise to new genetic strains (Dvorak and Harvey 1973). These effects of N_2O, and of the other inhalation anaesthetics including halothane, are almost certainly a consequence of their well-known property of dispersing the microtubular systems in the cell (Allison *et al.* 1970) and thus preventing proper chromosome separation. In the present context then, it is most certainly relevant to recall the fact (Boué and Boué, this volume) that a significant proportion of the spontaneous abortions in man are associated with the presence of an additional set of chromosomes in the fetus—i.e. polyploidy—or with the addition or loss of one or a few chromosomes—i.e. aneuploidy. In addition, and as we have seen, some of the congenital abnormalities seen at birth are also a consequence of such chromosome imbalance. Thus, although we, once again, have no direct evidence, there seems to be every likelihood that at least part of the increase in spontaneous abortion and congenital malformation associated with operating theatre environment could be a consequence of the known action of anaesthetic gases in interfering with the normal processes of cell division and chromosome separation.

Fetal Abnormalities in Patients Conceiving after Chronic Exposure to Known Mutagens

One of the striking advances in medicine in recent years has been the dramatic success obtained in the treatment of acute lymphoblastic leukaemia of childhood. Intensive treatments with cocktails of cytostatic drugs, including cyclophosphamide, 2-mercaptopurine, vincristine, etc., can now result in long-term survival in as many as fifty per cent of treated cases (Pinkel 1972), so that in the near future a not unappreciable number of survivors will mature and wish to take on the responsibility of producing children of their own. There have, of course, been a number of examples of mothers treated as adults with such toxic drugs, and who later produced children that were perfectly normal. The number of such cases is fairly low, and we really have very little information on miscarriages and possible abnormalities in such individuals.

In the last couple of years, however, there have been reports of children being born to mothers who had been receiving, or had received up to ten years previously, successful treatments for leukaemia and who had subsequently given birth to perfectly normal healthy offspring. To the author's knowledge, at least eight healthy babies have been produced by four successfully treated females (Bacon and Kernahan 1975, Wegelius 1975, Estiu 1977) and there is at least one report of paternity to a successfully treated male (Hinkes and Plotkin 1973). Now most of the successful drugs that are used in leukaemia therapy produce a very considerable amount of chromosome damage in all the dividing cells in the patient, and massive amounts of chromosome damage can be seen in dividing blood lymphocytes and in dividing cells from other tissues. In the case of the female, the oocytes in the gonad do not undergo division until the time of ovulation, and it may well be that they are spared from the bulk of the chromosome-damaging action of the drugs. Cells in the male gonad, however, divide continually from puberty onwards, and will suffer a considerable amount of chromosome damage. Chromosome damage in an early gonial cell in the male will result in the development of a clone of cells (and eventually a large population of sperm) containing the same chromosomal mutation, and a number of such clones might be expected to develop.

Animal experiments have shown that males or females exposed to some of the cytostatic drugs mentioned do indeed have a high frequency of mutant sperm or eggs and of abnormal offspring (Leonard 1973, Russell 1976). It is, therefore, important for us to be alert to the fact that increasing numbers of successfully treated leukaemic patients, or their spouses, will be turning up at departments of obstetrics and gynaecology and that these individuals may have an increased risk of producing fetal abnormalities. For the future then, we should pay particular attention to the reproductive performances of these individuals and be on our guard to the possibility of increased fetal abnormality.

Hair Dyes

Finally, a brief speculation about a possible hazard that may merit some investigation. Just over a year ago two reports, from the U.S.A. (Ames, Kammen and Yamasaki 1975) and U.K. (Searle *et al.* 1975), showed that many compounds that are major constituents of hair dyes are strongly mutagenic to bacteria. Moreover, considerable amounts of these compounds are applied to the scalp during dyeing, and significant amounts may be absorbed through the skin, so that metabolites can be detected in the urine many days after an individual has had a hair dyeing session. Later studies on two of these compounds, the aromatic animes 2-nitro-p-phenylenediamine (2-NPPD) and 4-nitro-o-phenylenediamine (4-NOPD), showed that they produced chromosome damage in human and mammalian cells exposed *in vitro*, and comparative studies in this Unit (Perry and Searle 1977) showed that they were more efficient in producing DNA damage than the potent mutagen ethyl methane sulphonate (figure 4).

As a consequence of all these studies, hair dye components are now

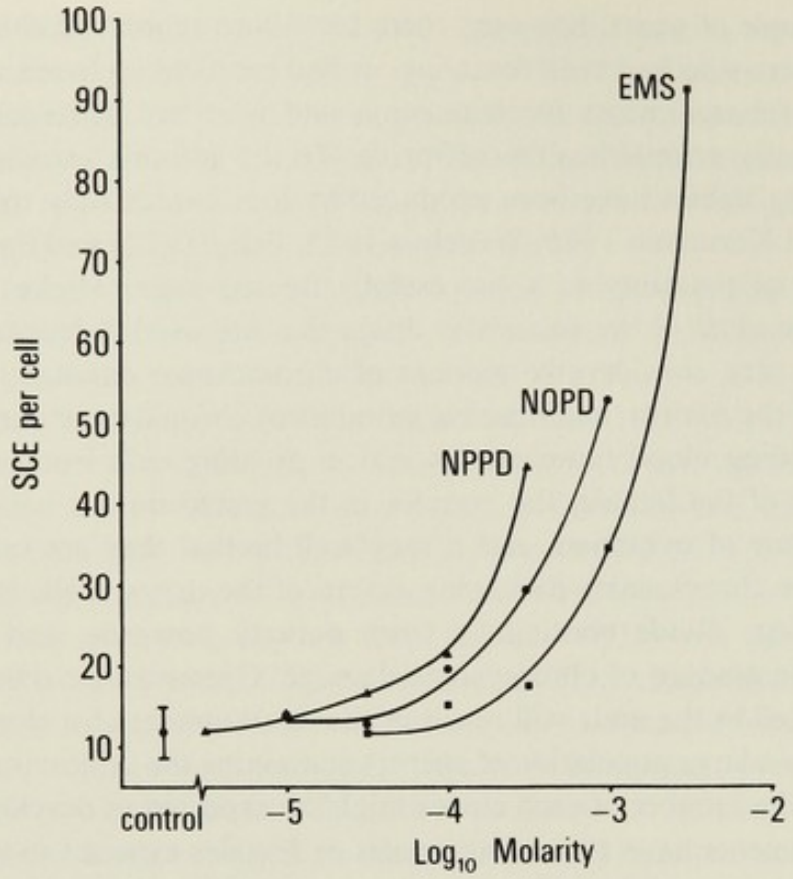

Figure 4. Incidence of DNA damage, measured as sister chromatid exchanges (SCE), in Chinese hamster cells exposed to the known mutagen ethyl methane sulphonate (EMS) and the two hair dye constituents 2-nitro-p-phenylene-diamine (NPPD) and 4-nitro-o-phenylenediamine (NOPD) (from Perry & Searle 1977).

being tested for carcinogenicity, since there is a very close correlation between the ability to induce chromosome damage and mutation, and carcinogenesis. The possibility that these and similar compounds may increase the risk of producing fetal abnormalities in female hairdressers, or in women who have dyed their hair at or around the time of conception or in very early pregnancy, is an obvious one that cannot be dismissed and would appear to merit study.

Conclusion

There can be no doubt that the affluence of modern society has brought with it a variety of environmental mutagens that are obvious hazards in terms of the normal development of the human fetus. Although there is much concern over iatrogenic factors influencing fetal malformation, as is properly witnessed by the number of contributions in this book, we must not forget that there are vast amounts of man-made chemicals being poured into our environment that are not manufactured for medicinal purposes; that we are exposed to many of these in our everyday lives; that many of them react positively in tests for carcinogenicity and mutagenicity; and that many have simply not been subjected to test. We would be complacent indeed if we were not aware of the possibility that many of these agents may provide significant risks to the normal development of the human fetus.

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Fetal Malformations and the Committee on Safety of Medicines

The Gin Acts of the eighteenth century were the first results of the recognition by the government of this country that it had some responsibility to prevent the adulteration and abuse of a drug. Until 1864, however, when the first *British Pharmacopoeia* was published, there was no control over medicines in the U.K. Indeed the counters of pharmacists' shops were piled with opium pills and their shelves with jorums of laudanum, freely offering oblivion for a few pence to those pouring out of the dark satanic mills of the industrial revolution. As regards standards for drugs it then sufficed always to purchase your infusion of digitalis leaves from Mistress Ford rather than from Mistress Page, her rival down the street, whose brews might differ as does the strength of a cup of coffee in different homes.

Since 1864 (Denston and Dunlop 1958) governments in the U.K. have been active in establishing the purity and strength of medicines, in preventing the deception of the public by quack nostrums for certain serious disorders, in minimising the danger of drug addiction by controlling the manufacture, sale and supply of potent narcotics and in requiring prescriptions—sometimes of a specified type—for the sale of potent drugs. In these matters the public were provided with considerable legal protection by a mass of legislation achieved within the context of a bewildering number of statutes controlled by a variety of government departments. Although this meant that legislation on medicines was somewhat chaotic and in need of consolidation, it worked wonderfully well until the therapeutic explosion of the last forty years rendered it inadequate to cope with modern conditions.

The Evolution of the Committee on Safety of Medicines

Before 1966, no statute required the official pre-marketing approval of a medicine on the grounds of its safety and, apart from those biological agents listed under the Therapeutic Substances Act, anyone could market any product without seeking official approval for its safety. It took the emotional reaction to the thalidomide tragedy to galvanise us out of our *laisser-faire*

attitude. 'Sweet are the uses of adversity . . .' Following the tragedy the Minister of Health—at the time Mr Enoch Powell—established the Committee on Safety of Drugs in 1963 as an interim measure, which lasted until the comprehensive legislation on medicines of 1968 could be planned, enacted and become operative in 1971 (Dunlop 1970). The Committee was a purely voluntary arrangement, official only in the sense that its members were appointed by the Minister who also provided its finance, accommodation and secretariat. It consisted of eleven very part-time, originally honorary, scientists, physicians and pharmacists, assisted by a small staff of civil servants who did most of the preparatory work. The Committee, however, took the decisions.

In spite of the absence of legal sanctions, manufacturers at the time were quite glad to share some of the responsibility for the safety of their products with an independent body. Thus, the Association of the British Pharmaceutical Industry and the Proprietary Association of Great Britain promised, before the Committee started to function on 1 January 1964, that none of their members would (a) put a new drug to clinical trial, or (b) market a new drug, without the Committee's approval—a promise loyally observed.

The Committee's remit did not include the cost or comparative efficacy of medicines. Although the safety and efficacy of medicines are often inextricably entwined, efficacy *per se* was not its function. To undertake its responsibilities three sub-committees were developed: the first to scrutinise the adequacy of the pharmaco-dynamic studies undertaken on a new medicine before its trial on patients was permitted; the second to scrutinise the adequacy of its clinical trials before marketing; and the third to monitor any adverse reactions to it after marketing and to feed the information back to the medical profession. This monitor was largely based on reports of suspected adverse reactions, which doctors were urged to send to the sub-committee on a post-paid 'Yellow Card' supplied for this purpose. Such reports often pinpointed a problem that was then investigated in depth by the Committee. This resulted in the discovery of some important adverse reactions: for example, the thromboembolic phenomena sometimes resulting from contraceptives, particularly from those with a high oestrogen content; deaths from the excessive use of bronchodilator aerosols in acute asthma; and jaundice from repeated halothane anaesthesia.

The Medicine Act of 1968 is a complex piece of legislation and it would take too long to describe its provisions in detail. It encompasses far more than the limited functions of the old voluntary Committee on Safety of Drugs. In it the Secretary of State for Health and Social Services and the Minister of Agriculture, responsible to Parliament, act as a Licensing Authority for drugs and are advised by the renamed, now statutory, Committee on Safety of Medicines and a similar Committee on the Safety of Veterinary Products. The Act also established a Medicines Commission to be an advisory body to Ministers on the broad aspects of policy regarding medicines, including the numbers, functions and personnel of the expert committees giving advice to the Author-

ity; to direct the preparation of the *British Pharmacopoeia*, hitherto the responsibility of the General Medical Council; and to act as an Appeal Tribunal against an adverse decision of the Authority taken on the advice of one of the Safety Committees. The Committee on Safety of Medicines has somewhat similar functions to its predecessor, but considers the efficacy of medicines as well as their safety.

Licences of Right were originally issued to all products already on the market prior to the commencement of the licensing system, but all these are now being reviewed not only for safety but for efficacy as well—a vast task. The Act provides for an inspecting system to try to ensure the best conditions for the manufacture, storage and distribution of medicines. It provides that all medicines must be sold from pharmacies, except for simple, relatively innocuous home remedies that appear on a General Sales List drawn up by the Commission. Lastly the Act requires that any promotional literature must be consistent with the terms of a data sheet, approved by the Safety Committee. It describes concisely the essential facts about the new drug: its generic and brand names, its dosage, its method of administration, its indications and contra-indications, and its main adverse effects.

Monitoring of Medicines outwith Britain

The statement that medicine is international and knows no frontiers is a platitude subject to many qualifications. The multitude of controls imposed on the pharmaceutical industry varies enormously in different countries. Such discrepancies are not surprising when one considers the rapidly changing conditions that exist and the variety of circumstances under which legislation on medicines has had to be enacted in different parts of the world. Nevertheless, international pharmaceutical companies encounter many frustrations and difficulties as they try to plan their production and marketing policies to conform to the widely diverse controls that exist.

The Food and Drug Administration in the U.S.A. (Dowling 1970) is the premier drug regulating organisation in the world, and since early this century has done much to protect the public, not only of the U.S.A. For its immense task of inspecting and licensing, throughout the U.S.A., the manufacture of foods, cosmetics and pesticides as well as human and veterinary medicines, the F.D.A. employs a vast staff of civil servants with whom ultimate power rests and whose careers depend on the correctness of their decisions, especially in avoiding licensing a drug that turns out to be unduly toxic. Few people will be aware of the opposite mistake of delaying the licence of a drug that might have been life-saving, and those whose lives might have been saved will not be there to protest. In Britain, as we have seen, ultimate power rests with very part-time professional experts, who advise Ministers responsible to Parliament. Their careers do not depend on membership of the Committees or Commissions, on which they serve largely as an altruistic chore. They are assisted by expert civil servants who do most of the work but do not make the decisions.

It is curious that in the U.S.A., the home of big business and free enterprise, they should, until recently at any rate, have been far more bureaucratically rigid in their control of drugs than we have been in this country with our so-called socialised medicine. On the whole, controls have been rather easier and more elastic here and new medicines have often been released in Britain a considerable time before they become available in the U.S.A. Indeed there are at present a few medicines that we consider to be of significant value that are not yet available there.

It would take too long to attempt to describe in detail the different drug control systems that operate on the continent of Europe. They vary considerably, and cause many frustrations and difficulties to international pharmaceutical companies as they attempt to plan their production and marketing policies to comply with the various controls that exist in the different countries. On the whole these tend to follow the pattern of the F.D.A. in America, and consist of a commission of full-time civil servants, often advised by a panel of academics and clinicians or by one or two appointed professors who have carried out research on the product under consideration. The civil servants, however, usually take the ultimate decision. On the Continent pharmacists tend to have a higher professional standing than in Britain and, particularly in Holland, are citizens of great credit and renown and of very considerable prosperity. Thus, pharmacists rather than physicians tend to form the majority on these commissions, and a pharmacist is often the President of the control authority.

There can be no dissent that some governmental control of medicines is desirable. The question is in the degree of control: inadequate regulation can prejudice public safety, but excessive legislation can also be prejudicial. The thoughtful legislator must direct his efforts between the two extremes: to protect the public from inadequately tested and dangerous medicines but at the same time to allow an orderly progress of research, development and marketing by the pharmaceutical industry. I believe that the harm done by the procrastination imposed by the very rigid F.D.A. regulations, and the vast expense involved in getting a new drug licensed, has outweighed the benefit from any increased safety that may possibly have been attained, and I am not alone in this view. Nowadays we tend in this country to do tomorrow what the U.S.A. does today: there is no doubt that since it came more under the law our control system in Britain has become more rigid and restrictive. It would be a pity if in our desire to protect the physical health of the public we fell into the same errors that long ago afflicted the Inquisition in their efforts to control spiritual health.

Detection of Teratogenicity

Though every new drug has now to be rigorously tested for teratogenicity on the developing mammalian embryo before being passed for clinical trial, we all know that such experimental data derived from animals can only give a very approximate indication of the effect likely to be produced on humans.

The results of animal trials have to be extrapolated to the human case with great care, and their importance should not be exaggerated.

There is no special teratogenicity sub-committee of the Safety of Medicines Committee, but its Adverse Reactions Sub-Committee is closely concerned with the subject. The sub-committee collaborates in a continuing study with the Office of Population and Census Studies and with the Registrar General in monitoring infants born with malformations (the study has already involved 800 of them) and checking the history of their mothers' drug intake during pregnancy. The potential dangers of drug administration during pregnancy are now well appreciated, and indeed there is a need for discretion by all women at risk of pregnancy, since they may be unaware of being pregnant at the very time when the risk from drugs is greatest. The so-called placental barrier is a relative one as far as drugs are concerned, and it is now recognised that the question is not so much whether a drug will reach the fetus but the rate at which it will do so and the mechanisms of transport involved.

Table 1. Possible side effects of drugs on the fetus.

Drug	Possible side effect on fetus
Alcohol	Growth retardation, microcephaly
Androgens and progestogens	Virilisation in female fetus
	Neoplasia in teenage vagina
Antineoplastic drugs	Deformities
Antithyroid drugs	Goitre and hypothyroidism
Chloroquine	Deafness, corneal opacities
Contraceptive agents	See Alberman, this volume
Corticosteroids	Adrenal atrophy
Cortisone	Cleft palate
Fertility drugs	See Alberman, this volume
Folic acid antagonists	Deformities
Narcotics	Respiratory depression
Smoking	Growth retardation
Streptomycin	Deafness
Sulphonylureas	Hypoglycaemia
Tetracycline	Discolouration of teeth
Thalidomide	Phocomelia, limb reduction deformities
Warfarin	Nasal bone hypoplasia

Types of Teratogenicity

Two major types of toxic effects can be produced by drugs on the fetus: (a) effects leading to structural abnormalities occurring during the stage of embryogenesis (a true teratogenic effect such as occurred with thalidomide), and (b) effects (produced by drugs given later in pregnancy or at delivery) that are largely similar to those produced in adults (table 1). Examples of these latter are chloroquine and streptomycin, which may cause deafness; anti-thyroid drugs, which may cause goitre and hypothyroidism; corticosteroids, which may cause adrenal atrophy; sulphonylureas, which may cause

hypoglycaemia; narcotics of all kinds, which effect the neonate similarly to the mother; and so on.

So far no drug has been found to exert the same appalling teratogenicity as thalidomide. Embryonic tissues, particularly between the third and eighth week of pregnancy, are similar in some respects to neoplastic tissues. It is therefore not surprising to find that they are particularly sensitive to antineoplastic drugs. Indeed aminopterin was the first drug shown to be teratogenic (Bongiovanni, Di George and Crumback 1959). The folic acid antagonists are the most likely to cause fetal abnormalities (Melnyk, Duffy and Sparkes 1971). Cortisone commonly produces cleft palates in some animals, and may in rare instances have this effect on the human fetus. Androgenic and progestational steroids, when given to the mother in large doses for some time for threatened abortion in early pregnancy, can cause virilising effects on female fetuses. The effect of the fertility drugs and contraceptives is discussed by Dr Alberman (below, this volume). Smoking during pregnancy is associated with retardation of intrauterine growth (Lowe 1959).

Many other drugs have been suspected of causing human fetal abnormalities but clear evidence is lacking. Nevertheless, the serious fetal abnormalities resulting from a disease as mild as rubella or a drug as generally safe as thalidomide exemplify the peculiar vulnerability of the fetus. Nelson and Forfar (1971) have shown that the mothers of children with congenital defects took, on average, far more medicines during their pregnancies than a comparable group with normal children. This did not necessarily mean that the drugs involved were teratogenic. They were mostly relatively simple remedies commonly used for the discomforts of early pregnancy; mild analgesics, iron, antacids, hypnotics, anti-emetics and so on. There could be many other explanations of Professor Forfar's striking figures besides the teratogenicity of such drugs. Yet when it is remembered how long it took to incriminate thalidomide, with its great teratogenicity and with the characteristic malformations it produced, drugs of very low teratogenicity producing occasionally common malformations are extremely difficult to recognise and may still have to be identified. Thus, it would appear wise to avoid giving any drugs during pregnancy unless that drug is specifically indicated, and pregnant women should be warned against self-medication with common household remedies.

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Fertility Drugs and Contraceptive Agents

There has been considerable concern about the possible teratogenic effects of synthetic sex hormones taken shortly before, or early in, pregnancy. This concern has extended to encompass the use of oral contraception and fertility drugs also. A substantial proportion of pregnancies in Great Britain now follow closely upon the use of oral contraception. Twenty per cent of mothers in 1970 conceived within, at most, eighteen months of such therapy (Chamberlain *et al.* 1975). The use of fertility drugs also is probably increasing. It is therefore of considerable importance to monitor the outcome of pregnancy after such exposure so that any increased risk of death or defect can be quickly ascertained.

This paper will concentrate on ways in which these hormones might increase these risks, and of methods of monitoring that could provide an early alerting system. It will include a brief survey of some recently published accounts to see what type of information is already available and what is lacking. This account cannot be fully comprehensive, but is intended to cover the largest published series.

Exogenous Sex Hormones and The Fetus

Oral contraceptives have a multitude of effects other than on ovulation, and it is recognised that many of these persist for some time after the cessation of medication. This may be because they become stored in adipose tissue, and continue to be released from this tissue until the supply is exhausted.

Such side effects include an interference with the motility of the Fallopian tubes, which could delay the passage of the ovum and give rise to the 'over-ripe' ovum that has been associated with an increase in the risk of chromosomal aberrations. The artificial stimulation of ovulation might also interfere with the optimal time sequence of events before fertilisation. There is also a hint from experimental evidence that such hormones might interfere directly with meiosis (Jagiello and Lin 1974). Thus we should be looking for a possible change in incidence of chromosomal aberrations.

Other effects include changes in vitamin A level (Gal, Parkinson and Craft 1971) or carbohydrate metabolism, and such biochemical effects in the

mother might indirectly affect fetal development. Alternatively, the hormones might have a direct effect on the fetus, as in the case of virilisation of a female fetus after the administration of some forms of progesterone, or the recently documented effect on vaginal epithelium, leading in rare cases to vaginal adenocarcinoma (Herbst *et al.* 1972). We should therefore also be looking for congenital malformations, or effects on offspring that may not manifest themselves for many years.

Methods for Detection of Such Risks

There are very considerable problems to be overcome before any such adverse effects can be identified. The major problems are due on the one hand to the fact that defects leading to very early embryonic or fetal death are virtually impossible to detect, and, on the other, that defects compatible with survival, but not easily detected in infancy, are difficult to monitor.

From a practical point of view one may argue that the only risks we need to be interested in are those that affect a viable fetus, lead to a handicapping disorder, and are therefore measurable. However, these may form only the tip of an 'iceberg' of total defect, and we should not be satisfied until we know that there is no substantial increase in the less easily measured risks.

Compounding these problems of detection are others, namely of the selection of groups to be studied. As in other similar situations, women exposed to oral contraceptives or fertility drugs are not a randomly selected group. Women taking oral contraceptives tend to be younger, healthier and to smoke more than those who do not. More seriously for the estimation of risk of defect, the offspring of women who do not ovulate without hormone stimulation cannot be compared without qualification with those of women who are fertile.

Reported Studies

Published work on the effects of these drugs falls into four main categories. There have been a few studies that have followed a defined population of mothers from before, or shortly after, pregnancy into early infancy of the offspring. There have been some studies of certain types of pregnancy outcome, abortions induced or spontaneous, or livebirths only; there have been systematic or, more often, sporadic reports of children with defects born to mothers who were exposed to fertility drugs or other synthetic hormones; and finally, there are some reports of changes in reported rates of malformations related to changes in exposure over a period of time.

Only the first of these methods can give a real indication of the risks to the offspring, but these longitudinal studies have the drawback that they have to be very large in order to include enough exposed mothers. The other methods can only give indications of possible risks, and may indeed be misleading if the samples studied are biased. The results of some of these studies will be discussed below, first those relating to specific case reports or retro-

spective enquiries, and then those of the prospective studies. An attempt will be made to assess the possible magnitude of the problem in each case.

Fertility Drugs

There appears to be no good estimate of what proportion of pregnancies occur following the administration of fertility drugs. However, since they are administered to only a very small proportion of wives of all infertile couples, who themselves make up only about ten per cent of all couples, it is unlikely that more than three or four per thousand pregnancies are involved. Probably, the true figure is much less. The drug in most common use currently in this country is clomiphene, although rarely pergonal or human menopausal gonadotrophin are used. Before discussing reported studies three comments should be made. Firstly, it appears that in the case of pregnancies following stimulation with these drugs it is quite common for further supportive hormone therapy to be used in early pregnancy, so further compounding the difficulty of interpreting any findings. Secondly, most published reports are based on earlier drug use, with different regimes, and may not be directly relevant to current use.

Lastly, in many reports it is impossible to distinguish between pregnancies arising while the patients are still under treatment, and those arising shortly after treatment has been discontinued.

Clomiphene. There have been quite a number of sporadic case reports of fetal birth defects in offspring resulting from ovulation induced by clomiphene. There have been at least twelve cases of neural tube defect reported in the literature (Dyson and Kohler 1973, Sandler 1973, Field and Kerr 1974, Nevin and Harley 1976) and several reports of other malformations (Ylikorkala 1975, Berman 1975). Moreover, Ahlgren and his colleagues (1976) quote two reports to the Swedish Adverse Reactions to Drugs Board, one with anencephaly and one with oesophageal atresia following clomiphene. Boué and Boué (1973) described the rate of chromosomal abnormalities in some spontaneous abortions that followed such therapy, and showed that it was higher in abortions conceived in the induced cycle itself than in the cycles following induction.

As stated above, such reports can do no more than raise one's 'index of suspicion', and direct attention to reports of longitudinal studies of exposed pregnancies. One large series was reported by Kistner in 1967. He collected reports of 1450 pregnancies following clomiphene induction, although he was not primarily concerned with the malformation rate. He reported that twenty-five per cent had ended as abortions *or* stillbirths, but that of the remaining pregnancies, which resulted in 1232 livebirths, twenty-eight had had birth defects, a prevalence of only 2.3%. MacGregor, Johnson and Bunde (1968) reported on 1744 pregnancies associated with clomiphene. Twenty-three per cent aborted and there were 38 infants out of 1506 with birth defects, including five with Down's syndrome. Goldfarb and his colleagues (1968) reported on 160 clomiphene-related pregnancies, and noted that in 166 exposed infants there were two with malformations, both haemangiomata. Murray and

Osmond-Clarke (1971) reported on 114 such pregnancies, which resulted in 78 livebirths of which one subsequently died with multiple malformations. Hack and colleagues (1972) reported on 104 such births, of which one had congenital heart disease, one was lacking two fingers, two had pyloric stenosis and two had suspected congenital dislocation of the hips treated. Other reports giving malformation rates include that by Ahlgren *et al.* (1976), who described 159 pregnancies after clomiphene, resulting in 148 infants. Amongst these were one case of Down's syndrome, one with a meningocele, one with oesophageal atresia and a congenital heart disease, and ten others with other major or minor defects. (This sample of 159 comprised all the pregnancies following clomiphene in two Swedish hospitals between 1967 and 1974.) This report would give a prevalence of five per cent for 'major' malformations and four per cent for 'minor' malformations.

A major difficulty with such studies lies in the estimate of the usual incidence of major and minor malformations in the general population from which the treated pregnancies were derived, and, particularly in the case of Down's syndrome, the possible confounding effect of maternal age distribution in treated cases. Few of these reports give maternal age distribution, but those that do suggest that the treatment of mothers aged forty or more is very unusual. In Ahlgren's series none of the mothers was over thirty-nine, and three-quarters were under thirty. The main findings are given in table 1, and for reference the incidence of 'major' malformations in some unselected series is given. On balance a study of these data, mostly based on malformations diagnosed at birth, suggests that there may be a slightly raised incidence of congenital heart defect, and possibly of Down's syndrome. In the most carefully, prospectively studied populations of a normal age distribution the

Table 1. Malformations reported after pregnancies associated with clomiphene: 'prospective' data compared with an unselected population.

Authors	No. of births	% defects reported[1]
Kistner 1967	1232	2.3
MacGregor *et al.* 1968	1506	2.5[2]
Murray & Osmond-Clarke 1971	78	1.3
Goldfarb *et al.* 1968	166	1.2
Hack *et al.* 1972	96	5.8
Ahlgren *et al.* 1976	148	9.5[3]
Total	3219	2.7
Leck 1974 (unselected pop.)		2.3–2.7

[1] Defects in stillbirths probably rarely reported.
[2] Included 5 Down's syndrome.
[3] Included 1 Down's, 1 meningocele and several minor defects.

incidence of congenital heart disease in total births is of the order of seven per thousand, and of Down's syndrome just under two per thousand.

On the other hand it should be noted that the manufacturers of clomiphene state that 'subcutaneous administration of clomiphene in pregnant rats on one day (the 12th) during the period of organogenesis resulted in a dose-dependent increase in the incidence of malformations in doses of 1.5 to 1000 mg/kg.' Malformations were also seen after administration to rabbits and dogs. One cannot be certain that there is not also such an effect in humans, independent of the risk status of the mother.

A practical suggestion by Ahlgren and his colleagues, which should be seriously considered, is that any pregnancy following clomiphene therapy should be investigated prenatally for chromosome defects or raised alpha-fetoprotein. Such a policy would enable fetuses with Down's syndrome or neural tube defects to be identified and their pregnancies terminated, and might lower the malformation rate to an average level.

Other fertility drugs. Reports of the outcome following other fertility drug use, mostly human menopausal gonadotrophin, present a picture of a worse prognosis. It is of course well known that the multiple pregnancy rate, particularly of those other than twins, is considerably raised after such treatment. However, from published accounts the malformation rate also seems to be high.

Four reports of the follow-up of pregnancies after gonadotrophin therapy have been found. An early report by Tyler (1968) described twenty-eight pregnancies going to term after human menopausal gonadotrophin, of which one had an extra digit, and no other malformations were seen. One is by Hack and colleagues (1970) and relates to 78 pregnancies that reached twenty weeks. These had a forty per cent multiple birth rate, including five sets of triplets, two of quadruplets and one set of sextuplets. Major malformations found included cyclopia in one of a pair of twins; one case of a single umbilical artery and multiple congenital malformations; and two cases of severe congenital heart disease, one fatal with tricuspid atresia and other defects, the other in a surviving triplet. It should be noted that we know now that cyclopia is frequently found in trisomy 13, so that one of these may well have been a chromosome defect. Another report is of twenty-six pregnancies resulting in thirty-six infants following the use of human menopausal gonadotrophin (Spadoni, Cox and Smith 1974). Amongst these there were two infants with severe defects, one with Down's syndrome, the other a twin with multiple malformations (table 2). A more recent report from Israel (Caspi *et al.* 1976) describes 157 births occurring after gonadotrophin therapy. There were five major malformations, three ventricular septal defects and one suspected supravalvular stenosis, and one child with a sacrococcygeal teratoma. Another child had hypospadias and several had orthopaedic defects.

Searle Laboratories (1977) have also kindly let me have the results of recent unpublished clinical trials on pergonal in which there were six malformations in 150 babies, two of the gut in multiple births, one Pierre Robin

Table 2. Malformations reported after gonadotrophins: 'prospective' data.

Authors	No. of births	% defects reported
Tyler 1968	28	3.6
Hack *et al.* 1970	122	4.9[1]
Spadoni *et al.* 1974	36	5.6[2]
Caspi *et al.* 1976	157	7.0
Total	343	5.8

[1] Included 1 cyclops.
[2] Included 1 Down's syndrome.

syndrome, one meningocele, one child with a double metacarpal and one twin with fragilitas ossium.

It is clear from these descriptions that there is a wide variation in the detection of malformations, and in the types described. Without a control series it is impossible to come to any definite conclusion as to whether the rates are really increased after therapy. Nevertheless, in the case of gonadotrophins, reports that describe a minimum of six cases of congenital heart disease in 493 cases must raise doubts about their effects. In very carefully examined series the incidence of congenital heart disease is usually about seven per thousand.

It has been suggested that the finding that malformations seem to be unduly common after both clomiphene therapy and the use of gonadotrophins may be additional evidence that the risk is due to the selection of these subfertile women, with all that implies, rather than a direct effect of the drugs. This seems a reasonable interpretation, especially if taking into account the previous investigations and therapy and the supportive treatment given in pregnancy that such women are often exposed to. Allowing for all of this, it is perhaps a pleasant surprise that as many as ninety per cent of infants after induced ovulation appear to be normal.

Exogenous Sex Hormones during Pregnancy

As in the case of fertility drugs, oral contraceptives are sometimes inadvertently continued into early pregnancy, probably when the embryo is most vulnerable to insults.

Again, there have been a number of sporadic reports of fetal defects in such pregnancies. Unfortunately, in most reports these pregnancies have been grouped with others in which the exposure has been to hormonal pregnancy tests, or to supportive hormone therapy. This grouping has presumably been made because many of the hormones concerned are the same, although the doses may be very different. For instance, the dose of norethisterone used in contraception is 1 to 4 mg, that in hormonal pregnancy tests 10 mg. Moreover the type of women who receive hormone pregnancy tests may be very different from those who conceive accidentally while using oral contraceptives, the

legend being that much of the popularity of the pregnancy test is due to its reputed effect as an abortifacient. Similarly the women who receive supportive hormone therapy in pregnancy are highly selected in favour of those with a previous history of miscarriages, or bleeding in the current pregnancy.

Another important difference between those taking oral contraceptives and those taking other exogenous hormones is that the contraceptives are likely to be consumed throughout the first month of pregnancy, in contrast to the other hormones, which are unlikely to be administered until some days or weeks after the first period has been missed.

All these differences make it desirable to separate those mothers with accidental 'breakthrough' pregnancies from the other exposures. One of the earliest of these reports, drawing attention to the possible hazards of oral contraceptives continued into pregnancy, was by Nora and Nora (1973). However, from their expanded report (1975) it is difficult to identify the relevant cases. From another report (Janerich, Piper and Glebatis 1974) it emerged that out of 108 cases of limb-reduction defects six pregnancies had been due to oral contraceptive failure, and that in another nine other types of hormone had been administered, six supportively and two as pregnancy tests. In the corresponding controls only one mother had experienced a 'breakthrough' pregnancy and three others had taken hormones for other reasons. The authors suggested that one possible explanation for the apparent excess of mothers of cases with breakthrough pregnancies was a functionally abnormal endocrine system or unusual fertility. An alternative suggestion by Kay (1975) was that the concurrent use of other drugs might explain both the contraceptive failure and the limb defects. However, in support of the theory that the sex hormones were associated with the defects, Janerich and his colleagues produced evidence from the New York State Register of defects, which showed that limb defects but not other defects had increased with pill use.

In a subsequent report on the pregnancies of 103 mothers who delivered babies with Down's syndrome, and of the same number of controls, Janerich and his team (1976) found that there was no excess of cases who had taken oral contraceptives in, or shortly before, the relevant pregnancy.

There are also several longitudinal studies in which mothers were asked early in their pregnancy about any exposure to drugs, including oral contraceptives, and the outcome of pregnancy was subsequently monitored. One such study was in West Jerusalem, involving 11 468 births, where an excess of malformations was found after exogenous hormone administration in pregnancy, but none had occurred after the consumption of oral contraceptives (Harlap, Prywes and Davies 1975).

In the longitudinal study of 9566 mothers by Spira and her colleagues (1972) no excess of malformations after exposure to hormones in pregnancy was found, and no malformation seems to have occurred after oral contraceptives taken in early pregnancy. However, the first of these studies related to the period 1966–68 and the second to 1963–69, when oral contraceptives were very little used.

Kay and his colleagues (R.C.G.P. 1976) were able to look at the outcome of pregnancy in 136 mothers who had inadvertently taken oral contraceptives early in pregnancy. 102 of their pregnancies went to term and two of these had malformations, one a congenital dislocation of the hip, and the other fatal urogenital and anal malformations.

Finally, the Committee on Safety of Medicines in this country has conducted a case-control retrospective study of 836 cases with certain congenital defects and 836 controls. As far as oral contraceptives were concerned there was no difference between cases and controls, thirty mothers of cases and thirty-two of controls having used oral contraception within three months of conception, eleven cases and ten controls having been exposed to these drugs in early pregnancy (Committee on Safety of Medicines 1977).

Pregnancies Occurring
after Cessation of Oral Contraceptives

It is in this area that the largest studies have been carried out. These have been of two main types, studies of abortions and studies of all other pregnancy outcomes. The abortion studies were mostly started after the observation by Carr (1967) that the chromosomal anomaly triploidy was unduly common when the pregnancy had been conceived within six months of oral contraception. Since over ninety per cent of all fetuses with chromosomal anomalies are aborted spontaneously only studies of abortions are likely to answer the question of whether the incidence of all, or some chromosomal anomalies is increased after the use of oral contraception.

Neonates. Klinger and his colleagues (1976) studied the chromosomes of a series of 1670 neonates, and one of 1233 induced abortions. In both they found a small but not significant excess of chromosome anomalies in the pregnancies that had followed oral contraception. This excess remained, but was still not statistically significant after correction for maternal age. No correlation was found with duration of contraceptive use, or interval between its cessation and conception.

Spontaneous abortions. There have now been numerous other reports following Carr's initial study, but no general agreement, in part because the type of abortions included differed considerably from study to study, and possibly because of differences in formulation of oral contraceptives over a period of time. Lazar and his colleagues (1973) found a small, non-significant excess of chromosome abnormalities in spontaneous abortions following the use of oral contraception, but they looked only at developmentally early specimens, and the rate of use of oral contraception was very low. Poland and Ash (1973) found a significant excess of developmental abnormalities in fetuses aborted by women who had used oral contraceptives within six months of conceiving compared with controls. Dhadial and her colleagues (1970) found a small excess of chromosomal anomalies in abortions following pill use, but this was not statistically significant.

Lauritsen (1975) also found such an excess, but the difference between the 'pill' group and the controls was not significant. Alberman and her col-

leagues (1976) found an excess of anomalies following pill use, and this was significant, particularly after allowing for associated maternal factors. In the latter series there was also a suggestion of a trend for the excess to increase with increasing duration of use.

Table 3. Spontaneous abortion series: chromosome anomalies following oral contraception.

Authors	No. of abortions karyotyped	% chromosome anomalies	
		after o.c.	not after o.c.
Dhadial *et al.* 1970	263	27	19
Lazar *et al.* 1973	847	66	63
Lauritsen 1975	246	60	49
Alberman *et al.* 1976	676	32	25

Taken together, these reports suggest that spontaneous abortions in mothers who have used oral contraception show a small excess of the proportion chromosomally abnormal (table 3), but the problem of the interpretation of these findings remains. In the case of spontaneous abortions there is no reliable denominator of the number of conceptions from which they are derived. A change in the proportion chromosomally abnormal may therefore reflect a real increase in incidence of abnormalities, and therefore an increase in abortions. Alternatively, such a change may reflect a real decrease in the incidence of chromosomally normal abortions, and therefore a decrease in abortions.

Total pregnancies. Because of these questions the results of the follow-up of total recognised pregnancies following cessation of oral contraception become more important. Here again there are now several such reports. The first study was by Peterson in 1969. He compared the outcome of pregnancy in 442 mothers of single pregnancies who had taken the pill prior to the study pregnancy, with that in 699 mothers who had not used such drugs. After further excluding mothers in whom the contraceptive had been used for therapeutic regulation of periods, he found that the incidence of spontaneous abortions in the pill series was similar to that of controls, slightly more than 9% compared with 8.6% in the controls. The rate of malformations in the two groups was also similar, 3.7% in the cases, 4.8% in the controls. Another study of birth outcomes by Robinson (1971) showed no significant difference between malformations after the pill and in controls. The largest study of this type is that by the Royal College of General Practitioners (1976), in which the rate of spontaneous abortion in ex-pill users was found to be slightly lower than that in controls, and the overall rate of malformation in births much the same, even when age and parity were allowed for. It is worth noting that there was a small excess of children with either Down's syndrome or another autosomal chromosome anomaly, but the numbers were too small to draw any valid conclusions. There has been a report from Germany suggesting that the

spontaneous abortion rate in ex-pill users may be lower than in controls (Döring, Kanka and Netzer 1976) but no allowance was made for age. The long-term follow-up study of women using different methods of contraception (Vessey *et al.* 1976) has shown no excess of either miscarriage or malformations in pregnancies after oral contraception, but no allowance has yet been made for maternal age. Lastly, preliminary results from a study of pregnancies of women doctors (Alberman *et al.*, in preparation) also show a slight overall decrease in spontaneous abortion after oral contraception, after allowing for age. A small excess in those pregnancies that occurred within one month of pill use was not statistically significant.

Table 4. Incidence of spontaneous abortion in recognised pregnancies and of reported malformations following oral contraception.

	No. of pregnancies		% spontaneously aborted		% malformations reported	
Authors	after o.c.	not after o.c.	after o.c.	not after o.c.	after o.c.	not after o.c.
Robinson 1971	1250[1]	1250[1]	—	—	minor 2.1 major 1.0	1.3 0.5
Peterson 1969	442	699	9	9	3.4	4.3
R.C.G.P. 1976	5530	11009	8.7[2]	9.1[2]	3.1[3]	3.4[3]

[1] Births only. [2] Pregnancies after 1970 only.
[3] Special enquiry in 2000.

The balance of this evidence is in favour of a very small reduction of incidence of spontaneous abortion in recognised pregnancies following pill use (table 4). This would be compatible with a small decrease in the proportion of chromosomally normal abortions, and a reciprocal increase in those chromosomally abnormal, which was postulated earlier. However, with frequent changes in the dose and formulation of oral contraceptives constant vigilance is necessary to ensure that this apparently beneficial change is not translated into one that is adverse, for it does seem that these drugs may affect future pregnancies. The size of this effect seems to be very small. This suggests that the relatively few studies that have looked at outcome after the consumption of oral contraceptives in pregnancy were probably not based on large enough samples to detect any change of a similar magnitude.

Conclusion

In conclusion, there is no evidence up to the present that the use of oral contraception has any important adverse effect on subsequent pregnancies, or that its inadvertent consumption in early pregnancy has such an effect. On the other hand, taking published evidence from several sources together, there is some evidence that pregnancies following oral contraception may be slightly less liable to abort spontaneously. The fact that there may be such an effect,

albeit of small magnitude, makes it even more important that we continue to monitor the outcome of large numbers of pregnancies exposed to such therapy.

With regard to fertility drugs there may be a relatively high malformation rate in resulting births, but we are not able to say whether this is due to the drugs or to the high-risk status of the treated mothers. Again, constant vigilance is called for.

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Antenatal Infections associated with Fetal Malformations

Infectious disease is still extremely common in the general population, and Dingle *et al.* (1953) found that 72% of the morbidity observed in a study of families was attributable to infection. Only one of the protozoal diseases, toxoplasmosis, and none of the bacterial diseases other than syphilis, has been incriminated in the causation of fetal malformations. Maternal virus disease has been studied, and the role of rubella virus as a teratogenic agent is undisputed, but the relationship of other viruses to fetal anomalies is not unequivocally established.

Statutory notification of infectious disease is not uniform in the United Kingdom and the Republic of Ireland, and, by and large, acute specific viral infections that have, or may have, teratogenic or crippling effects on the fetus are not notifiable. Infection as a cause of maternal death has declined, and only recently has attention been focused on viral disease other than rubella as a cause of maternal and perinatal morbidity. The overall perinatal mortality figure for England and Wales was 22.3 per 1000 total births (H.M.S.O. 1973) and is currently about 21.0 per 1000 (D.H.S.S. 1976), but many deaths *in utero* and in the perinatal period are unexplained; some may be consequent on viral diseases that have been relatively little studied. Observations on the association of fetal or perinatal death or morbidity with maternal viral disease are often inadequate because of incomplete reporting of early pregnancy, when fetal deaths occur most frequently. Prospective studies on cohort populations are rare, for the logistics of such studies are complex, but without them our knowledge will remain both incomplete and controversial.

Two large-scale studies have been undertaken. Information was obtained on maternal infections occurring from 1950–52 in pregnant women having antenatal care in Great Britain (Manson, Logan and Loy 1960). 1068 single live births were studied, following maternal rubella, poliomyelitis, measles, influenza, chickenpox and mumps. A control population of 5455 single live births was also studied, these children having been born to women without overt viral disease. Rates of congenital malformation in excess of the expected 2.3% were recorded for poliomyelitis (3.3%), influenza (3.7%) and measles (7.0%). The malformations were too few and too varied in type to base

evidence of causal relationships on the viral infections, and the results were considered inconclusive. A second long-term study was conducted in New York, based on maternal infections occurring from 1957–64, and using matched controls. The study is notable for its careful design and long follow-up (Siegel, Fuerst and Peress 1966; Siegel 1973).

372 single live children born to women with viral disease (150 chickenpox, 128 mumps, 66 measles, 65 hepatitis) were compared with 393 controls (Siegel 1973). There was no apparent difference in the overall incidence of congenital defects among virus affected and control subjects, the rates being 2.2% and 2.3% respectively. When grouped by period of gestation at the onset of maternal viral disease, the rates appeared to increase following chickenpox (7.4%), measles (6.3%) and hepatitis (10.0%) in the first trimester, compared with controls (3.4%). Cases of major malformations were few and the spectrum of defect was wide; thus, *the observed differences could have been due to chance and have no causal significance.* In both studies, the rates of congenital malformations were higher than expected following maternal viral disease, but no pattern emerged, and chance association could not really be excluded. Clearly, even larger studies are needed.

Nearly a fifth of all perinatal deaths are ascribed to congenital malformations, especially those of the central nervous system (Butler and Bonham 1963). The defects are tabulated by Lorber (1972) from the data of Butler and Alberman (1969). Overall (1972), reviewing the relationship of intrauterine virus infections and congenital heart disease, made proposals for future investigations that apply to all types of congenital defects. He suggested that the epidemiology of specific defects should be studied, with particular reference to the association of maternal viral infection with abnormal offspring. Virological studies of infants with specific defects should be made, and experimental animal models of specific defects should be developed.

Although the majority of maternally acquired viral infections seem to cause little harm to mother or fetus, some may result in severe damage, usually to the fetus. A good account of these diseases and of their general mode of operation in damaging the fetus is given by Banatvala (1971), and an account of the pathogenesis of fetal viral infections is given by Mims (1968).

Rubella in Pregnancy

For nearly two hundred years rubella was regarded as a benign disease of childhood and the least serious of all the exanthemas. In 1941 Gregg published an observation of historic importance, completely altering our concept of rubella as a mild disease of childhood, and reporting that women who had had rubella during the first trimester of pregnancy gave birth to infants with cataracts and other ocular defects, microcephaly with subsequent mental retardation, heart disease, and low birth weight with failure to thrive. An association with deafness and other congenital defect was soon published by Swan and others (1943). Up to thirty per cent of fetuses suffered from con-

genital abnormalities if exposed to maternal rubella *in utero* during the second month of gestation.

Rubella virus was first isolated in 1962 by two groups of workers (Parkman, Buescher and Artenstein; Weller and Neva). This discovery, and the development of serological techniques that followed, provided the basis for laboratory diagnosis, and led to more accurate assessment of the risk of rubella in early pregnancy. The spectrum of congenital defects, not all of which are recognisable at birth, was found to be broader than those of the rubella triad (ocular defects, deafness and heart disease), and the concept of arrested development during organogenesis yielded to recognition that the virus exerts a continuing effect upon the developing fetus. Thus, teratogenicity, death *in utero*, acute neonatal infection, or late postnatal disease may all result from fetal infection. Dudgeon (1967) reported that the incidence of defects may approach fifty or sixty per cent following exposure in the first month of gestation, and that such defects may be multiple, while Hardy *et al.* (1969) called attention to the danger of rubella contracted even as late as the thirty-first week of gestation, noting that poor physical growth, poor communicative ability and developmental retardation might follow.

The impetus towards the development of a rubella vaccine followed an epidemic that swept across the U.S.A. in 1964, leaving many thousands of deformed children in its wake. Soon afterwards, an experimental live vaccine was grown in monkey kidney cells and was subjected to clinical trial (Meyer, Parkman and Panos 1966). Its progeny, adapted to duck embryo (Buynak *et al.* 1968) was licensed for distribution in the United States. Meanwhile, a virus vaccine strain (Plotkin *et al.* 1967) grown on human diploid cells, and the Cendehill strain (Peetermans and Huygelen 1967), were developed, becoming available for general use in Britain in 1970. Since rubella is a mild disease only occasionally implicated in serious disease such as encephalitis (Rose and Mole 1976), or rheumatoid arthritis (Martenis, Bland and Phillips 1968), the aim of vaccination is to protect the developing embryo.

The approach to the problem differs in the United States and in Britain. In the former, young children of both sexes are protected by mass vaccination programmes, since they are believed to be the main disseminators of infection in the community. The wholesale acquisition of herd immunity should reduce the risk of natural infection among pregnant women, but suffers the drawback that a high proportion of vaccinated female children might again be susceptible to rubella by the time they reach childbearing years, *for the duration of immunity after vaccination has not yet been established* although it is likely to be long lasting.

Mass vaccination in Britain is offered to young girls between the ages of eleven and fourteen years (D.H.S.S. 1970), the immunological basis being to select out those who would be a risk to future offspring as near to the age of childbearing as possible, and to leave unimmunised males to be exposed to naturally occurring wild virus, thus maintaining, in some measure, the immunising potential of the natural disease (Dudgeon *et al.* 1971). In support of

the latter view, Enders (1970) believed that elimination of wild virus from the community might remove the most effective means of maintaining vaccine-induced immunity, and there is some evidence that immunisation, although productive of long-lasting antibody (Schiff *et al.* 1974), does not produce the same solid immunity as the natural disease, and that the antibody response may be qualitatively and quantitatively different. Thus, Horstmann and her colleagues (1970b) reported that the incidence of reinfection in a selected group, as indicated by a rise in antibody titre, was 80% in vaccinated individuals compared with 3.9% in those who were naturally immune. None of the reinfected had symptoms, but a few excreted virus from the nasopharynx. Reinfection was more commonly encountered in those with low HI antibody titres with no detectable complement fixing antibody. As Dudgeon and his colleagues (1971) observe, it is important to know whether an individual sensitised to rubella virus antigen, either as a result of natural infection or from vaccine, can respond to re-exposure by specific recall and rise in antibody production, without subclinical infection with local virus multiplication in the nasopharynx, or viraemia. If immunological response without virus multiplication or invasion is possible, reinfection, rather than being a hazard, would be a safeguard that would boost immunity.

Both in the United States and in Britain, individual patients may be selected for immunisation during the childbearing years, provided that pregnancy is avoided. Immunisation may be given during the puerperium (Horstmann, Lieblaber and Kohorn 1970a), to patients attending family planning clinics (Yellowlees 1976), or during a menstrual period (assuming no recent intercourse or the use of a reliable contraceptive method). Immunisation of individuals is usually preceded by serological testing to ascertain those who are susceptible, but this is not necessary, and may be counter-productive in certain circumstances (Rose and Mole 1976). Beazley *et al.* (1971) decided to screen the maternal population antenatally before offering rubella vaccination in the puerperium on grounds of cost-effectiveness and because they reasoned that the baseline value obtained would be useful should patients later be exposed to rubella-like illness. Serological testing is not undertaken on young girls in the national vaccination programme in Britain, although 46–80% were found to be immune in clinical trials (Dudgeon *et al.* 1971).

Guidelines for the use of live rubella virus vaccine have been laid down in the United States by the Public Health Service Advisory Committee on Immunisation Practices (1971), which also chronicles the likely side effects. Rash from lymphadenopathy may occur but the principal side effects are on the joints. Arthralgia and arthritis, mainly of the small peripheral joints, occur in one to fifteen per cent of vaccinated children, although usually in no more than five per cent. The joint symptoms are of greater severity and longer duration than those caused by other vaccines. These symptoms, or non-joint associated pain and parasthesiae in the arms, hands or popliteal fossae, begin from two to ten weeks after vaccination, persisting for from one to three days. Rarely, they recur, but they do not result in permanent damage. The symp-

toms are more frequent and more severe in mature females, and are more marked with HPV-77 derived vaccines such as those used in the United States. The vaccine virus has been recovered from knee joint effusions of children three to four months after administration of vaccine (Ogra and Herd 1971). Postvaccination arthralgia is not a problem with the vaccines used in Britain (British Medical Journal 1972). Brachial or lumbosacral radiculoneuritis—'the arm syndrome' or 'the leg syndrome'—is seen with equal frequency after HPV and Cendehill vaccines (Gilmartin, Jabbour and Duenas 1972). Diffuse myelitis associated with rubella vaccination has been reported (Holt *et al.* 1976) but it is not certain that the neurological illnesses were directly attributable to the use of the vaccine.

Virus may be shed from the pharynx from one to four weeks after vaccination and transmission to susceptible contacts is theoretically possible. However, only a handful of several thousand susceptible persons deliberately exposed to rubella vaccines developed antibody, and most of these had been exposed to natural rubella also. Only in very rare instances has transmission of vaccine virus been thought to occur. Vaccine virus has not been sought in breast milk. Were it present, it would be unlikely to act as an immunising agent in the recipient newborn.

Up to eight per cent of volunteers may not develop antibody (Dudgeon *et al.* 1969) and reinfection, as adjudged by a rise in antibody titre, may occur, as discussed previously. Forrest *et al.* (1972) reported the occurrence of typical rubella in a woman eleven months after vaccination. We have twice seen typical rubella occur within two to three weeks of vaccination in the puerperium; the balance of evidence suggested that the patients had been exposed to wild virus at about the time of vaccination, since the vaccine virus, on examination, was undoubtedly an attenuated strain. *Should clinical rubella reinfection occur during pregnancy, viraemia should probably be assumed* whether or not rubella specific IgM can be demonstrated (Lancet 1973), but if subclinical reinfection should be demonstrated, the fetus is unlikely to be at risk, provided that only rubella IgG antibody can be demonstrated.

Rubella virus has been isolated from the uterine cervix six days after the onset of rubella infection, and vaccine virus has been isolated after fourteen days. Local rubella infection poses a risk of intrauterine infection in early human embryos, which may not be so great as that of maternal viraemia. Nonetheless, it is wise to regard preconceptual rubella or rubella vaccination as dangerous to the fetus. Rubella vaccine virus can produce chronic placental infection and can infect the conceptus (Larson *et al.* 1971); proof of its teratogenicity is not available (Giles 1973). *The vaccine is contra-indicated during pregnancy.*

The preliminary report on the first year's operation of the National Congenital Rubella Surveillance Programme indicated that the estimated figure of 200–250 affected children born annually in non-epidemic years was approximately correct (Dudgeon *et al.* 1973), emphasising the paramount need of prevention. It is now about sixty a year. Rubella vaccines became available in

the United Kingdom in 1970, and in that year a selective vaccination programme was introduced whereby young girls aged eleven to fourteen years were offered vaccine without preliminary serological test (D.H.S.S. 1970). Further recommendations relating to women of childbearing years emphasised that *reliable contraceptive measures should be used for at least two months after vaccination*; priority is given to women at special risk of contracting rubella, including school teachers, nurses, doctors and others involved in community care (D.H.S.S. 1972, 1974). 287 children with congenital rubella were identified by the National Congenital Rubella Surveillance Programme between 1971 and 1974, most having been born between 1969 and 1973 (Marshall *et al.* 1976). 44% had been born to primiparae, emphasising the need for rubella vaccination before a woman's first pregnancy.

Vaccination before the first pregnancy offers the best hope of avoiding congenital rubella, but it will be at least another four years (a decade since the beginning of mass vaccination) before maternal rubella in the population will decline. The methods currently available for prevention were summarised by Harley (1971) in the Swift Memorial Lecture. They are: 'isolation' of seronegative pregnant women; passive immunisation of the pregnant woman who has had contact with rubella; termination of pregnancy when distinct seroconversion has occurred in the first trimester; and active immunisation, as previously discussed. Ideally, seronegative pregnant women should not work in hospitals, doctors' surgeries, schools or any other situation with a high risk of rubella exposure, and should avoid known contact with persons with rubella, in particular, with infants with congenital rubella most of whom are infectious at birth, and some ten per cent of whom are infectious at twelve months. Menser *et al.* (1971) reported a case, suggesting that the patient had remained chronically infected for thirty years. These precautions are particularly important during a rubella epidemic (Carne, Dewhurst and Hurley 1973) but are, of course, difficult to implement in practice. The value of gammaglobulin prophylaxis remains controversial, but there is no contraindication to giving it to pregnant women who have been exposed to rubella, after the first sample of blood for antibody estimation has been withdrawn. High titre globulin, if available, may protect (Schiff 1969).

Termination of pregnancy for rubella or after rubella contact is widely practised, 2504 abortions being performed for this reason in England and Wales between 1971 and 1973. Interference with pregnancy could be made more selective if direct methods relevant to diagnosis in the fetus, rather than inferential tests on the mother, were in use. Virus has been isolated from the liquor amnii (Alford 1965, Alestig *et al.* 1974, Levin *et al.* 1974), and Alford detected neutralising antibody in sera from embryos aged eleven to sixteen weeks. Levin *et al.* (1974) rightly observed that serological tests on the mother leave the question of transmission to the fetus unanswered. Viral isolation is lengthy and exacting, and blood from living fetuses in the first trimester is unobtainable. Rapid diagnostic tests, such as specific immune fluorescence, have not been applied to cells of liquor amnii.

Cytomegalovirus

Cytomegalovirus infections were reviewed by Lamb (1971), who outlined the features of classical cytomegalic inclusion disease. Intense jaundice, widespread purpura, haemolytic anaemia and hepatosplenomegaly occur, together with episodes of respiratory distress. Involvement of the central nervous system is recognised by neonatal convulsions, spasticity, microcephaly, choroidoretinitis, and radiological evidence of cerebral calcification. Osteitis of the long bones may also be detected radiologically. The illness runs a prolonged course, in those who survive the neonatal period, and hepatosplenomegaly may persist into the second year of life. Biliary atresia has been reported, and microphthalmia may be an associated finding. The majority of those who do not have neurological signs at birth are not mentally retarded. The incidence of subnormality following congenital infection has not been established, but McCracken *et al.* (1969) described varying degrees of mental retardation in nine of twenty infants with cytomegalic inclusion disease.

The virus shows a high degree of species specificity, so that it is unlikely that animals act as a reservoir of infection. It is probably transmitted in urine and saliva, but close and prolonged contact is probably required, for the virus loses infectivity rapidly at ambient temperature. These conditions are met during pregnancy, and the fetus is probably at risk during the viraemic phase of maternal infection, possibly during passage through the birth canal, and in the puerperium, since virus has been isolated from cervical excretions and from breast milk. Infections of the female genital tract may play an important role in intrauterine or neonatal infections, but only recently have they received study (Montgomery, Youngblood and Medearis 1972). Identification of cytomegalovirus inclusions in the cells of endocervical glands has been reported (Ross 1966). Like other herpesviruses, cytomegalovirus may persist in latent form following a primary infection. Montgomery, Youngblood and Medearis (1972) speculated that cytomegalovirus infections of the cervix during pregnancy represent activation of latent infection by hormonally-induced local changes to which older, multiparous women are resistant. Nunazaki *et al.* (1970) also believe that latent virus is reactivated and multiplies in the genital organs of pregnant women after the second trimester, appearing in the cervical secretion. They could not demonstrate excretion of virus in the newborn, but recovered it from mouth and urine in sixty per cent of healthy infants between five to nine months of age. Excretion ceased abruptly at twelve months and they were unable to advance an explanation for this. They considered the possibilities of horizontal or vertical transmission, favouring the latter as the likely route of infection, and believing that it occurred during passage through the infected birth canal.

Viruses present in the cervical secretions near term are thought to pose a forty per cent risk of neonatal infection (Reynolds *et al.* 1973). McCormack (1975) describes the spread of cytomegalovirus infection from mother to infant in two ways: first, the intrauterine route, and, second, the perinatal.

About one per cent of newborn infants in the United States experience intrauterine infection with the virus (Melish and Hanshaw 1973), and virus can be isolated from twelve per cent of women, more frequently in the third trimester. Primary infection during the first trimester, with recovery of virus from the liquor amnii, has been described (Davis *et al.* 1971), and placentitis has been reported (Hayes and Gibas 1971), sometimes occurring in the absence of fetal infection. Structural malformations of the type seen in congenital rubella are rare, and Dudgeon (1971) suggests that this may argue that fetal infection usually occurs after the period of organogenesis. The chances of primary disease coinciding with pregnancy are high, for about half the population of the Western hemisphere is without antibody on reaching the child-bearing years.

Congenital infection with cytomegalovirus is common, since 0.5–3% of infants excrete the virus at birth (Stern and Tucker 1973, Walker and Tobin 1970). The majority of babies excreting virus escape serious disease or even outward signs of infection, although in the past it was thought that intrauterine infection invariably resulted in damage to the central nervous system in a high proportion of cases. Stern *et al.* (1969) found a significant association between cytomegalovirus infection and microcephalic mental deficiency, and believed the virus might account for ten per cent of cases of mental retardation compared with two to three per cent of cases of mental deficiency associated with rubella and toxoplasmosis. Walker and Tobin (1970) identified thirty-six cases of congenital cytomegalovirus infection in Manchester in a two-year period, also finding an association with mental retardation. Elek and Stern (1974) estimate that congenital cytomegalovirus infection causes some 200–600 mental cripples a year in England and Wales, comparing this with an estimated figure of 1300 for Down's syndrome. They regarded the relationship between intrauterine infection and severe brain damage with mental retardation as sufficiently well established to warrant the development of preventive measures, and describe experiments with live vaccine in susceptible volunteers. They surmise that the eventual goal might be the vaccination of all young adolescent girls, as in the case of rubella vaccine.

Little purpose would be served, other than the important one of advancing knowledge of the pathogenesis of the damaging variant of intrauterine cytomegalovirus infection, by screening for the disease during pregnancy, since, at present, our knowledge is insufficient to assess the risk of brain damage in the individual case. Should the case for vaccination be proven, and a safe vaccine become available, it is probable that its administration would be preceded by screening, as is the case with rubella.

Miscellaneous Virus Diseases

The viral infections known to affect the newborn were discussed by Sever (1968), who distinguished rubella and cytomegalovirus as viruses that can produce congenital malformations and mental retardation from the other viruses (herpesvirus hominis, measles, Western equine encephalitis, varicella,

variola, vaccinia, poliovirus, hepatitis, and Coxsackievirus B) that are associated with severe tissue involvement of the developing fetus, but not with congenital malformation. He regarded the virus of herpes simplex (herpesvirus hominis types 1 and 2) as next in importance to rubella and cytomegalovirus. Bradford-Hill *et al.* (1958) had emphasised that determination of the probability that illness in the mother during pregnancy will give rise to congenital malformation in the infant demands a prospective method of inquiry, and, in a small series, had been unable to adduce evidence of significant association, other than in rubella. They studied thirty-five cases of mumps (epidemic parotitis), thirty of chickenpox, and ten of measles, finding that the proportion of liveborn children with low birthweights was relatively high, though not statistically significant, after maternal chickenpox.

Siegel, Fuerst and Peress (1966), in a prospective study, implicated mumps and hepatitis, as well as rubella, in the occurrence of fetal deaths. Their sample was too small to yield conclusive findings for measles (nineteen cases) and chickenpox (thirty-two cases), but, in individual cases they discerned a likely causal relation between these diseases and fetal death. Measles is a declining disease, in consequence of vaccination, the number of cases in the United Kingdom in 1973 being 54000, and in 1974, 30000, compared with the half to three-quarters of a million that used to occur in epidemic years. Elizan and Fabiyi (1970) reviewed the experimental data relating to the linkage of congenital and neonatal anomalies with viral infections, summarising the major findings in a table. The results of experimental infections in animals are conflicting, and no coherent pattern emerged, even for rubella. Associations between virus disease and congenital malformation or disease are not necessarily those of cause and effect, although Roberts and Powell (1975) believed, however, that the interrelationships observed amongst congenital defects militated against the notion that multiple external factors are involved in the causation of human malformations.

Many defects have been described in association with maternal virus disease but, other than those associated with rubella or cytomegalovirus, none has been established through long-term, prospective population studies.

Herpesvirus Hominis (HVH)

Tobin (1975) referred to six cases linking HVH to the congenital defects of microcephaly, intracranial calcification, diffuse brain changes, chorioretinitis, retinal dysplasias or microphthalmia associated with vesicular rash at birth. Only one case was associated with type 1 virus, the other five being associated with type 2. South *et al.* (1969), describing two cases, suggested that the virus had neurotropic teratogenic potential. Hanshaw (1973) believed that these observations supported the thesis that HVH is able to infect the fetus during the period of organogenesis, and induce anomalies such as microcephaly and microphthalmia. There is no doubt that transplacental transmission can occur (Boué and Loffredo 1970) and, in view of reports of congenital malformations following maternal infection in early pregnancy, it is important to follow the development of infants born to mothers with herpetic

infections in the first half of pregnancy over a period of five to ten years (Hanshaw 1973).

Varicella-Zoster Virus (chickenpox-zoster)

Neonatal varicella and herpes zoster both occur, and teratogenic potential has been ascribed to the varicella-zoster virus. Savage, Moosa and Gordon (1973) described a case, reviewing previous reports. The defect appears to be specific and the case for teratogenicity, although unproven in the final sense of the word, seems sound. The constant features of fetal structural malformation consequent on maternal infection by chickenpox in early pregnancy are reduction deformities of limbs and skin scars, sometimes causing contractures. Additional features include low birth weight, Horner's syndrome, choroidoretinitis, dysphagia and meningoencephalitis. The v-z virus is known for its neurotropic properties, and the limb deformities may be consequent to denervation, as is known to occur in the peripheral neuropathy caused by thalidomide. Gangrene of the digits has been recorded after chickenpox (Gyde and Beales 1970). Further work on the effects of varicella-zoster during pregnancy seems mandatory, and studies are in progress. There is as yet no licensed varicella vaccine, although live vaccine has been used (Asano *et al.* 1976). Passive immunisation by specific or non-specific immunoglobulin has been practised (British Medical Journal 1969).

Influenza Virus

Influenza during pregnancy, particularly during the 1957 'Asian' influenza epidemic, has been associated with childhood leukaemia (Hakulinen *et al.* 1973). Neurological complications of influenza are not infrequent (Lancet 1970) and, after the 1957 epidemic, Hakosalo and Saxën (1971) reported a significant increase in malformations of the central nervous system in children exposed during the fifth to eleventh weeks of gestation. Coffey and Jessop (1963) also noted that anencephaly, encephalocele, and meningocele tended to occur in those who had been exposed to influenza in the first trimester, although not all studies have shown a relationship (Leck and Steward 1972). Interpretation of data has been vexed because of the belief that viraemia does not occur in influenza, and that transplacental transmission is, therefore, unlikely to arise. Khakpour, Saidi and Naficy (1969) showed that viraemia can occur during the incubation period, and Yawn *et al.* (1971) showed that the virus is able to infect the developing fetus in the last trimester of pregnancy. Inactivated influenza vaccine, properly used, can prevent influenza A and B, and a live influenza-virus vaccine has been licensed in Britain; others are in the development stage (Lancet 1975). The prevention of influenza is particularly recommended in certain high-risk categories, of which pregnancy, during pandemics, is sometimes regarded as one; the inactivated vaccine would be used.

Although other associations between maternal virus disease and congenital malformations have been suggested as a result of serological studies, or of the *post hoc ergo propter hoc* argument, there is little substantive evidence to support them. The case report of Leary, Welt and Beckett (1949),

linking infectious mononucleosis with a fatal congenital anomaly, is one such. Variola was formerly a major disease, and has now been virtually eradicated in consequence of the programme of the World Health Organisation. The study of Naderi (1975) indicated that smallpox vaccination was not likely to be followed by malformation, although women who were vaccinated in the first trimester of pregnancy more often had children with clubfoot ($p=0.05$).

Virus Vaccines and the Developing Fetus

The real and theoretical risks of the administration of live virus vaccines have been considered by Levine, Edsall and Bruce-Chwatt (1974), against the background of epidemic situations and overseas travel. They concluded that immunisation against poliomyelitis and yellow fever is indicated in women contemplating travel to affected areas; that pregnancy is a contra-indication to rubella and mumps vaccination and routine smallpox vaccination; that measles vaccination is not indicated in pregnancy; and that smallpox vaccination with immune globulin cover is mandatory for the pregnant women travelling to a smallpox endemic area. The guidelines they established are useful in resolving the dilemma of whether or not to immunise a pregnant woman who intends to travel into a epidemic area when the risk to maternal, and thus to fetal, health is clear. Some further discussion is, perhaps, necessary in considering the risks of inadvertent vaccination during pregnancy.

Among the human viral infections, only the virus of rubella is indisputably teratogenic and infection by the viruses of mumps, measles, poliomyelitis or yellow fever is unlikely to cause congenital deformities. There is some case for considering that the viruses of chickenpox and of influenza may be causally related to specific defects. Theoretically, introduction of an attenuated virus might harm the fetus, despite the lack of adverse effects in the mother, and Levine, Edsall and Bruce-Chwatt (1974) cite the increased frequency of damaged piglets following administration of hog cholera vaccine to sows in the first month of pregnancy. Irrespective of congenital malformation, smallpox vaccination can result in death of the fetus from vaccinia. However, Levine *et al.* (1974) were able to find only twenty reported cases of intrauterine vaccinia in over forty years, although many, many thousands of women were vaccinated; there was no reported example of teratogenesis. Cumulative experience thus attests the relative safety of smallpox vaccination during pregnancy. Smallpox vaccination poses a very slight risk of disseminated disease in the fetus, a risk that both obstetrician and mother may well be prepared to accept. Naderi (1975) found a barely significant correlation between smallpox vaccination and clubfoot. Neither risk is of consequence in an epidemic situation.

Rubella vaccine is unique amongst immunising agents in that its purpose is the protection of the conceptus from the teratogenic and other damaging effects of the virus. The living attenuated virus can be transplacentally transmitted, and vaccine virus has been recovered from the fetal eye twenty weeks after administration of vaccine at thirteen weeks of gestation, the eye showing

histopathological changes considered to be typical of congenital rubella (Fleet *et al.* 1974). Although it is as yet impossible to quantitate the risk to the fetus of rubella vaccine, the vaccine must be regarded as a hazard to the fetus. It is contra-indicated during pregnancy, and for a period of eight weeks before conception.

It would seem unjustifiable to add iatrogenically induced malformations to the disasters that can overtake the fetus, and the risk of rubella vaccine, though it may prove to be slight, is likely to be unacceptable.

Poliomyelitis vaccines exist in two forms, the oral living attenuated vaccine (Sabin) and the killed injectable vaccine (Salk), of which the former is used in the United Kingdom, and has resulted in the virtual elimination of paralytic poliomyelitis. The relative risks of the two vaccines are discussed in editorials (British Medical Journal 1977, Lancet 1977). Theoretically, since they are capable of multiplication, live virus vaccines may be shed and could infect other, susceptible individuals, but, in practice, most do not behave in this way and neither rubella nor measles vaccines spread to household contacts or into the environment. Live polio vaccine virus is a striking exception, for the virus multiplies in the wall of the gut and is excreted in the faeces, whence it infects other persons. Dissemination is inevitable. We have isolated poliovaccine virus from the throat of a child attending a postnatal clinic within the maternity unit. Wherever immunisation against poliomyelitis is practised, pregnant women will encounter the vaccine virus, and will acquire it. Thus, the sugar lump is but one of two ways in which pregnant women inadvertently receive active immunisation against poliomyelitis. Fortunately, the pathological and teratogenic potential of the attenuated strains for the conceptus has been the subject of many retrospective studies. Other than a single case report, where virus isolation from the fetus was not attempted and the postulated relationship was unsubstantiated, there is no evidence that oral polio vaccine poses a risk during pregnancy (Levine *et al.* 1974). Between 1961 and 1964 in the U.S.A. approximately 100 million doses of each of the three types of oral poliomyelitis vaccine were distributed; no data emerged to contra-indicate the use of the vaccine during pregnancy (Special Advisory Committee 1964). The vaccine must be regarded as safe.

Hundreds of millions of doses of yellow fever vaccine have been administered in mass vaccinations, but no adverse effects on pregnant women or the fetus have been reported despite passive surveillance, which has revealed a side effect of the vaccine in young infants. Measles vaccine might, conceivably, be indicated during an epidemic in a susceptible isolated population, but has not been so used. Protection by immune globulin would be a more likely expedient. There is no indication for mumps vaccination during pregnancy, most adults being immune, but the mumps vaccine virus crosses into the placenta (Yamauchi, Wilson and St Geme 1974). Maternal and perinatal mortality seemingly rises during a pandemic of influenza, and, in these circumstances, pregnancy could be regarded as a high risk situation. Inactivated vaccine is used in the United Kingdom, and is not contra-indicated during

pregnancy. Vaccines are not available for cytomegalovirus, varicella-zoster, herpesvirus hominis, or hepatitis viruses, although some are under consideration and are in the experimental stage. Rabies is so dangerous a disease that vaccination would have to be attempted, whether the patient were pregnant or not.

Congenital Syphilis

Formerly a very important cause of neonatal illness, with subsequent stigmata such as 'saddle nose' or 'sabre shins' and Hutchinson's triad of deformed teeth, interstitial keratitis and eighth nerve deafness, congenital syphilis has become a rare disease. Serological screening for treponemal disease is an important aspect of antenatal care, and in a ten-year period, bearing in mind the relative risk of transmission to offspring at different stages of the disease, we have estimated that the congenital disease has been averted in about forty-six instances through case detection of previously undiagnosed maternal disease and its treatment. There is, thus, no case for relinquishing these important screening tests at present. Indeed, if sexually transmitted disease continues to rise, they may become more important. More recently introduced and widely used screening tests are discussed by O'Neill (1976); Hare (1973) describes the tests in use over the ten-year period mentioned above.

Toxoplasmosis

Although there had been intimations previously that parasites resembling toxoplasmas were implicated in congenital disease, the report of Wolf, Cowen and Paige (1939) established the fact of congenital toxoplasmosis before knowledge of the acquired disease in man was gained. The tetrad of clinical signs that characterises congenital toxoplasmosis, although it may be seen in whole or in part in other diseases, is: internal hydrocephalus or microcephaly, choroidoretinitis, signs of involvement of the central nervous system (usually convulsions), and evidence of cerebral calcification. A diffuse rash, hepatosplenomegaly, thrombocytopaenia with purpura and microphthalmia may also be present. The mother usually has no recognisable disease during pregnancy, and the great majority of babies born to women who acquire toxoplasmosis during pregnancy are uninfected or, at least, not severely affected. The stage of pregnancy in which maternal infection is acquired is an important determinant of the outcome for the fetus. Desmonts and Couvreur (1974) reported an acquisition rate of toxoplasmosis during pregnancy of 6.3 per hundred and the disease occurred in 59 of the non-aborted offspring of the 378 pregnant women studied. Of these, two babies died and seven had severe congenital disease; all were born to women who had acquired the disease during the first two trimesters. The placenta yielded parasites in twenty-five per cent of those who had acquired toxoplasmosis during pregnancy, and no infected infants were born to those who had had antibody before becoming pregnant. There are undoubted differences in the rates of acquisition of the primary infection in different populations, and these data pertaining to French

women should not be extrapolated to the population of Britain. Fleck (1965, 1971) showed that the inhabitants of Tristan da Cunha and the Hadza tribe in Tanzania showed steep rises in acquisition of antibody (five per cent per year) before the childbearing age. The degree of association with domestic cats, and with preparation of foodstuffs for domestic pets, as well as ingestion of raw meat, are all factors relevant to acquisition of the primary infection, and Fleck (1971) reported an overall incidence of congenital toxoplasmosis in England and Wales of one in 20000 live births. In Germany, seventeen infants with congenital toxoplasmosis were born to 3213 obstetric patients studied, and in New York, two in 4048 (Kimball, Kean and Fuch 1971). The latter authors, in a prospective study, failed to find evidence of an association between habitual abortion and toxoplasmosis.

Toxoplasma gondii seems to be a coccidian parasite, closely related to the genus *Isospora* (Hutchinson *et al.* 1970). For many years the mode of transmission and the life cycle were obscure, although the disease had been recognised in rabbits in 1908, and sixty years later the infection was still present in the same laboratory colony of animals (British Medical Journal 1970). Although the parasite had been seen in man in 1914, the widespread infection that occurs was not recognised until the introduction of the dye test (Sabin and Feldman 1948). A large proportion of the world's population, including about thirty-three per cent of the adult population in England, seems to be infected. Apart from congenital disease, and the consumption of raw meat (Desmonts *et al.* 1965), the origin of the infection was mysterious until Hutchinson (1965) showed that *Toxoplasma* could be transmitted by the faeces of cats that had been fed on toxoplasma-infected mice. The faeces remained infectious for a year or more, and disinfection did not interrupt the cycle of transmission. Later, cysts were seen in infective faeces, and a typical coccidian life cycle of schizogony and gametogony was shown to occur in the intestines of germ-free cats that had been fed brains containing cysts of the parasite. The association of cats with the human disease had long been known (Price 1969), and there is highly suggestive evidence that the cycle of infection in man follows ingestion of highly infective and resistant oocysts from the excreta of domestic animals (Fleck, Chessum and Perkins 1972).

Intimate contact with domestic animals, and the consumption of raw meat, would seem unwise during pregnancy, although hand and other hygiene would, undoubtedly, decrease the risk of the former. The risk of congenital infection in Britain, on balance, seems to be about half that of phenylketonuria, a disease for which national screening is undertaken. As with the latter, screening might show the disease to be more prevalent than is supposed. As Dudgeon (1975) has observed, control of toxoplasmosis presents a problem: spiramycin may be of value in chemoprophylaxis of a primary infection in pregnancy, to reduce the risk of fetal damage; but control of the disease by epidemiological means would require one European nation to alter its cooking habits, and another to give up keeping domestic cats!

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THE DIAGNOSIS

Ultrasonography in the Diagnosis of Fetal Malformations

Sonar has an important role to play in the prenatal diagnosis of fetal malformation. It is still too crude in resolution, however, to define any but the grossest skeletal malformations. Improvements will doubtless come but in the meantime its main contribution is in facilitating amniocentesis by locating suitable pools of liquor amnii, outlining the placenta, excluding the presence of multiple pregnancy and, above all, in estimating maturity accurately. This is necessary for the correct interpretation of alpha-fetoprotein levels in amniotic fluid and particularly in maternal serum. No other method of examination can achieve all these objectives in the earlier half of pregnancy.

In later pregnancy it provides a ready means of further studying intrauterine development.

The ultrasonograms reproduced in this paper were all taken with Diasonograph apparatus (Nuclear Enterprises Ltd, Edinburgh). Some are in grey scale, and others are from a bistable storage oscilloscope.

Introduction

The contribution of sonar, as I prefer to call diagnostic ultrasonic echo sounding, may be a modest one in the prenatal diagnosis of fetal malformation but it is nevertheless important. In this respect it has more to offer than radiology since the latter is only applicable in the second half of pregnancy, preferably the last trimester, cannot be frequently repeated and is not without recognised hazard. It may seem a far cry from the method developed in 1917 for detecting U-boats in the ocean to studying intrauterine development both normal and abnormal, but such are the maritime origins of the subject (Langevin 1921).

Properties of Ultrasound

Sonar stands for 'sound navigation and ranging' and is akin to radar. The reason for using ultrasound instead of ordinary sound is that ultrasonic energy, because of its short wavelength and very high frequency, can be propagated as a beam with very little divergence. The precise point of origin of an echo can therefore be determined. When dealing with a multiplicity of echoes,

such as occurs from within the human body, the relative positions of each can be mapped with geometrical precision and displayed by cathode ray oscillography (Donald, MacVicar and Brown 1958).

Both ultrasonic and X-ray energy can penetrate tissues far more efficiently than light or heat, which are absorbed more extensively from the surface inwards. Both ultrasonic and X-ray energies are transmitted in wave form but thereafter there are no points of similarity. X-rays belong to the electromagnetic spectrum. They provide shadow pictures that depend largely upon the mineral content of the tissues studied. Ultrasound, on the other hand, is simply mechanical vibrational energy of such a high frequency that it is beyond the range of animal hearing.

For medical diagnosis we use frequencies in the megahertz range, commonly 2.5 MHz, which provides a wavelength in tissue of the order of 0.7 mm. It is therefore much too coarse for histological diagnosis. This is an inherent limitation, since any attempt to improve resolution by increasing the frequency and consequently reducing the wavelength is thwarted by loss of penetration in contrast to X-rays.

Ultrasonograms are 'echo reflection maps' (Donald and Brown 1961), and the visualisation of even a very early fetus *in utero* is simply a scaled-down version of submarine detection, and copies the miniaturisation techniques of metal flaw detection in modern engineering practice (Firestone 1946).

Ultrasonic echoes are generated in accordance with Rayleigh's law, which determines the fraction of reflected energy. This depends on the differences in specific acoustic impedance in contiguous tissues providing a reflecting interface to the passage of an ultrasonic beam. These differences in the very early embryo, when tissue differentiation is not far advanced, are at first very slight.

The first and most obvious echoes occur between liquor and the fetal body as a whole, so that a 'fetal pole' is visible after six weeks' amenorrhoea (Donald 1969a) and the crown-rump length can thereafter be measured (Robinson 1973a). It is only later in pregnancy that tissue differences become sufficiently marked to make it possible, for example, to distinguish between brain and ventricles or heart muscle, including the interventricular septum and blood. Skeletal differentiation appears much earlier, in the second trimester, and consequently cranial and neural tube defects are revealed at an earlier stage than congenital organ defects.

Developments in Sonar

One cannot study abnormal development without also studying the normal, especially with a new modality such as sonar (Campbell 1968). Extensive work on these lines has been done and departures from normal growth rates can now be readily recognised (MacVicar and Donald 1963, Robinson 1975b, Willocks 1963, Willocks *et al.* 1964, 1967). This new facility has been backed up in the course of the last twenty years by a consistent improvement

in technology that has graduated first from simple A-scan to B-scan with compound sector scanning; time motion display methods, which can now demonstrate the movement patterns of the fetal heart, for example, from the seventh week of amenorrhoea onwards (Robinson 1972, 1973b); and more recently, in the last two years, full grey tone scaling, thanks to the principle of scan conversion. This last employs a Lithicon storage tube in which electrons emitted as in a standard cathode ray tube impinge, not on a luminescent and therefore visible phosphor, but on a storage target that is invisible and is then discriminatingly scanned by an electron reading beam and displayed with all the qualities of black and white television (Donald 1975, 1976a).

Attempts at three-dimensional display by the use of acoustic holography have been found to be too time-consuming and expensive to be justified by a minimum additional information yield (Donald 1969b), but the new developments in real-time scanning, whereby moving pictures of living structures can be made, as and where they occur, provide an exciting new prospect.

Limitations

It is important to recognise the present day limitations of sonar (Donald 1973), not only the constraint of two-dimensional display but also its uselessness in revealing enzyme and metabolic deficiencies and consequent mental retardation, except insofar as it facilitates amniocentesis. However, it is about as inappropriate to run a prenatal handicap diagnostic service without sonar back-up as to have no facilities for tissue culture.

Types of Deformity Identifiable by Sonar

Certain specific malformations can be demonstrated with varying degrees of exactitude. Hydramnios, even in subclinical extent, is easily recognised (Sunden 1964) and raises the suspicion of malformation.

Only gross cranial defects can at present be diagnosed with confidence by routine sonar examinations, but it is safe to assume that if a head cannot be found the fetus has not got one (Donald and Abdulla 1967). The diagnosis of anencephaly by sonar is, therefore, not difficult (Campbell *et al.* 1972). Even in late pregnancy, when the question of therapeutic abortion does not arise, the knowledge of gross fetal abnormality may modify the obstetrician's management both as regards induction of labour and Caesarean section. Confirmatory radiography is likely to be invoked, if possible, before embarking on Caesarean section in solely fetal interests (figures 1 and 2).

The dangers of allowing an anencephalic pregnancy to become postmature, as may easily happen, are very considerable, and we have had a maternal death in our hospital from ruptured lower uterine segment due to the shoulders in such a case.

Hydrocephaly (and microcephaly) are more likely to be diagnosed only in later pregnancy, always bearing in mind the possibility of error from mistaken dates or maturity or actual intrauterine growth retardation. A biparietal diameter exceeding 11 cm, however, makes the diagnosis more than likely,

Figure 1. Hydramnios and anencephaly. Blob-like limb echoes towards the left of picture, fetal trunk and unformed head to the right.

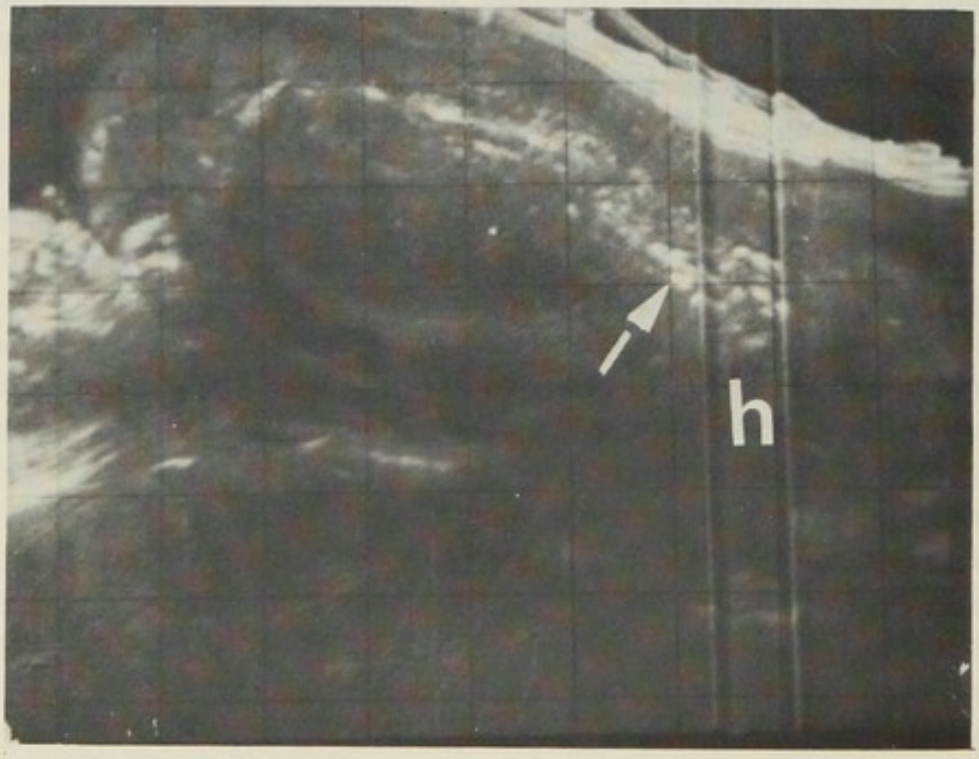

Figure 2. Anencephaly and spina bifida. Malformed head to right (h) and disorganised spine (arrow). Placenta is posterior. Grey scale picture.

and 12 cm makes it a certainty (figure 3). Ultrasonic mensuration of the fetal head can with advantage be combined with transthoracic and transabdominal measurements at the level of the ductus venosus. This may help to distinguish between microcephaly and immaturity, since the ratio of these diameters does

Figure 3. Hydrocephalus. Biparietal diameter 12.32 cm. Sector of placenta antero-lateral to right of picture.

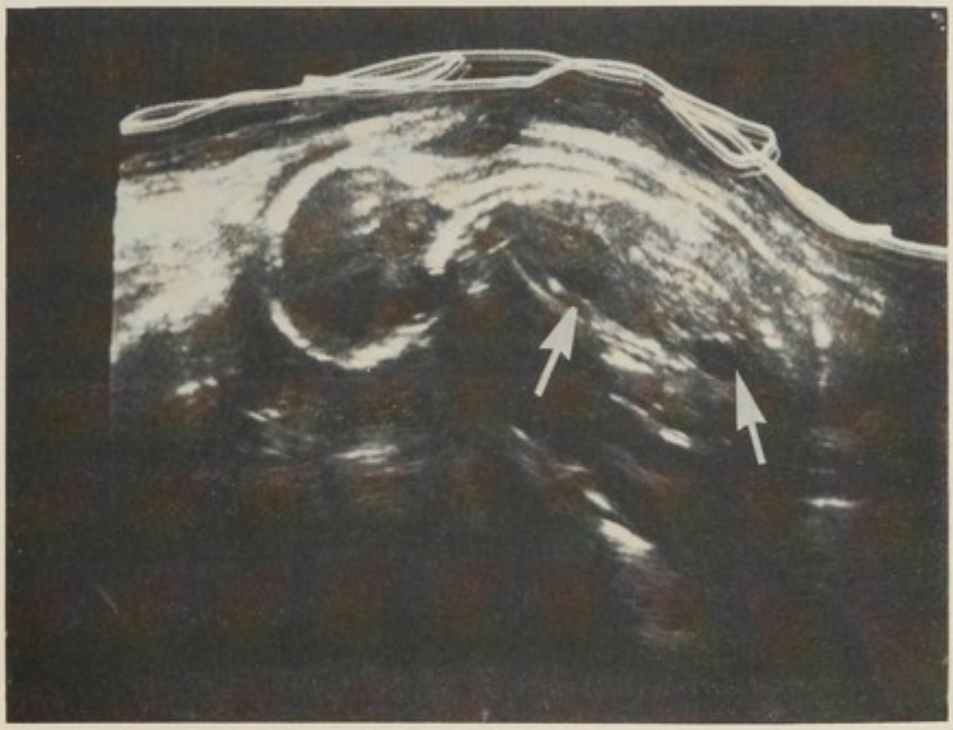

Figure 4. Normal fetal spine in diabetic pregnancy. Note heart (left arrow) and full fetal bladder (right arrow) (Courtesy of Dr Mary Pont).

not fall to unity under 36 weeks (Campbell and Wilkin 1975, Hansmann 1974, Higginbottom *et al.* 1975).

The recognition of spina bifida, although calling for considerable expertise and patient positioning of the scanning plane, is important because it may

define the level of the neural tube defect and help to determine the possibility of successful surgery after birth. The normal ultrasonic appearance of the vertebral column in longitudinal section is that of a 'tramline' consisting of two parallel lines (figures 4, 5), whereas in transverse section the spinal canal should have a circular shape. The pictures, however, are still rather crude. Campbell *et al.* (1975), in seeking to confirm the provisional diagnosis of neural tube defect in three cases because of a raised alpha-fetoprotein level in the liquor, failed to diagnose a lumbo-sacral meningocele in one, correctly found a meningomyelocele in another and were right in failing to find anything wrong in the third who was found, after termination, to be normal. The AFP result was possibly due to heavy contamination of the liquor amnii specimen with blood.

When it comes to the diagnosis of malformation of organs the task may be even more difficult, especially if there is oligohydramnios as well, as is often the case with genito-urinary abnormalities. Nevertheless kidneys can be identified (figure 6), even polycystic kidneys with high grade grey-scaling, and the bladder can be seen to fill and empty (Campbell, Wladimiroff and Dewhurst 1973, Garrett, Robinson and Gruenwald 1970, Garrett, Kossoff and Osborn 1975, Wladimiroff and Campbell 1974).

Commonly, babies with multiple malformations grow badly even though maternal oestriol excretion rates may appear normal. The dysmature baby, however, demonstrates, as a rule, the double phenomenon of a low oestriol level in the mother's urine as well as poor ultrasonic measurements.

The successful diagnosis of some of the grosser cardiac lesions while still *in utero* is likely to develop with the introduction of real-time scanning techniques.

Amniocentesis

It is in amniocentesis that sonar has most to offer. In fact it would not be an exaggeration to say that sonar is essential, not only for the safe performance of the operation but also for the correct interpretation of the results of liquor amnii studies.

There are three very good reasons for this:—

1. The need to locate the placenta to its very margins (Donald and Abdulla 1968, Kobayashi *et al.* 1970, Gottesfeld *et al.* 1966).
2. To diagnose or to exclude the presence of twins.
3. Assessment of maturity to within one week, preferably half a week (Campbell 1969, 1970, Campbell and Newman 1971, Hellman *et al.* 1969).

No other diagnostic technique can achieve these three objectives. Not only can sonar enable one to avoid traumatising the placenta, but it also demonstrates where best to tap a pool of liquor (figures 7 and 8). After all, a specimen of fluid that clots on standing is not much use to the cytogeneticist, nor for that matter is one that reacts to urease! The patient may have an unexpectedly full bladder due to apprehension.

The method of Bang and Northeved (1972) involves the use of a trans-

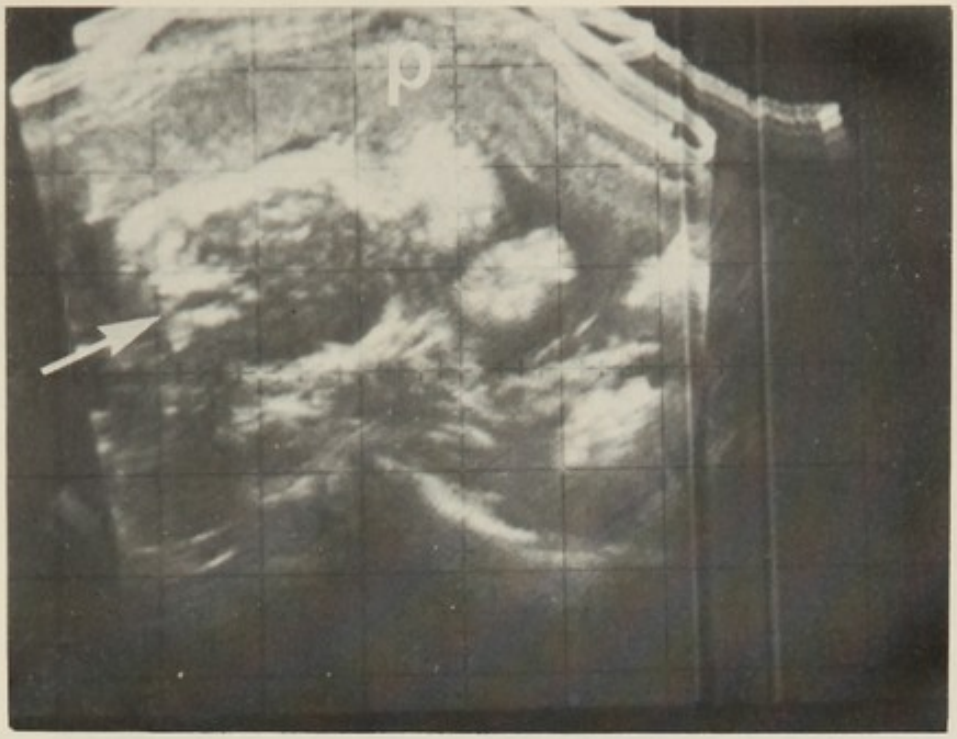

Figure 5. Spina bifida. Transverse section. Incomplete neural arch (arrow). Anterior placenta (p).

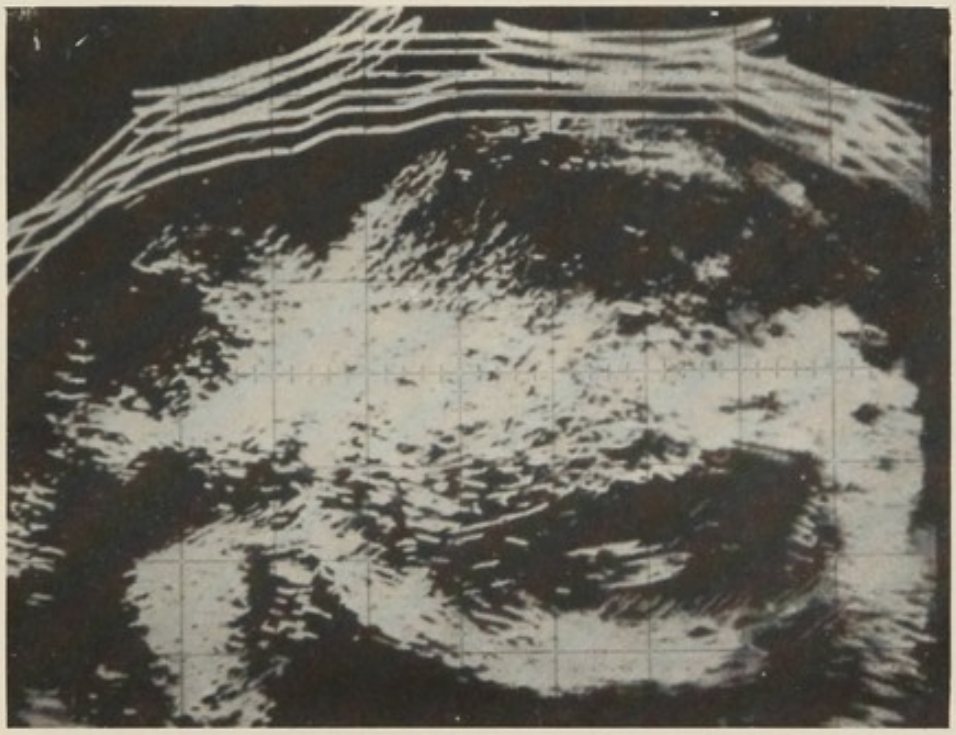

Figure 6. Enlarged view of both fetal kidneys (normal) on either side of spine. History of previous neonatal death from polycystic kidneys (Courtesy of Dr Mary Pont).

ducer with a hole for the exploring needle passing down its centre but we ourselves prefer to locate a safe and suitable site and angle of penetration with reference to our scanning gantry. A 'bloody tap', apart from its unserviceability, means a likely feto-maternal transfusion of significant proportions. It

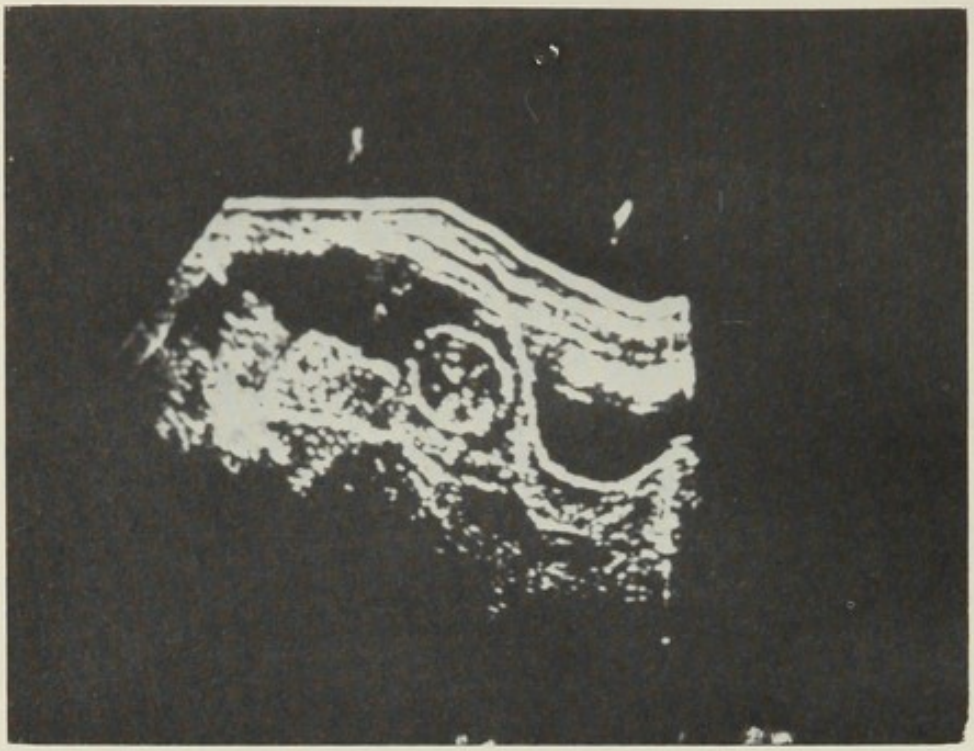

Figure 7. Posterior placenta and fetal head at sixteen weeks' gestation in a diabetic pregnancy with previous intrauterine death at 32 weeks. Easy amniocentesis.

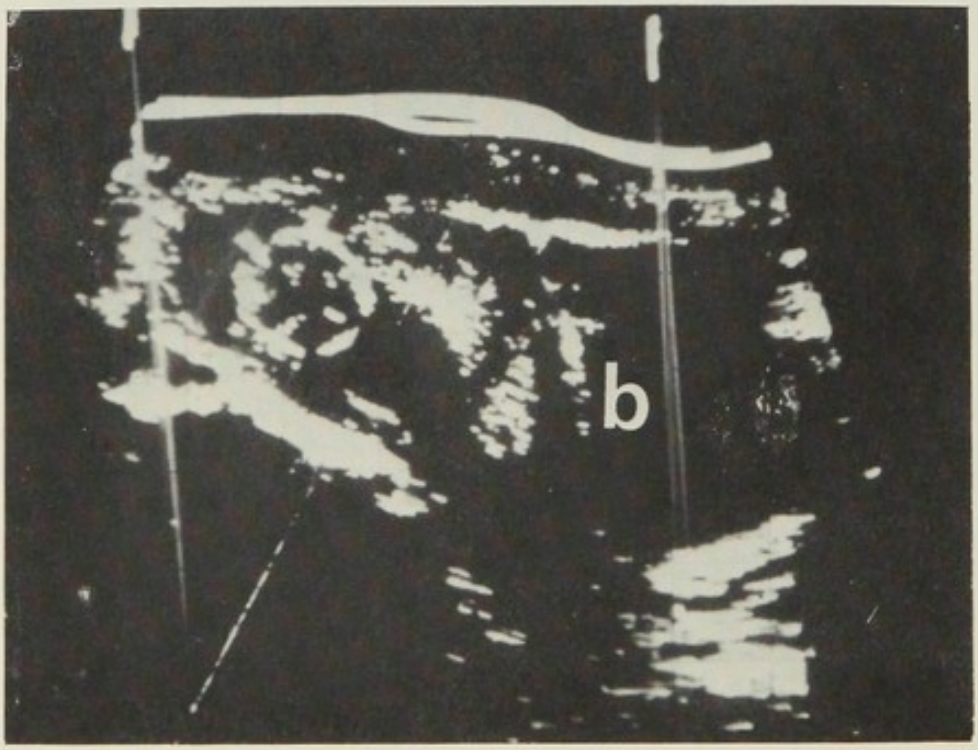

Figure 8. Fourteen weeks' gestation with anterior placenta and poorly accessible pool of liquor amnii to left of and above fetal head. Previous spina bifida. Note full maternal bladder (b).

might be argued that, in the case of Rh-negative women, the hazard of isoimmunisation can always be countered by the prompt administration of anti-D immunoglobulin, but this is a reckless view that takes no account of

other complications such as fetal intra-amniotic bleeding (from which I have had one fetal death shortly after amniocentesis), infection, maternal intraperitoneal bleeding from puncture of the vascular placental site (again, I have had such a case), abortion and maternal death.

The diagnosis of multiple pregnancy is important, because the finding of a high serum alpha-fetoprotein (Wald, Brock and Bonnar 1974), which may have provided the indication for amniocentesis in the first place, may have been due to twins and not the suspected neural tube defect (Wald *et al.* 1975). In one of our own cases we failed to diagnose twins and merely satisfied ourselves about the position of the placenta. Following amniocentesis the patient aborted. Admittedly she had threatened to abort earlier in the pregnancy, but the operation can have hardly contributed to the welfare of the pregnancy.

Maturity

The question of maturity is perhaps the most important of all. Using Robinson's technique of measuring the crown-rump length in very early pregnancy and demonstrating the fetal heart both as to rate and dimensions, an accuracy to within a few days is possible (Robinson 1973a, Robinson and Fleming 1975). Even the size of the fetal sac can be estimated and is related too to fetal growth (Hellman *et al.* 1969, Robinson 1975a: figure 9).

After the twelfth week of gestation (menstrual age) the head can be seen and measured. At this early stage the head growth is so rapid and predictable, up to the thirtieth week at least, that mistaken estimates of maturity should not occur in good hands (Campbell 1968, 1970). I defy any other method, clinical, biochemical or anamnestic, to give comparable accuracy in measuring maturity (Campbell and Kurjak 1972; Robinson *et al.* 1974; Underhill, Beazley and Campbell 1971), and if routine screening of all pregnant patients by serum alpha-fetoprotein levels early in pregnancy is to become a standard method of searching out neural tube defects in the population at large it will have to be backed up by sonar on a much more extensive scale than is available at present to make any such programme worthwhile and to prevent a large number of unnecessary amniocenteses. I know of one hospital in Scotland, at least, where little notice is taken of the patient's menstrual history, since so many of their patients have recently abandoned the contraceptive pill and their ovulatory cycle is unpredictable.

Very Early Pregnancy

The cases of demonstrable fetal malformation that continue into the last trimester are numerically only a fraction of the large majority that mercifully fall by the wayside in very early pregnancy.

This phenomenon of biological wastage, which occurs in all species, including the human, although probably to a lesser extent, can now be revealed by sonar employing the full bladder technique (Donald 1963; Donald, Morley and Barnett 1972; Hellman, Kobayashi and Cromb 1973), and

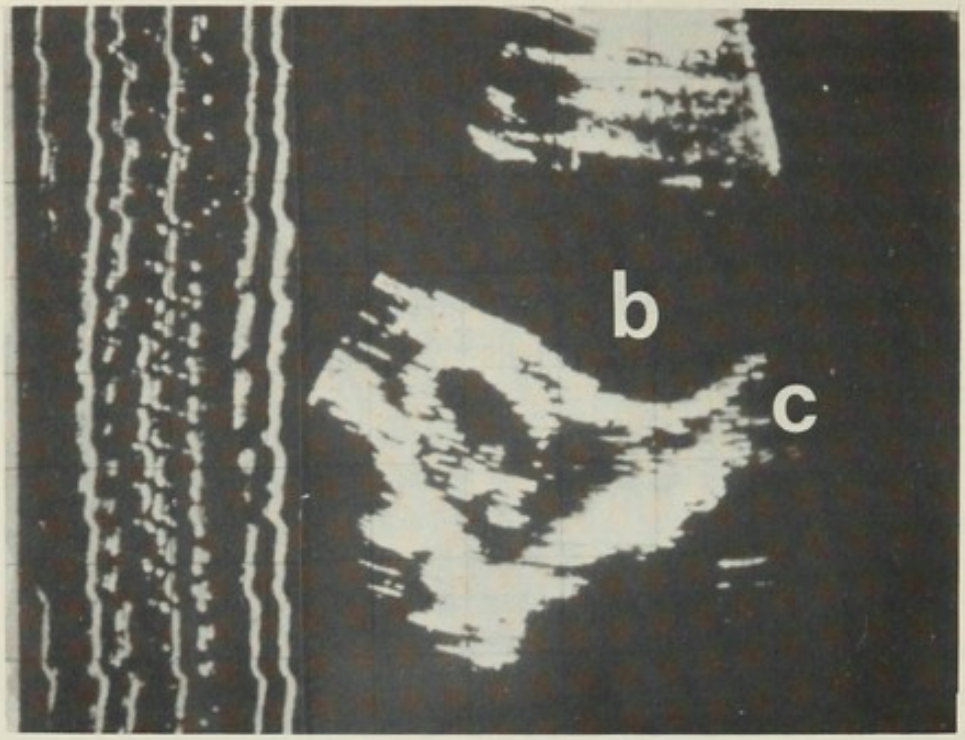

Figure 9. Very early gestation (menstrual age 7.5 weeks). Fetal pole visible in lower part of sac. Full bladder (b) and cervix to right (c). Fetal heart motion pattern superimposed to left of picture. Two previous spontaneous abortions.

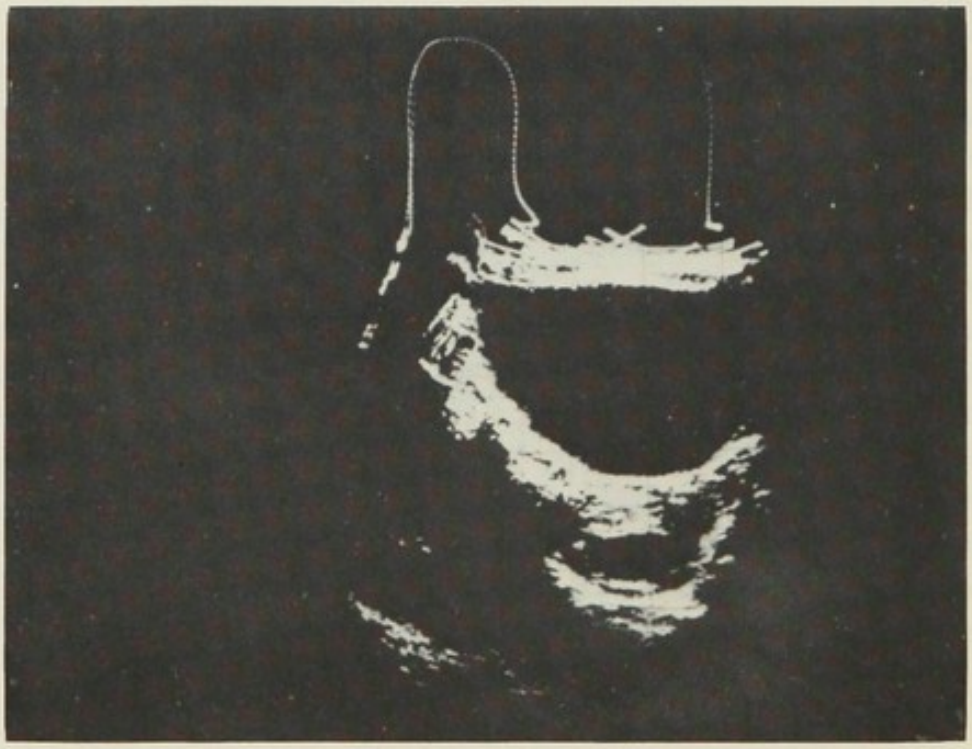

Figure 10. Blighted ovum at eleven weeks' gestation. Fifth pregnancy with no living children. Cervical cerclage a week previously. (Aborted again spontaneously three days after ultrasonography, which should have preceded cerclage.)

further amplified by grey scale presentation and, in the case of blighted ova that are virtually anembryonic by the absence of a fetal heart, by Robinson's method. Such cases of early pregnancy failure are far more common than

hitherto supposed and may account for a loss of as many as a quarter of all conceptuses. Often the patient is unaware of her blighted pregnancy and merely thinks that she has had a belated but heavy menstrual loss. The passage of fetal tissue, more or less disorganised, confirmed our suspicions on the few occasions when we were able to obtain the specimen passed. Occasionally tissue culture was possible and a chromosome defect found. This would appear to be a natural phenomenon whereby Mother Nature casts off what might have been hideously malformed had it survived (figure 10).

Intrauterine Growth Retardation

The association of poor intrauterine growth with multiple fetal abnormalities has already been mentioned. I am not sure whether it is appropriate here to mention intrauterine growth retardation as an entity in its own right without physical deformity, but most obstetricians and paediatricians too fear the possible relationship with mental retardation made manifest in later childhood (Davies and Stewart 1975). After all, there can be few handicaps more to be feared by parents and society at large than mental deficiency.

Our present techniques of prenatal fetal monitoring (Campbell and Dewhurst 1971, Davison *et al.* 1973, Lancet 1973, 1974) are still in their own infancy, but it is more than likely that sonar will add increasingly to the recognition of adverse factors affecting fetal development, including later intellectual capacity as well. There is certainly a need in the first place to spot the 'losers'.

The Future Contribution of Sonar

In the present state of the art we can only hope to do better in the future, not worse, and if already dilated cerebral ventricles can be displayed by high-grade grey scale scanning one can only anticipate better yet to come (Kossoff, Garrett and Radovanovich 1974).

In my view the real future lies in real-time scanning. This can be achieved either by mechanical or by electronic means. Mechanical methods are unlikely ever to present echo pictures above the human optical flicker rate, i.e. above 16, preferably 20, frames per second. Electronic scanning methods can achieve almost infinite speed and are likely to displace mechanical methods, but instead of single transducer operating systems they require sequentially fired multi-element transducers or phased array systems producing electronic sector sweep scans with great rapidity. I have had a limited personal experience with both techniques and reckon that they cannot equal the resolution quality of current single transducer systems, but they will ultimately score because of the speed with which the fetal vertebral column, for example, can be latched on to and followed down its entire length. However, transverse views of the neural canal can be made to appear unnaturally oval if the sectional view is in any way oblique. I have myself videotaped movements of arms, legs, chest wall, pulsating structures and whole body movements, which I half suspect may be exaggerated during such an ex-

amination. This phenomenon should be looked into, but there is no doubt that considerable skill is needed in manipulating the apparatus (Donald 1976b).

Real-time scanning in effect extends the ability, discrimination and depth of manual palpation by reaching what cannot be reached manually and revealing what is beyond the range of the clinician's touch.

Hazards

The question of hazard remains to be answered. It would be tragic if in our attempts to eliminate one hazard we introduced another. I have reviewed all the experimental evidences to date and the findings are reassuringly negative (Donald 1976). I can only review the future in a limited way.

Within the next few years I can foresee resolution so improving that it may be possible, by this non-invasive technique, to determine the sex of the child at an early stage. This may provide information so unwelcome to the parents that requests for termination of the pregnancy may arise in the prevailing ethical climate of today; and worse still, I can only gloomily predict the attitude of certain gynaecologists. I can see no good coming of it—only another slide down the slippery slope. The gynaecologist is what the Americans call the 'fall-guy'.

Sir John Brotherston and Professor Polani (below, this volume) have the unenviable task of looking into the future, and many of you will have the less enviable task of living with it.

Conclusion

It is one thing to prevent, by genetic counselling, a baby from ever starting its handicapped existence. It is another thing to do something about it once it has started, and it is sad to reflect that, apart from the direct treatment of Rh haemolytic disease *in utero*, all medical therapy has, as yet, to offer is basically negative and at worst destructive. Like the poor, the problem of fetal abnormality will be always with us, and in putting my shoulder to the ultrasonic wheel I may, in the last years of my clinical life, have contributed to modern therapeutic nihilism.

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Indications, Technique and Limitations of Amniocentesis

The obstetrician is confronted with the problem of human malformations over many years of practical work, by observing malformed newborns and by contact with their parents. It was a great breakthrough when amniocentesis for prenatal genetic investigation was established. In Göteborg we started performing amniocentesis for genetic purposes in 1970. The experience to which reference will be made in this paper has not been gathered by a single person. Prenatal investigation means teamwork. At the centre to which I belong, geneticists, psychiatrists, biochemists, paediatricians and obstetricians have built up a fruitful collaboration. This paper is written from the obstetrician's point of view. It is his task to perform amniocentesis and to provide the material for the laboratories. But the matter is not as simple as that, because genetic counselling is necessary prior to amniocentesis.

In Sweden, as in most countries, there is a shortage of geneticists and genetic advice has often to be given by other doctors, e.g. paediatricians and obstetricians. All physicians dealing with patients with an increased risk of having a baby with congenital disease should have a basic knowledge of prenatal diagnosis. Amniocentesis, however, should only be performed by a limited number of well-trained gynaecologists.

Indications for Amniocentesis

In this paper, the aspiration of amniotic fluid will be considered as the main purpose of amniocentesis. After some general remarks the indications recommended by a European study group, and then the experiences of the centre in Göteborg, are discussed.

Generally speaking, prenatal diagnosis of congenital abnormalities by amniocentesis is possible in cases with qualitative or quantitative changes in the constituents of amniotic fluid. In qualitative changes the fluid either contains a component not normally present or it lacks one of the normal components. In cases with quantitative changes the level of the normal components may be decreased or increased. This includes also structural chromosome abnormalities. Thus, one has to know the normal composition of the amniotic fluid, especially at the age of gestation at which amniocentesis is

performed. It is well known that the concentration of many constituents of the amniotic fluid varies with gestational age. One has of course also to know whether a certain congenital disorder exerts an influence upon the constituents of the amniotic fluid or not. If these facts are known regarding a certain abnormality, and if there is an increased risk of a particular fetus being abnormal, then amniocentesis is indicated.

It is a common view that the statistical risk of a fetal abnormality should be greater than the risk of the procedure. However, one has to bear in mind that the parents wish to exclude rather than to confirm an abnormality. The following list of indications for amniocentesis is mainly based upon the recommendations of the Stockholm Conference (1975) on prenatal diagnosis (table 1).

High maternal age is generally accepted as an indication for amniocentesis, since the frequency of autosomal trisomies and of some sex chromosome aneuploidies increases with maternal age. But there is still some disagreement about the borderline of age from which amniocentesis should be recommended. This controversy seems to depend partly upon different figures re-

Table 1. Indications for genetic amniocentesis. Modified recommendations of the Stockholm Conference (1975).

1. *Medical indications*
   - 1.1 *Chromosomal:*
     - High maternal age
     - Familial structural abnormalities
     - Mosaicism or aneuploidy in one of the parents
     - Abnormalities in a previous child
     - Sex determination (X-linked disorder)
   - 1.2 *Biochemical:*
     - Autosomal and X-linked recessive conditions where prenatal diagnosis is possible
     - Alpha-fetoprotein examinations in pregnancies at risk for neural tube defects
   - 1.3 *Linkage:*
     - In informative matings where the deficient mutant gene is linked with a marker gene

2. *Psychological indications:* parental anxiety

3. *Other possible indications:*
   - teratogenic virus infection
   - possible exposure to mutagens
   - repeated abortion
   - forensic paternity problems
   - ovulation induction (clomiphene)

4. *Risk cases detected by population screening*
   - e.g. elevated serum AFP, both parents heterozygote for metabolic hereditary disease

garding the frequency of chromosome aberrations in babies and fetuses of elder mothers (Christiansson 1976, Ferguson-Smith 1976, Hook 1976, Lindsjö 1974, Polani *et al.* 1976, Simpson *et al.* 1976).

Everybody agrees that amniocentesis should be offered to mothers 40 years of age and more and most workers recommend amniocentesis in mothers of 38 and 39 years of age too. The age between 35 and 37 has been called a 'grey zone' (Goldstein, Dumars and Kent 1976). The frequency of aneuploidies in the fetus for each single year of maternal age is not sufficiently known. What is needed are studies of large groups of patients in which the frequency of fetal chromosome abnormalities can be reliably determined for each single year of maternal age, especially the years from 35 to 39. However, many centres recommend a borderline age of 35 years, and the results from amniotic fluid investigations in the age group between 35 and 39 years have shown a frequency of major fetal chromosome aberrations above one per cent (Ferguson-Smith 1976; Simpson *et al.* 1976; Wahlström, Bartsch and Lundberg 1973).

Familial structural abnormalities, mosaicism and aneuploidy in one of the parents are high risk groups, and thus strong indications for amniocentesis.

When chromosome abnormalities in a previous child have been detected, the parents' karyotypes ought to be determined. Even if no parental aberration has been found, the fetal karyotype should be determined during a subsequent pregnancy. For example, a younger mother who already has a child with Down's syndrome has an increased risk of having another child with a trisomy (Burton, Gerbie and Nadler 1974; Golbus 1976; Littlefield, Milunsky and Atkins 1974; N.I.C.H.D. study group 1976; Passarge, this volume).

The determination of the fetal sex is recommended in sex-linked disorders as, for example, haemophilia and Duchenne's muscular dystrophy. If the mother is a carrier of the disease fifty per cent of her sons will inherit the mutant gene. Consequently legal termination of pregnancies with male fetuses results in the abortion of fifty per cent healthy fetuses.

There is a great number of hereditary metabolic diseases, but the particular biochemical defect is known only in some of these. About fifty of the latter can be detected prenatally by investigation of the amniotic fluid. Since no laboratory is able to perform all the different kinds of analyses, international collaboration has been established between different centres. For those disorders that can be detected prenatally and the laboratories in question one has to refer to the literature or, even better, to the special laboratories.

For neural tube defects and analysis of alpha-fetoprotein (AFP) in the amniotic fluid, see the paper by Brock in this volume.

Most workers accept psychological indications, i.e. parents without increased statistical risk but with extreme fear of having a baby with congenital abnormalities. Genetic counselling is of the utmost importance in these cases.

There are some debatable indications for amniocentesis and prenatal

investigation of the amniotic fluid, for example maternal thyroid dysfunction, diabetes, the intake or abuse of certain drugs early in pregnancy, exposure to chemical agents and environmental poisons, infectious diseases and irradiation during the earliest stage of pregnancy. Much research work is necessary to clarify these indications.

It has also been discussed whether amniocentesis should be performed in women with habitual abortion and in mothers who have lost a previous child perinatally where the cause is unknown. In the opinion of the geneticist of our group, in these cases the karyotype of the parents should be determined. If no parental abnormalities are detected, amniocentesis is not indicated.

Finally, amniocentesis is indicated in population screenings when the screening test has shown an increased risk of fetal abnormality detectable in the amniotic fluid.

The Göteborg Centre

The studies on prenatal genetic amniocentesis published from different centres show remarkable variations between the frequencies of the different indications. The reasons for these differences are often local ones, and not evident for the reader of an article. Therefore, it is necessary to say a few words about the background of the figures quoted in this paper.

In the city of Göteborg prenatal chromosome investigations have been feasible since 1970 and were offered to pregnant women 35 years of age and over and to other risk groups who attended the antenatal care units. At about the same time prenatal investigation of hereditary metabolic diseases was established, but determination of AFP in the amniotic fluid was not performed before 1974.

The city of Göteborg has nearly half a million inhabitants. During the years 1970–76 the number of deliveries has decreased from 7000 to 5000 a year, and the number of legal abortions was between 1000 and 2000 a year.

The number of genetic amniocenteses increased rapidly and during the last year about 400 patients attended our centre. By October 1976 the total number of pregnancies investigated by amniocentesis was 1000. About half of the cases came from the city of Göteborg. The other half were from other parts of Sweden, sometimes from as far away as the northern areas, which means a journey of more than 1000 km by aircraft. The patients living far away from Göteborg had, as a rule, very strong indications for amniocentesis. Table 2 shows the distribution of the single indications; in about half of the cases this was advanced maternal age. Nearly all mothers in the group 'previous child with aneuploidy' had a previous baby with Down's syndrome.

The case of structural chromosome aberration in a previous child was a deletion of a long arm of chromosome 2. In nine cases prenatal chromosome analysis was performed because one of the parents was a carrier of a balanced translocation. Five of these persons showed a D/D-translocation and the other four had other reciprocal translocations between two autosomes. In the five cases of X-linked recessive disease the purpose of the investigation was sexing of the fetus, which was done by karyotyping. The group with an in-

Table 2. Genetic indications for amniocentesis, Göteborg 1970–76.

Indication	Patients
Mother's age 35–39 years	383
Mother's age 40 years and over	212
Previous child: aneuploidy	105
Previous child: structural chromosome aberration	1
Balanced translocation in one of the parents	9
Mosaicism for trisomy-21 in the mother	2
X-linked recessive disease	5
Increased risk of neural tube defect	46
Previous child: metabolic hereditary disease	16
Anxiety about congenital abnormalities in the fetus	221
Total	1000

creased risk of neural tube defect is relatively small. This depends partly upon the fact that determination of AFP in amniotic fluid was not done in our centre before 1974 and partly upon the relatively low frequency of neural tube defects in Sweden. The group 'metabolic hereditary disease' contains families with a previous child with Gaucher's disease, Krabbe's disease and some other rare metabolic disorders.

The group 'anxiety about congenital abnormalities in the fetus' is relatively large. In this group there are women with a previous child with malformations of unknown origin, often multiple malformations, patients with relatives with some kind of congenital disease, e.g. aneuploidy, but also diseases that cannot be detected in the amniotic fluid. Many of the patients in this group had close contact with malformed children, e.g. nurses and teachers from schools and institutions for handicapped children, social workers and colleagues. Their worry and anxiety was often so great that they intended to terminate the pregnancy if they could not have a prenatal investigation carried out.

Technique of Amniocentesis for Prenatal Genetic Investigation

Table 3 shows the various stages of management. It is essential to provide genetic counselling before amniocentesis. Both parents should be informed about the statistical risk of having an abnormal fetus and about the indications, limitations and risks of amniocentesis.

Preliminary Ultrasound Examinations

Ultrasound scanning seems to me essential prior to amniocentesis, but it is obvious that amniocentesis is performed in many places without preceding ultrasonic investigation.

Ideally, the same person should perform the ultrasound investigation and the amniocentesis. By ultrasound, errors in determination of the gestational

Table 3. Prenatal genetic diagnosis. Management of amniotic fluid sampling by amniocentesis.

Genetic counselling	including indications, limitations and risks of amniocentesis
Ultrasound:	placental localisation
	gestational age
	fetal life
	multiple pregnancy
	hydatidiform mole
	extrauterine pregnancy
	acrania
	optimal puncture site
Transabdominal amniocentesis	
Confirmation	of fetal life after amniocentesis
Administration	of immunoglobulin anti-D in Rh-negative, non-Rh-immunised mothers

age can be avoided. According to most workers the optimal gestational age for amniocentesis is the sixteenth week, that is between the 105th and 112th day of the pregnancy calculated from the first day of the last menstruation with a 28-day cycle. By ultrasound fetal life should be confirmed before the puncture. Multiple pregnancy, hydatidiform mole and extrauterine pregnancy can be detected. There has been some discussion about the value of localising the placenta and whether the frequency of haemorrhages can be diminished by use of ultrasound. It is much easier to determine an optimal puncture site with the help of ultrasound. It is helpful to indicate the puncture site on the skin with a coloured pen. Furthermore, the distance between the skin and the amniotic cavity can be measured by ultrasound. The use of a puncture transducer and the visualisation of the needle by ultrasound is said to improve the safety of the puncture and to diminish the frequency of failed punctures and of bloody taps (Bang and Northeved 1972).

It is of great psychological value to confirm fetal life after amniocentesis with the help of Doppler ultrasound equipment.

All the details of the puncture have been discussed by others (Fuchs and Cederqvist 1970; Huddleston and Carney 1976; Jacobson 1972; Milunsky 1973; Schwarz 1975; Scrimgeour 1973). A few special points should, however, be emphasised. For example, a full bladder might be advantageous, since it gives a valuable reference point during ultrasound scanning. It also gives a better access to the uterus. If there is the slightest doubt about the nature of the aspirated fluid, a rapid test demonstrating the presence of protein and glucose excludes urine.

Amniocentesis in Twin Pregnancies

The management of twin pregnancies is an intriguing problem. When twins are detected by ultrasound, the special problems of this situation have to be explained to the parents and the indications should be reconsidered. Bang,

Nielsen and Philip (1975) recommended the following technique: the needle is inserted near a fetal head with the help of a puncture transducer. After aspiration of a sample of amniotic fluid, Congo red is injected into the amniotic sac. Thereafter the needle is withdrawn and inserted again near the second head. The colour of the second sample should be normal if the puncture of the second sac was successful.

My own experience comprises eight twin pregnancies. In the first two cases, which occurred at the very beginning of our study, the diagnosis was missed due to my inexperience in ultrasound. Since then, we have detected all twins and we have tried to puncture both sacs. In the first diagnosed case the puncture of the second sac was unsuccessful. Therefore in the following cases methylene blue was injected into the first sac. Thereafter we tried to puncture the second sac. In three cases the colour of the second fluid was not blue stained, in two cases it was. Thus, out of six diagnosed twin pregnancies, we apparently succeeded in getting material from both fetuses in three cases. With respect to these unsatisfactory results we have asked for ultrasound equipment that permits the visualisation of the needle during the puncture.

Complications of Amniocentesis

Another technical question is how to avoid fetomaternal haemorrhage and admixture of blood with the amniotic fluid.

Fetomaternal Haemorrhage

The problem of fetomaternal haemorrhage due to amniocentesis was investigated in our centre by two series, each consisting of approximately 200 consecutive amniocenteses. The first study was performed during the years 1970–73, shortly after we had started performing amniocentesis, and the second study during 1975–76, i.e. after some years' experience (table 4). The placental site was determined by ultrasound (Vidoson Siemens, rapid B-scan). Before and after amniocentesis a venous blood sample was taken for investigation of haemoglobin-F cells according to the technique described by Kleihauer, as modified by us (Bartsch 1972). The frequency of significant fetomaternal haemorrhages (i.e. more than 0.1 ml of fetal blood in the mother's circulation after amniocentesis) was about 5.5% in both series. It was calcu-

Table 4. The relationship between fetomaternal haemorrhage and placental site at the time of amniocentesis. Göteborg: series 1, 1970–73; series 2, 1975–76.

		Placental site:			
	Series	posterior wall	< 50% anterior wall	> 50% anterior wall	Total
total number of amniocenteses	1	52	72	89	213
	2	49	60	102	211
transplacental haemorrhages > 0.1 ml	1	0	0	9	11
	2	2	0	10	12

lated that the largest amount of fetal blood in the maternal circulation did not exceed 10 ml. There was a clear relationship between the site of the placenta as determined by ultrasound and the frequency of fetomaternal haemorrhages, most of which were observed when the placenta covered the anterior wall of the uterus. Thus, it is recommended that the placenta is avoided, by performing an ultrasound scan, in order to avoid fetomaternal haemorrhage.

Anti-D-Prophylaxis

Since it seems impossible to avoid fetomaternal haemorrhages completely, we decided to treat Rh-negative, non-Rh-immunised mothers with immunoglobulin anti-D. A dose of 250 μg anti-D was administered intramuscularly immediately after amniocentesis. The follow-up of 64 cases is shown in table 5. Forty-one mothers delivered a Rh-positive baby, twenty-three mothers a Rh-negative. Up to ten weeks after amniocentesis seventeen mothers were investigated serologically. A weak anti-D was found in six out of these seventeen mothers. The antibody was usually detectable by two-stage-papain technique only. Later in pregnancy antibody tests (papain technique and indirect antiglobulin technique) were performed in all 64 mothers, the last test being done either at delivery or a few weeks before. No anti-D was detected later than ten weeks after injection. The direct antiglobulin test in the cord blood was negative in all cases, with the exception of one case of ABO-immunisation. Thus, no case of Rh-immunisation or of fetal haemolytic disease due to Rh-antibodies was observed. Since there are no untreated controls, nothing is known about the frequency of Rh-immunisation due to amniocentesis. Therefore, it is difficult to measure the prophylactic effect of the anti-D treatment. However, a dose of 250 μg anti-D, administered to Rh-negative mothers at sixteen weeks, did not seem to be of any harm to the Rh-positive fetus. With reference to the size of fetomaternal haemorrhages in our series, a dose of 100 μg anti-D should cover even the largest observed fetomaternal haemorrhage (W.H.O. scientific group 1971).

Table 5. Genetic amniocentesis and anti-D prophylaxis in Rh-negative, non-Rh-immunised mothers.

Number of cases	64	
15th–20th week: amniocentesis + 250 μg anti-D (KABI, Stockholm) intramuscularly	41 mothers with Rh-pos babies	23 mothers with Rh-neg babies
anti-D in the maternal serum (positive/total)		
$\leqslant$ 10 weeks after amniocentesis	5/13	1/4
> 10 weeks after amniocentesis	0/41	0/23
Direct antiglobulin test in the cord blood	1 positive[1] 40 negative	23 negative

[1] Due to ABO-immunisation.

Intra-amniotic Haemorrhage

Occasionally the aspirated amniotic fluid is macroscopically bloodstained. In this series 5.5% of the samples of first amniocentesis were registered as 'bloodstained' (table 6). However, the frequency of bloodstained samples seems to a high degree to depend upon subjective factors in the observer. We therefore determined the amount of red cells in the amniotic fluid in 200 consecutive cases of first amniocentesis. As can be seen from the results (table 7) a few red cells were very often present. In thirty cases the number of red cells per μl of amniotic fluid exceeded 1000. The macroscopic classification of these thirty samples, done immediately after amniocentesis, was as follows (table 8): up to 2200 red cells per μl, the amniotic fluid was always classified as not bloodstained by macroscopic observation. If the content of red cells was 8000 or more, the macroscopic classification was always bloodstained. Between 2900 and 7700 red cells per μl of amniotic fluid there was an overlapping area with varying classification.

An important question is the nature of the blood in the amniotic fluid: is it adult or fetal blood? 94 samples (out of 200) that contained more than ten red cells per μl were investigated regarding the type of red cells (table 9). Forty-five samples contained only Hb-A cells. In the others varying concen-

Table 6. Macroscopically bloodstained amniotic fluid at the first amniocentesis, Göteborg 1970–76.

Weeks of gestation	14	15	16	17	18	19	20	>20	14–22
No. of amniocenteses	20	395	338	157	50	19	16	5	1000
No. of bloodstained amniotic fluids	0	17	23	9	4	0	1	1	55

Table 7. Amniocentesis and intra-amniotic haemorrhage. Concentration of red cells in amniotic fluid, Göteborg 1975–76.

Number of red cells per μl of amniotic fluid	Number of cases
0	18
1–10	88
11–100	30
101–1000	34
1001–10000	24
>10000	6
total	200

Table 8. Relationship between macroscopically bloodstained amniotic fluid and number of red cells per microlitre of amniotic fluid.

Case no.	Number of red cells per μl of amniotic fluid	Macroscopically bloodstained
1–9	1200–2200	no
10	2900	yes
11	3200	yes
12	3500	no
13	4000	no
14	4000	no
15	4200	yes
16	4800	no
17	5300	no
18	7000	yes
19	7700	no
20–30	8000–40000	yes

Table 9. Amniocentesis and intra-amniotic haemorrhage. Rate of adult and fetal red cells in amniotic fluid.

Number of red cells per μl of amniotic fluid	Adult cells only	% of fetal red cells			Total
		< 5	5–50	> 50	
11–100	19	4	2	5	30
101–1000	11	8	9	6	34
1001–10000	11	6	4	3	24
> 10000	4	1	1	0	6
Total	45	19	16	14	94

trations of Hb-F cells were found. From the total amount of red cells and the percentage of Hb-F cells, the concentration of Hb-F cells can be calculated. It is worth noting that the largest amount of Hb-F cells in this series never exceeded 4000 per μl of amniotic fluid. The content of AFP in the samples was always within the normal range.

The relationship between the admixture of fetal blood with the amniotic fluid and the content of AFP was investigated in a case of legal abortion in which the fetus was removed by laparotomy (table 10). The length of the fetus was 21 cm. The amniotic fluid contained 10 mg/l AFP and the fetal blood contained 1190 mg/l AFP. The number of red cells per μl of fetal blood was 3190000. Mixtures of amniotic fluid and fetal blood between 1/20 and 1/1000 were prepared. The cell count and the AFP-content were about the expected values. When the relation between fetal blood and amniotic fluid was 1/1000, the count was 4000 red cells per μl of amniotic fluid and the AFP-content was raised by ten per cent. As mentioned earlier, more than 4000

fetal red cells per μl of amniotic fluid were never found in this series. It is of course not permitted to draw too many conclusions from one single experiment, and more studies of this kind are planned.

Table 10. Experimental mixture of amniotic fluid and fetal blood. A case of legal termination: fetus 21 cm (crown–heel).

	Number of red cells per μl	AFP mg/l
fetal blood	3 190 000	1190
amniotic fluid	0	10
fetal blood/amniotic fluid		
1/20	145 000	78
1/50	53 000	33
1/100	29 700	22
1/200	14 000	15
1/1000	4 000	11

The following recommendations can be given regarding bloodstained amniotic fluid: in bloodstained samples, even if the bloodstaining is very slight, the number of red cells should be counted and the percentage of Hb-F cells should be determined. From these investigations, the content of Hb-F cells per μl of amniotic fluid can be calculated. From this figure the amount of fetal blood in the sample can be estimated within certain limits (Brock, this volume).

Limitations of Amniocentesis

Within the realms of prenatal genetic diagnosis amniocentesis is mainly carried out in order to aspirate amniotic fluid. Thus its use is limited to the study of the constituents of the fluid. Since it is impossible within the limits of this review to give a detailed list of disorders that *can* be detected by investigating amniotic fluid, it is even more impossible to mention all abnormalities that can *not* be detected in the fluid.

Samples of fetal blood are obtainable by means of fetoscopy (Valenti, this volume). In my opinion, the term amniocentesis should not include fetoscopy, since different instruments are used in the procedures. Neither should biopsies from the fetus or the placenta be called amniocentesis. These procedures are much more complicated than simple aspiration of amniotic fluid.

As far as puncture technique is concerned there are no limits, providing that one accepts repeated amniocentesis in cases of failed first attempt. Table 11 deals with the problem of unsuccessful amniocentesis in the first 1000 cases of this series. At the patient's first visit 47 punctures were unsuccessful. In fifty further cases no cytogenetic diagnosis was made due to laboratory difficulties. In four cases the AFP-concentration was around the borderline. Thus, there were indications for repeated amniocentesis in 101 cases. In eight

Table 11. Repeated amniocenteses, Göteborg 1970–76.

		total	repeated	not repeated
First amniocenteses	1000			
Failed puncture		47	45	2
Cytogenetic reason for repetition		50	44	6
Borderline α-fetoprotein		4	4	0
		101	93	8
Second amniocenteses	93			
Failed puncture		1	0	1
Cytogenetic reason for repetition		2	2	0
Borderline α-fetoprotein		1	1	0
		4	3	1
Third amniocenteses (success)	3			

cases it was not repeated for the following reasons: in five cases of failed cell culture, which occurred at the very beginning of our study, the patients were not asked to return. In a further case of failed cell culture the patient did not want another amniocentesis. The AFP determination in this case, however, was successful and the amniocentesis had been performed because of increased risk of neural tube defect. In the seventh case, which also occurred during the early time of our study, a total anterior wall placenta was detected by ultrasound and the puncture was unsuccessful. Since the gestational age was twenty weeks, the patient was not asked to return. The eighth case was an unsuccessful puncture too, and this patient did not want a second amniocentesis.

In the 93 cases of second amniocentesis a diagnosis was not possible in four patients. In three of these four cases a third amniocentesis gave the diagnosis. The fourth case was the only one in which no amniotic fluid was obtained despite repeated puncture. This patient had fibroids of the uterus and was punctured twice on different occasions without success.

After being very cautious at the beginning of our study, amniocentesis has been performed even in cases of threatened abortion. If there is a vaginal haemorrhage it may be wise to wait some days before performing amniocentesis.

As described earlier, the puncture of both sacs of twins may be limited by technical difficulties. It is perhaps possible to overcome these difficulties with adequate ultrasound equipment.

There is a time limit for amniocentesis. Prior to 91 days of gestation it is likely to fail due to the interference of the extraembryonic coelom with the

aspiration. Besides that, the amniotic cavity is relatively small and the number of viable cells in the amniotic fluid is low (Wahlström 1973).

During the later stages of gestation there may be ethical limits if the consequence of a pathological finding is legal abortion. Similar limits are given by the law of many countries. In our centre genetic amniocentesis after twenty weeks has been performed in only a few cases.

The most limiting factors for amniocentesis are lack of resources and insufficient knowledge. In Sweden, for example, the number of pregnant women 35 years of age and over is about 5000 per year. But the country has not the resources today to offer all these women a prenatal cytogenetic investigation. In the city of Göteborg, however, there are 250–300 such pregnancies a year and it is feasible to investigate them all. However, during 1976 only about a quarter of all pregnant women 35 years and older had a genetic amniocentesis.

Resistance from the patient may be a limiting factor too. The patient should never be persuaded into an amniocentesis.

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Complications of Amniocentesis

Although clinical experience indicates that amniocentesis in the first half of pregnancy is a relatively safe procedure, it is necessary to quantify any risk as accurately as possible, because this small risk has to be balanced against what may also be the relatively small chance of a positive finding. When I agreed to write this brief paper, as Chairman of the M.R.C. Committee that has organised the multi-centre controlled study of amniocentesis in this country, I planned of course to present the findings relating to amniocentesis risks. However, a decision to extend the U.K. study has delayed final analysis and the results will not be published until the end of this year. Until the M.R.C. has considered the final report, no part of the findings can be reported. In these circumstances I propose to review the relevant findings of the two comparable investigations carried out in the United States and Canada and published at the end of 1976. Both these multi-centre studies appear to indicate that amniocentesis is a highly accurate and safe procedure, when properly performed.

Study Designs

The United States Study was designed and directed at the National Institute of Child Health and Human Development (N.I.C.H.D.) by Charles U. Lowe and Duane Alexander, and the research was performed by contract with investigators in nine American centres between 1 July 1971 and 30 June 1973. The goal was 1000 subjects and 1000 controls. In fact, 1040 subjects and 992 controls were investigated, the indications for amniocentesis being either cytogenetic (91.3%) or for possible metabolic disorder (8.7%). The results were published in the Journal of the American Medical Association (N.I.C.H.D. study group 1976).

The Canadian Study followed the establishment by the Medical Research Council of Canada of a working group on the prenatal diagnosis of genetic disease. Fourteen Canadian centres took part, investigating 1020 pregnancies between early 1972 and mid 1975. A decision was made at the outset against using matched pregnancies as controls because of difficulties in obtaining suitable matching, as well as the costs of such a study. Data on the outcome of pregnancy in women of 35 years or more were obtained from vital

statistics records of the provinces of British Columbia and Manitoba, and the incidence of abortion between sixteen and nineteen weeks in the study population was compared with that in several hospital populations.

Amniocentesis was performed increasingly for possible neural tube defects as the study progressed, as well as for cytogenetic studies and for possible biochemical defects. The results were published in the Canadian Medical Association Journal (Simpson *et al.* 1976).

Results

It can be said at once that the incidence of both maternal complications and fetal loss was low in both series of subjects investigated, and compared favourably with results in control groups. In both studies, subjects tended to come more often from a professional or well-educated background than controls, and the N.I.C.H.D. subjects were older (for example 25% were over 40, compared with only 10% of controls), but corrections were made for some of these differences when the results were compared. A striking feature of both studies was that the use of ultrasound monitoring and placental localisation did not reduce fetal or maternal risks, although the Canadian study did show that ultrasound increased the chances of obtaining amniotic fluid, and of doing so on the first attempt.

Maternal Complications

The incidence of early complications was low, and although amniotic fluid leakage occurred within seven days in twelve of 1040 N.I.C.H.D. cases, for example, abortion occurred in only one case during that time. For the remainder of pregnancy (between one week after amniocentesis and delivery), the N.I.C.H.D. study showed no difference between subjects and controls with regard to toxaemia, vaginal bleeding, placenta abruptio or praevia, or infections. The American investigators noted, however, a significantly ($P < 0.01$) greater Caesarean section rate in subjects (17.9%) than in controls (12.4%), without being able to account for the difference.

The Canadian study revealed that gestational age at amniocentesis significantly influenced the maternal complication rate, the incidence being 7.3% in pregnancies tested at fifteen weeks or less, but only 2.8% at sixteen weeks or more ($P < 0.001$). The N.I.C.H.D. group reported that the incidence of vaginal bleeding was significantly increased by the number of needle insertions required for one amniocentesis, and this factor seems to be of general importance in amniocentesis complications. On the other hand, the N.I.C.H.D. study found no relation between the incidence of maternal complications and maternal age, parity, gestational age, volume of fluid removed, or size of needle.

Perhaps the most serious potential maternal complication of amniocentesis is rhesus sensitisation due to fetomaternal bleeding in a rhesus negative woman with a rhesus positive fetus. Surprisingly, neither the U.S. or Canadian study makes any reference to this hazard. An indication of the very real potential danger has been provided by Henrion (1976), who found evidence of fetomaternal haemorrhage in 38 of 273 amniocenteses cases investigated,

an incidence of 14%. This occurred despite his use of ultrasound. Henrion stated that intravenous injection of 85 μg of anti-D immunoglobulin was always effective in preventing rhesus sensitisation.

Fetal Loss or Complications

In both the U.S. and Canadian studies there was a low incidence of fetal loss, defined as abortions, intrauterine deaths and stillbirths. In the N.I.C.H.D. series the incidence of fetal loss was 3.5% in subjects and 3.2% in controls (a non-significant difference), but if the rates were adjusted to allow for the higher proportion of older mothers in the subjects, the figures were 3.3% and 3.4%. Evaluation of the neonates at birth and at discharge from hospital showed no significant difference in the incidence of abnormalities, which were found in twenty subjects and eighteen controls. No trauma from needle puncture was detected. The incidence of 'prematurity' (birthweight less than 2500 gm) was 7.7% in subjects and 6.2% in controls. Assessment during the first year of life also showed no deleterious effect on subject cases, and although death in the first year occurred in infants of seventeen subjects and eight controls, this difference was not significant. Physical assessment of the infants of subjects was satisfactory in comparison with controls and the Denver Developmental Screening Assessment showed more favourable results in infants of subjects, mainly because of the excess of infants with Down's syndrome in controls (seven) compared with subjects (three).

The Canadian study also reported very satisfactory pregnancy outcomes, and when the results in the women of 35 or more were compared with those in comparable women aged 35 or more reported from certain provincial records, comparison was reassuring. The fetal loss rate (abortions and stillbirths) was 4.7%, the neonatal death rate 0.5% in these older mothers. It was clear that in some hospitals a lower stillbirth rate was sometimes reported, but this was due, it was considered, to variations in reporting a fetal loss as a stillbirth, or an abortion, around the twentieth week of pregnancy.

Both the U.S. and Canadian investigators noted that fetal loss tended to be increased by multiple needle insertions for a single tap. The Canadian study found this to be a statistically significant effect, and although the N.I.C.H.D. study could not demonstrate statistical significance, the fetal loss rates found with one, two, and three or more needle insertions for one tap were, respectively, 2.9%, 4.3%, and 8.1%. On the other hand, both studies found relatively little effect from repeating amniocentesis, say a week later.

Both studies also found an effect of needle size on fetal loss, although the Canadian group investigated this factor at greater length. The N.I.C.H.D. study simply mentioned increased fetal loss if needle size greater than 18 gauge was used, while the Canadian group found fetal loss increased (and fewer successful amniocenteses) with needle sizes larger than 19 gauge: they suggest that the optimum needle sizes are 20 or 21 gauge.

The N.I.C.H.D. study found no effect on fetal loss rate whether or not there had been a previous fetal loss, and whether or not ultrasound was used; also there was no difference in relation to the volume of amniotic fluid re-

moved, or whether amniocentesis was performed in hospital or a physician's office. In the Canadian study, almost all the tests were performed in hospital.

Conclusions

The findings of these two large and carefully conducted studies appear to justify the reassuring conclusions which have been reached. The N.I.C.H.D. study concludes that 'Mid-trimester amniocentesis is a highly accurate and safe procedure that does not significantly increase the risk of fetal loss or injury'. The Canadian study concludes that 'Second trimester amniocentesis at about 16 weeks gestation is a safe, accurate and reliable procedure for the diagnosis of certain classes of genetic disease when it is monitored by ultrasound, performed by a trained obstetrician and carried out in a major health sciences centre'.

It is possible that the excellent results in the amniocentesis subjects might have been influenced by their somewhat better socio-economic status, and it is difficult to allow for the difference, although it was perhaps less marked in the N.I.C.H.D. series than in the Canadian study. It is also probably true that amniocentesis must usually have been performed by doctors skilled in the technique and that poorer results would be likely if the procedure was to be done by less experienced investigators, although the N.I.C.H.D. study found no difference in the results of tests done in hospitals or physicians' offices.

Neither of the studies has actually quantified the risks of amniocentesis —no deleterious effect has been demonstrated. There must be some effect, but it may be small enough that for practical purposes it can be disregarded, provided the amniocentesis is performed with every regard for its safety.

From these publications, these safety factors would seem to be as follows: gestation sixteen weeks or more; expert ultrasound monitoring; expert amniocentesis technique; needle gauge 20 or 21; not more than two needle insertions for one tap; anti-D immunoglobulin for rhesus negative women.

Even with these safety factors, another complication of amniocentesis is erroneous diagnosis, but this is discussed on pages 148–9, 158–9, and 171–2. However, both the N.I.C.H.D. and Canadian studies show accuracy rates of 99.4% and 99.3% indicating that this is a small, although still reducible, hazard.

On all these counts, therefore, it seems reasonable to conclude that amniocentesis, properly performed, has a very low complications rate, and that its use could be extended in the management of pregnancies at increased risk of a detectable fetal abnormality.

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Current Problems of Amniotic Fluid Cell Culture

Amniotic fluid cells have been grown in culture and their chromosomes studied for at least ten years (Steele and Breg 1966), while the use of amniotic fluid cells for the antenatal diagnosis of inborn errors of metabolism dates from 1968 (Nadler). At present cultured amniotic fluid cells are generally used for the antenatal diagnosis of chromosome abnormalities, usually in women over thirty-five years of age. However, their use in biochemical disorders continues to increase as it becomes possible to diagnose more enzyme deficiencies.

Experience gained over the last ten years has helped to improve culture techniques, and the aim of every laboratory is to achieve a success rate approaching 100% after a minimum length of time in culture. A number of factors may affect this success. These include the condition and size of the amniotic fluid sample, its transport to the laboratory and the method of culture used.

Amniotic Fluid Sample

Volume and Cell Content

The volume, cell content and bloodstaining of amniotic fluid samples relate to the gestation at which amniocentesis is performed. The total volume of amniotic fluid and the number of viable cells per millilitre of fluid (Nelson 1973) increases after twelve or thirteen weeks' gestation and an adequate number of viable cells must be contained in the sample if it is to be successfully cultured. Amniocentesis is usually performed between thirteen and twenty-five weeks' gestation. However, fluids taken before about fifteen weeks tend to be rather acellular; therefore cultures grown from these samples have few patches of cells and, although complete failure of growth between thirteen and fifteen weeks is unlikely, the chances of producing sufficient growth quickly are reduced. Many more cells are required for the detection of inborn errors of metabolism than for the investigation of chromosome abnormalities, so that it is even more important that these samples contain plenty of viable cells.

The volume of fluid taken should be at least 10 ml, and, for biochemical

studies, 20 ml or more is advisable. This allows duplicate cultures to be initiated. Whereas chromosome analysis has been achieved from the cells contained in 1 ml of fluid, cultures containing cells from less than 5 ml of fluid have a decreasing likelihood of producing satisfactory growth. Samples of suitable size and cellularity are less likely to be obtained when amniocentesis is performed before fifteen weeks' gestation. Therefore, the optimum time for amniocentesis, when cell culture is required for cytogenetic or biochemical investigation, is around sixteen weeks; some laboratories will not accept samples taken before sixteen or seventeen weeks.

Contamination with Blood

Slight contamination of amniotic fluid samples with blood is not very detrimental providing amniotic fluid, containing cells, is also present. However, the most common cause of failure of cell growth is bloodstaining sufficient to cause blood clots to form in the sample. Table 1 shows cell growth failures in our laboratory in 1976, half of which are attributable to gross bloodstaining. If a sample contains blood it should not be centrifuged before being sent to the laboratory as this also removes the amniotic fluid cells (table 1).

Table 1. Failures in culture (402 specimens). Two failures possibly attributable to laboratory technique (0.5%).

Description of specimen	No. of specimens	Repeated	Successful
Urine	2	1	1
Previously centrifuged	1	1	1
Very bloodstained	5	2	2
Clear	2	2	1
Total	10	6	5

Maternal Urine

Maternal urine is occasionally obtained at amniocentesis and, although it rarely grows in culture, it still remains a possible source of error and failure (table 1). Samples can be tested for the presence of urea and potassium (Guibaud *et al.* 1976) but urine samples are usually noticed by their lack of alpha-fetoprotein and by their odour and appearance.

Transport of Samples

Samples should be put in a clean, sterile, plastic or glass universal or similar container and sent with full details, including date of last menstrual period and reason for amniocentesis. Under *no* circumstances should a specimen, even if bloodstained, be centrifuged; it is vital that the specimen is sent to the laboratory without delay.

Except in exceptional circumstances, and then only if the sample is very large, these samples should not be divided between laboratories as this increases the chance that neither laboratory will achieve satisfactory cell growth.

Ideally, samples should arrive at the laboratory within 24 hours of amniocentesis. However, provided they are not frozen or subjected to excessive heat, they can be sent by post, rail or air, but they should not be sent such that they might spend a weekend in transit. In table 1, one of the clear samples that failed to grow in culture had been several days in transit, but cells from a repeat sample, sent more rapidly, grew satisfactorily.

An overall success rate of 97% was achieved, but nine out of the ten failures shown could perhaps have been prevented if suitable samples had reached the laboratory quickly. Therefore, including repeats, there were two failures from 402 samples that were possibly attributable to laboratory technique (0.5%). Both specimens were from the same patient, and a normal child was subsequently delivered.

Amniotic Fluid Cell Culture

Samples are centrifuged at 1000 rpm for five minutes and the supernatant is removed for the determination of alpha-fetoprotein. The cell pellet is resuspended in Hams F10 medium (12 ml) containing 25% fetal bovine serum and crystamycin, and divided between two plastic petri dishes (5 cm diameter) each containing seven 6×22 mm coverslips. The dishes are each placed in separate anaerobic jars, gassed with 5% CO_2 in air to maintain the correct pH and then placed in separate incubators. After 18–24 hours, cultures are examined for the presence of macrophages, many of which settle in cases of open neural tube defect (Sutherland, Brock and Scrimgeour 1973). Cultures are then left to incubate for a further four or five days and thereafter examined three times weekly, when half the medium is changed.

Culture Problems

In all tissue culture pH and temperature are very important, particularly during the first few days, so for this reason duplicate cultures are independently incubated to reduce risk of failure. This also reduces risk of failure due to infection with fungi or bacteria.

The fetal bovine serum used to supplement the medium is of variable quality, and while most sera obtained from reputable firms will allow cell growth they are not equally suitable for use in initiating amniotic fluid cell cultures. Batch samples of serum must therefore be pre-tested for their suitability for this purpose. Even then variation is experienced. For example, the average time taken to obtain sufficient cells for chromosome analysis using one batch of fetal calf serum was 11.8 days (measured over six months) while the time taken using the subsequent batch was 14.3 days (measured over two months). However, the additional 2.5 days may not seem too excessive when compared with the time it may take between discovery of a chromosome abnormality and subsequent abortion. It must be remembered that these are average times, and that there is a wide spread for individual samples.

After seven to ten days it is usually possible to say whether a culture will be successful and, provided that there is close collaboration between labora-

tory and clinician, it should then be possible to warn the obstetrician that a repeat will be necessary.

Harvesting Cells for Chromosome Analysis

Amniotic fluid cells settle and attach individually and each divides to form a patch of cells. Each patch is therefore a clone derived from a single cell (Fujimoto *et al.* 1968). To obtain metaphases for chromosome analysis, coverslips are removed when they show small patches of dividing cells, incubated for four to six hours in fresh medium containing colchicine, treated with equal volumes KCl (~.075M) and Hanks basic salt solution (1 g/l) as hypotonic solution and fixed with methanol and acetic acid (3:1). The cells are therefore harvested *in situ* and metaphases examined still attached to the coverslips in clones.

Problems in Interpreting Abnormal Karyotypes

Problems arise when the expected fetal phenotype cannot immediately be predicted from its karyotype. This may occur in such cases as: (a) unexpected, apparently balanced rearrangements; (b) mosaics; or (c) unidentifiable chromosome material. In these cases it is usual to examine the chromosomes of both parents; this can be done within two days of blood being collected.

Rearrangements. If a translocation or inversion seen in amniotic fluid cells is subsequently found in one of the parents and appears identical, then it can be assumed to have no phenotypic effect. However, a new translocation is sometimes detected; some caution is then required, since an abnormally high proportion of apparently balanced translocations has been found in the mentally subnormal (Jacobs 1974).

Mosaics. Chromosome mosaicism in amniotic fluid cells may reflect the fetal karyotype but it may also arise in culture. Since each patch of cells is a clone, the karyotype of cells in the same patch is the same and, in chromo-

Table 2. Chromosomal mosaicism in specimen no. 74/85 (Sutherland, Bowser-Riley & Bain 1975).

	Karyotype			
	46,XY		47,XY + metacentric	
Culture	colonies	cells	colonies	cells
Amniotic fluid:				
27 weeks: coverslips	1		1	
29 weeks: coverslips	1		2	
34 weeks: coverslips	9		8	
Tissues (at birth):				
blood		100		0
skin		9		21
cord		10		20
amnion		26		4

somal mosaics, patches rather than cells are scored according to their karyotype. There are two ways in which mosaicism in cultures may be shown to reflect true fetal mosaicism.

If amniocentesis is repeated and cultured amniotic fluid cells with different karyotypes are again found, the mosaicism can be assumed to be real. Table 2 shows a case in which amniocentesis and cell culture was performed three times; each time two chromosomally different cell types were seen, and the mosaicism was subsequently confirmed in the child (Sutherland, Bowser-Riley and Bain 1975).

If two or more different cultures are established independently from the same initial sample and each culture shows mosaicism, this can be considered to reflect chromosomal mosaicism in the fetus (table 3). This is another very important reason for establishing duplicate cultures. Unfortunately, having established that mosaicism is real rather than an artifact may not solve the problem, because the abnormal karyotype often includes an extra chromosome that cannot be identified.

Table 3. Chromosomal mosaicism in specimen no. 76/1030.

		No. of colonies scored	
	Culture	46,XX	47,XX + ring
1st harvest	Mixed	16	6
2nd harvest	I	4	1
	II	16	4
	III	1	1
Total		37	12

Chromosomes of unknown origin. Small extra chromosomes of unknown origin are sometimes inherited, so parental chromosome analysis may help to establish the likely phenotype (Watson and Scrimgeour 1977). Other extra chromosomes are not inherited, such as that referred to in table 2. This chromosome is metacentric and seen in skin cord and amnion as well as amniotic fluid cells, but not in blood. The child appears to be normal.

Shown in figure 1 is the abnormal extra chromosome from the case referred to in table 3. It appears to be a small, satellited ring, as shown by its double appearance in some cells. Some of it stains darkly with C-banding, indicating the presence of centromeric heterochromatin. This, therefore, is a case in which the mosaicism appears real but the extra chromosome cannot be identified or its significance predicted. Parental blood was not made available and the phenotype has yet to be established.

However, some of these small marker chromosomes do have a phenotypic effect (Speed, Johnston and Evans 1976), including a ring very similar to that in figure 1. In such cases of unidentified chromosomes, parental karyo-

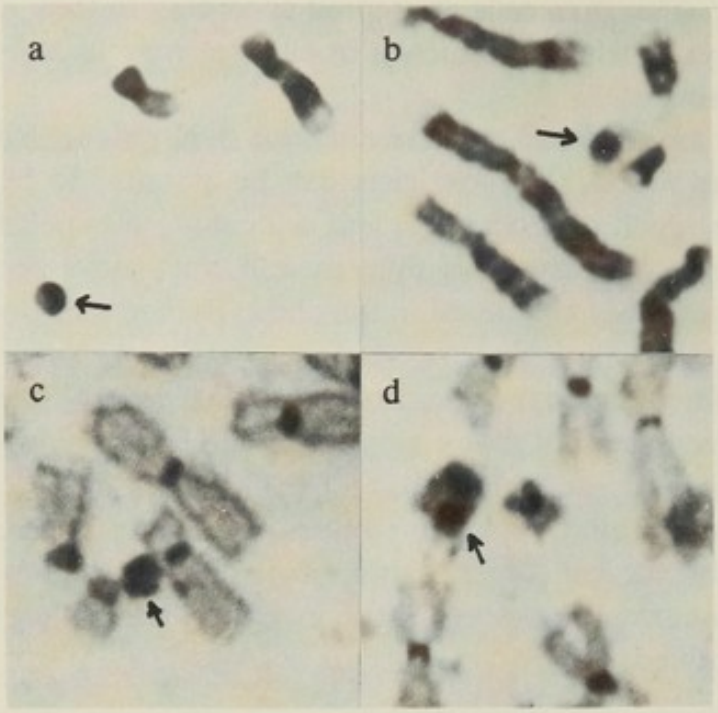

Figure 1. Supernumerary ring chromosomes: a & b) G-banding, showing single and double rings respectively; c & d) C-banding, showing single and double rings respectively.

types and fetal phenotypes must be ascertained so that more information can be accumulated to allow more accurate prediction of the phenotypic outcome.

Figure 2 shows a chromosome 15 with extra material. It is dicentric and has satellites between the centromeres. Most of the extra material is centromeric heterochromatic, as shown by its dark staining with C-banding, but without further information the phenotype is in doubt. Blood from parents was requested but was not immediately obtained, and when consulted the parents requested termination before chromosome analysis could be performed. The mother was subsequently found to have the same abnormal chromosome. This emphasises the need for urgency in obtaining, processing and reporting on specimens. Some laboratories ask for parental blood to be sent with all amniotic fluid samples, but this would put an intolerable load on most laboratories. However, it may be advisable in cases where it may be difficult to recall the patient quickly.

In cases of recurrent first trimester abortion where a parental chromosome abnormality is suspected, parental chromosomes should be examined before amniocentesis and preferably before the patient again becomes pregnant.

Maternal Cell Contamination

Cells of maternal origin occasionally grow in amniotic fluid cell cultures, but the risk of error can be reduced by ensuring that several patches of cells are analysed and checking that they are all the same sex.

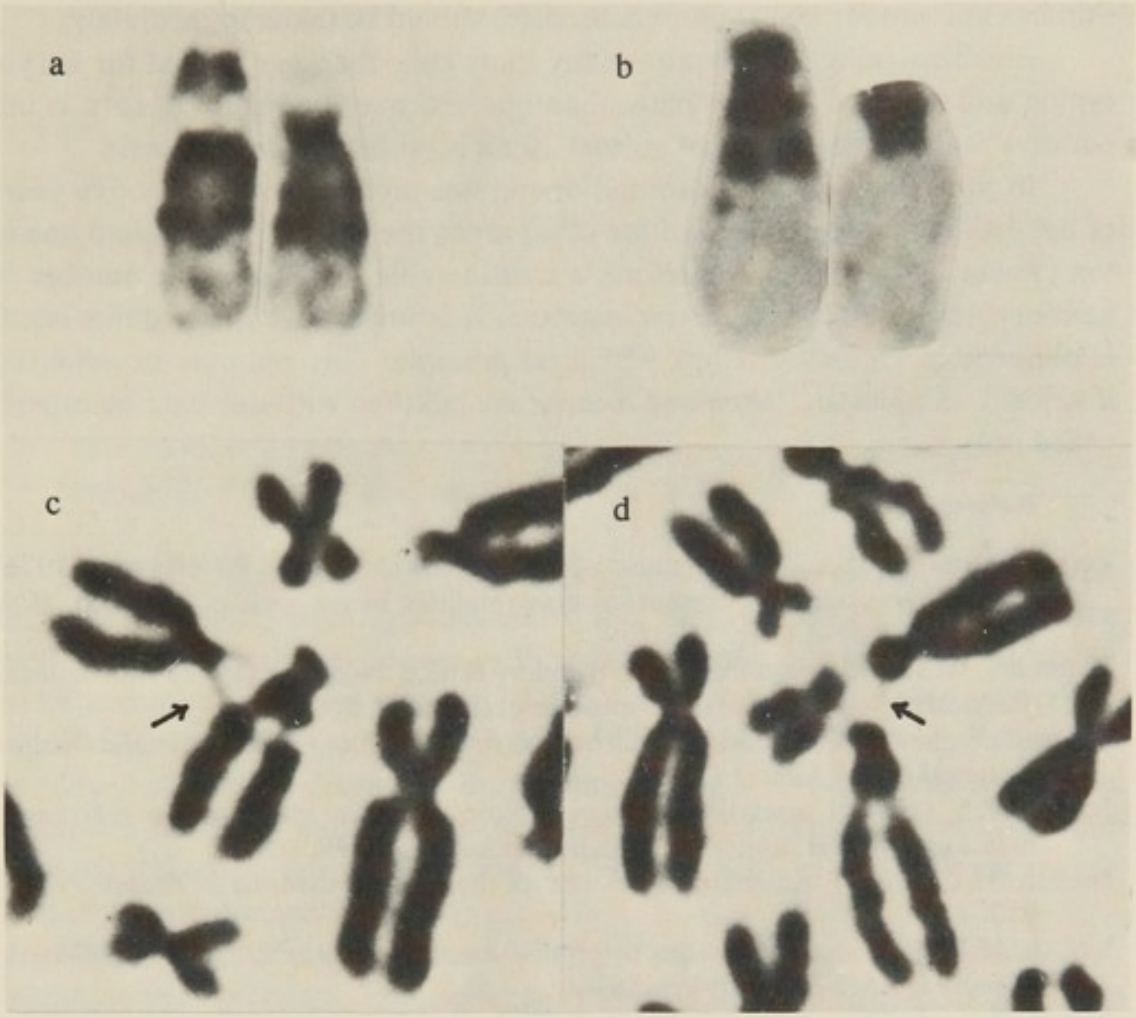

Figure 2. Abnormal chromosomes 15: a) G-banding of chromosomes 15; b) C-banding of abnormal 15 with another D chromosome: c & d) satellite associations at interstitial and terminal regions (Watson & Scrimgeour 1977).

Cultures for Diagnosis of Biochemical Disorders

Many inherited inborn errors of metabolism that can be diagnosed antenatally are initially diagnosed on blood leucocytes, and it is at this time that preparations should be made for diagnosis during future pregnancies.

When cultured amniotic fluid cells are assayed, skin fibroblasts from the previous affected child and from the parents should also be assayed. Therefore, when a child is found to have an inborn error and there is any possibility of further pregnancy, skin should be taken from the affected child and the parents, before the mother becomes pregnant. The cultured skin fibroblasts are assayed to confirm that antenatal diagnosis is possible and the cells are stored in liquid nitrogen until required. When the patient becomes pregnant, the laboratory that has stored the cells (and that which is to perform the enzyme assay, if different) must be notified as soon as possible and told when amniocentesis is planned. It usually takes longer to obtain sufficient cells from a skin biopsy than from an amniotic fluid sample, so it is not possible to take skin samples at the time of amniocentesis. If a woman becomes pregnant and

skin has not already been cultured, biopsies should be taken immediately.

Biochemical assays require many more cells than are needed for karyotyping and repeat assays or more than one test may be required, so it is imperative that a clean sample of at least 20 ml is taken at amniocentesis.

In the Edinburgh area, around one in five mothers over thirty-five years of age have amniocentesis, and for other areas the figure is lower than one in ten (Polani *et al.* 1976). Therefore, a considerable increase in the number of amniocenteses performed can be expected. It is imperative that a quick result is obtained at the first attempt, whenever possible. This can only be achieved if suitable samples are taken and if these are cultured with due care by experienced people.

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Biochemical Studies on Amniotic Fluid and its Cells

Although individual genetic metabolic diseases are relatively rare, with incidences ranging from approximately one in 1500 to one in 100000 live births, their collective incidence in European populations has been estimated to be about one per cent (Carter 1970). Some of these disorders appear benign while, for others, effective methods of management and therapy can be employed after early diagnosis. But in many cases severe handicap will be the inevitable outcome, and it is for these disorders that genetic counselling and prenatal diagnosis provide new opportunities to achieve a significant reduction of genetic disease.

At present, the exact nature of the primary biochemical abnormality, including in most cases the specific enzyme deficiency, is known for approximately 130 genetic diseases, and for about sixty of these the abnormality has been demonstrated in cultured skin fibroblasts from patients. Similar diagnostic tests can also be applied to cultured amniotic fluid cells, since their basic complement of enzymes is, for the most part, qualitatively similar to that of skin fibroblasts, and to date these tests have been successfully used for the prenatal diagnosis of thirty metabolic diseases. These diseases are listed in table 1. More than half are lysosomal disorders, reflecting the rapid elucidation of this group of severe diseases in the last ten years by many laboratories whose research interests were entirely devoted to these aspects of metabolism. With few exceptions, no other metabolic diseases have received such concentrated attention and the view has often been expressed that this has led to over-optimism about the future expansion of biochemical prenatal diagnosis. This is probably true, but the lysosomal diseases did provide the important cornerstone of knowledge and technical skill from which prenatal diagnosis could be confidently initiated as part of a genetic advisory service. This experience has served to establish and promote improvements in technical and organisational methods, and has identified some problem areas requiring a greater awareness and much further research.

This paper summarises some of these aspects, and is based on experience of monitoring a total of sixty-six pregnancies for twelve metabolic disorders at the Institute of Child Health, London.

Table 1. Diseases for which biochemical prenatal diagnoses have been made. (Figures in parentheses refer to numbers of pregnancies monitored solely at the Institute of Child Health, London.)

Lysosomal diseases	*Other diseases*
Cystinosis (6)	Argininosuccinic aciduria
Fabry	Citrullinaemia
Fucosidosis	Galactokinase deficiency
Gaucher	Galactosaemia
Glycogenosis, type II (1)	Homocystinuria
GM_1-gangliosidosis (2)	Hypophosphatasia
Hunter	Lesch-Nyhan
Hurler (2)	Maple syrup urine (3)
I-cell	Methylmalonic aciduria
Krabbe (9)	Propionic acidaemia
Maroteaux-Lamy	Severe combined immunodeficiency
Metachromatic leucodystrophy (3)	Xeroderma pigmentosum
Niemann-Pick (1)	
Sandhoff (5)	
Sanfilippo A (4)	
Sanfilippo B	
Tay-Sachs (29)	
Wolman (1)	

Identification of the Disease

An obvious, yet often not fully appreciated, requirement for prenatal diagnosis of a metabolic disease is a precise knowledge of the specific enzyme deficiency or metabolic abnormality for which the fetus will be at risk. A presumptive diagnosis of an index case based on clinical and histopathological findings is not acceptable, since different enzyme defects may result in remarkably similar manifestations, particularly in early infancy. Every effort should therefore be made to establish an early, precise biochemical diagnosis; only then can the family receive accurate genetic counselling.

Most of the diseases currently amenable to prenatal diagnosis can be detected reliably by tests on the patient's blood, usually the freshly separated leucocytes, but tissues obtained at biopsy and autopsy are also important, particularly for possible retrospective biochemical diagnosis. To avoid loss of these valuable specimens through unsuitable methods of handling, it is essential that they should be treated strictly according to biochemical requirements, usually by rapid freezing and storage below $-30°C$. Increasing use is also being made of skin fibroblast cultures in the study of index cases and their parents. These provide an on-going source of fresh tissue for characterisation of the specific expression of enzymic deficiency in a given family, and can be stored in a frozen cell-bank until required for control purposes when monitoring a subsequent pregnancy.

Diagnosis may be difficult, however, where the index patient has died and no specimens have been preserved for biochemical analysis. Tests for the

heterozygosity of the parents are then the only means of confirmation, and can be accepted as a reliable indication where the biochemical assay has been shown to differentiate accurately between normal and heterozygous states. With the notable exception of Tay-Sachs' disease, serum has found little application to carrier detection, but for many disorders this can be achieved satisfactorily using separated mixed leucocytes for enzymic assay. For certain enzymes where there is overlap of normal and heterozygous ranges, it appears that these might be better differentiated using a more homogeneous leucocyte population. For example, levels of β-glucosidase and aspartylglycosylamine amidohydrolase activity of lymphocytes separated in Ficoll were reported to differentiate carriers for Gaucher's disease (Beutler and Kuhl 1970) and aspartylglucosaminuria (Aula, Raivio and Autio 1976), respectively, while for type II glycogenosis, heterozygous levels of α-glucosidase activity were found in lymphocytes after stimulation with phytohaemagglutinin (Hirschhorn *et al.* 1969).

In general, owing to the wide fluctuations found in cultured cells, the use of cultured skin fibroblasts cannot be relied on to distinguish between normal and heterozygous levels of enzymic activity. Nevertheless, skin fibroblast cultures from obligate heterozygotes should be established whenever possible, for use as control specimens in helping to differentiate homozygous recessive from heterozygous levels of enzymic activity in cultured amniotic fluid cells from a subsequent pregnancy.

Other specimens employed in attempts at carrier detection, particularly for the lysosomal diseases, have included hair follicles and tears. In our experience, at least for the detection of Tay-Sachs' carriers, assays on tears provided reasonable discrimination, whereas selected hair follicles showed wide variation in enzymic activity.

Genetic Heterogeneity

The elucidation of the primary enzyme deficiency in a particular metabolic disease, and the wider application of the appropriate test, have usually led to the discovery of genetic variants of the disorder. It would be surprising, in fact, if variant forms of genetic diseases were not found, since the number of possible gene mutations is so great. Many are known to occur in human populations, e.g. the numerous haemoglobin variants. The clinical expression of such genetic heterogeneity may be extremely diverse, and, in the main, will be governed by the different effects the individual mutations have on the physicochemical and biological properties of a given enzyme. The structural change in the protein may lead to its catalytic activity being lost entirely or modified to varying degrees with respect to kinetic properties or substrate specificity. An additional complicating factor is that the polypeptide chains of an enzyme, coded at one or more gene loci, may associate or be further modified in a variety of ways to give isoenzyme patterns that show complex changes due to gene mutation. In view of these possibilities of genetic variation, it is essential that the enzymic phenotype of an index case should be fully charac-

terised, to provide accurate information for genetic counselling and the evaluation of results of prenatal tests.

The variant forms of hexosaminidase deficiency illustrate this requirement. Profound deficiency of hexosaminidase A in classical Tay-Sachs' disease, and of hexosaminidases A and B in the clinically identical Sandhoff's disease, should present no problem in enzymic diagnosis by appropriate methods, but later onset forms of GM_2-gangliosidosis have been reported in which hexosaminidase A deficiency may be either similar to that found in Tay-Sachs' disease or only partial, with residual activity towards artificial substrate falling in the heterozygous range (Brett *et al.* 1973). Only for the former phenotype would prenatal tests reliably detect an affected fetus. A further variant had the typical clinical features of Tay-Sachs' disease but was found to have normal activities of hexosaminidases A and B with artificial substrates; a deficiency was observed only when the natural substrate GM_2-ganglioside was used for enzymic assay (Sandhoff *et al.* 1971). Finally, cases of so-called 'pseudodeficiency' have been described, in which a healthy parent of a child with Tay-Sachs' disease (Navon, Padeh and Adam 1973; Vidgoff, Buist and O'Brien 1973) or Sandhoff's disease (Dreyfus, Poenaru and Svennerholm 1975) was found to have hexosaminidase deficiency similar to that of the affected offspring when tested against artificial substrate, but possessed heterozygous activity towards the natural lipid substrates.

These examples give an idea of the heterogeneity that may be found in the phenotypic expression of enzymic activity and of the consequent, though rare, problems this presents in diagnosis, particularly as applied to prenatal tests. For hexosaminidase and several other lysosomal enzymes these problems can only be solved by the development of sensitive assay methods using natural substrates. For other enzyme variants, methods of kinetic analysis will be required to characterise differences in, for example, substrate affinity and cofactor binding. In all these areas there is a need for further research.

Variability of Enzymic Activity in Cultured Cells

The levels of enzymic activities in cultured cells are markedly affected by culture conditions (Ryan, Lee and Nadler, 1972; Butterworth *et al.* 1974), and even when the conditions are strictly controlled they may show wide variations, which reflect the particular state of growth at the time the cells are harvested for assay (Heukels-Dully and Niermeijer 1976). It is therefore essential that laboratories attempting prenatal diagnosis should have experience of this variability and of the control measures taken to minimise the attendant risk of mistaken diagnosis, particularly where there might be difficulty in deciding whether a low activity represents a heterozygous level or residual activity in an affected homozygote. This difficulty can be reduced if the enzyme assay is performed whenever possible on two different subcultures, with appropriate growth-matched controls, and if the activity of the deficient enzyme is expressed relative to that of a second unaffected enzyme (Young *et al.* 1975). Also, in many cases the volume of amniotic fluid ob-

tained at amniocentesis is sufficient for cell cultures to be established in two different laboratories, thereby providing not only a safeguard against total loss of the specimen, but also an increased chance of obtaining unequivocal results from the duplicate enzyme assays.

Where early cultures of amniotic fluid cells, particularly primary cultures, are used for enzymic assay, the problem of low activity may be further complicated by variability due to the presence of different cell types with quantitative and qualitative differences in their enzyme content (Gerbie *et al.* 1972, Hoehn *et al.* 1974). The cellular morphology of early cultures should be characterised so that appropriate control data can be applied to their biochemical evaluation.

Biochemical Techniques

The basic methods of biochemical diagnosis applied to amniotic fluid cells are those devised for testing leucocytes and cultured skin fibroblasts and, conventionally, may require several million cells and necessitate culture periods of up to eight weeks. This delay is clearly undesirable for several reasons, and has been considerably reduced by the further adaptation of assay techniques to a micro-scale, particularly for some of the lysosomal diseases. Thus, the few thousand cells obtained after two to three weeks' culture were tested successfully in assay volumes up to 5 μl for the prenatal diagnosis of Pompe's disease (Niermeijer *et al.* 1975), metachromatic leukodystrophy and GM_2-gangliosidosis (Neiermeijer *et al.* 1976), GM_1-gangliosidosis (Kleijer, van der Veer and Niermeijer 1976) and mucopolysaccharidosis I, IIIB and VI (Kleijer, Sachs and Niermeijer 1975; Kleijer *et al.* 1976).

These methods have been scaled down even further to make use of 10–100 amniotic fluid cells obtained after one to two weeks in primary culture. The cells are grown on thin plastic film from which the initiating colonies can be dissected after freeze-drying, and incubated in an assay volume of 1 μl or less. The enzyme activity is measured with a microfluorimeter and related to a single cell. In this manner, pregnancies at risk for Pompe's disease (Niermeijer *et al.* 1975), Fabry disease (Galjaard *et al.* 1974) and GM_1-gangliosidosis (Kleijer, van der Veer and Niermeijer 1976) have been monitored, and the method appears capable of further general application. For example, it should be possible to measure in this way many of the enzyme reactions that can be linked to a nicotinamide adenine dinucleotide (NAD) system.

However, except for such applications as the radio-autographic detection of Lesch-Nyhan syndrome (Halley and Heukels-Dully 1977) and xeroderma pigmentosum (Ramsay *et al.* 1974), and in ^{35}S-cystine-uptake studies for cystinosis (Schneider *et al.* 1974, Willcox and Patrick 1974), micromethods are not generally suited to the use of radioactive substrates, nor to the detection of those neurolipidoses requiring natural substrates. For these and other tests it is still necessary to culture a relatively large number of cells over a period of several weeks. Nevertheless, during this period it might be possible in some cases to use the cell culture itself as an informative bio-

chemical experiment. The spent culture medium obtained at the various stages of feeding before harvest could be analysed for relevant metabolites by a variety of sensitive techniques, including the use of radiochemicals with high specific activity, and might provide valuable early evidence of abnormality. It might also be possible to accentuate an abnormality by substrate or hormonal induction of enzymes that normally develop full activity only in the later stages of gestation. The timing and cell requirements for the methods of biochemical diagnosis outlined above are summarised diagrammatically in figure 1.

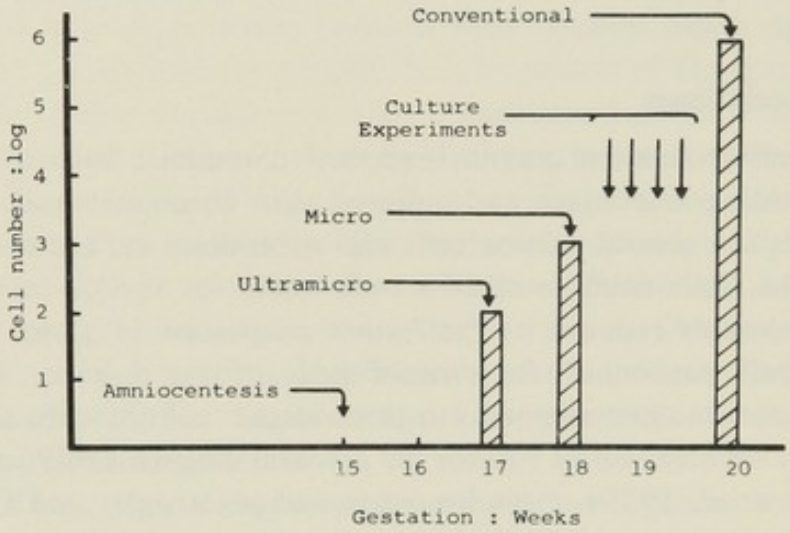

Figure 1. Biochemical analysis of cultured amniotic fluid cells: approximate culture periods and cell numbers appropriate to the different experimental scales employed.

Of course, the problem of delay inherent in all methods requiring cultured cells would be avoided if amniotic fluid and its uncultured cells could be used immediately in reliable tests. One might expect, however, that any index of direct metabolic assessment, including enzymic activity, would show wide variations since amniotic fluid is a complex, rapidly-changing mixture in which the majority of cells are in various stages of decay. In spite of these limitations, which must impose caution in the interpretation of results, the analysis of cell-free amniotic fluid and uncultured cells may provide valuable information and should be investigated further, at least as a source of corroborative data. While the absence of a particular enzymic activity would not be accepted as positive proof of a deficiency, its presence, even at very low levels, may reliably indicate an unaffected fetus, provided of course that the enzyme tested is unmistakably that related specifically to the disease being monitored. This has been our experience in testing twenty-nine pregnancies at risk for Tay-Sachs' disease. Results of hexosaminidase A analysis by automated diethylaminoethyl-cellulose chromatography of amniotic fluid and extracts of uncultured cells have in every case agreed with the subsequent findings on cultured cells.

Analysis of amniotic fluid may also give an early indication of fetal

abnormality when increased concentrations of metabolites are detected, as reported for methylmalonic aciduria (Morrow *et al.* 1970) and argininosuccinic aciduria (Goodman *et al.* 1973). The reliability of chemical analysis of glycosaminoglycans in amniotic fluid for the prenatal diagnosis of mucopolysaccharidosis has been questioned (Brock *et al.* 1971; Matalon, Dorfman and Nadler 1972), and it is doubtful whether the methods employed were sufficiently sensitive to detect abnormalities at fourteen to sixteen weeks' gestation. It is essential to have a reliable means for the discrete separation of heparan-, dermatan- and chondroitin sulphates, and of hyaluronic acid, the major glycosaminoglycan constituent in early pregnancy. This can be achieved using micro-analytical techniques (Whiteman 1973, Henderson and Whiteman 1976) that involve the isolation of glycosaminoglycans as Alcian blue-complexes, their separation by two-dimensional electrophoresis and individual colorimetric estimation after staining with Alcian blue. Present experience at this Institute suggests that this is a valuable ancillary method, supplementing enzyme and $^{35}SO_4$-uptake studies, and capable of giving an accurate diagnosis at fourteen to sixteen weeks' gestation. Examples of its application are shown in figure 2.

Organisation

There is a tendency to consider the provision of facilities for the prenatal diagnosis of metabolic diseases as applying only to the actual investigation of pregnancies at risk. But clearly, the major part of the biochemical work involved is not concerned with the relatively few pregnancies monitored, but with the identification of the specific genetic defect in index patients and carriers. For example, in this laboratory, the number of pregnancies tested for a neurolipidosis is currently about fifteen per year, but the annual number of patients investigated for these conditions averages 250. This requires the full-time commitment of experienced scientific staff, with access to the comprehensive facilities of an established paediatric biochemistry department having a main research interest in metabolic diseases. Close cooperation with hospitals and genetics centres on a supra-regional basis is essential and has been established successfully by a number of laboratories. What is now required is the incorporation and secure funding of these improvised arrangements into a coordinated scheme providing national cover within a comprehensive genetics service.

In particular, these issues should be resolved in anticipation of the need to provide a genetic service dealing with cystic fibrosis, the commonest inherited disorder in our population with a carrier frequency of approximately one in twenty. New lines of research have been developed in the past few years, which hold the promise of final elucidation of this disease and the ability to detect carriers. In this event, the provision of prenatal diagnostic facilities would need to be broadened to include not only the monitoring of pregnancies in previously affected families but also a scheme for voluntary mass-screening of carriers.

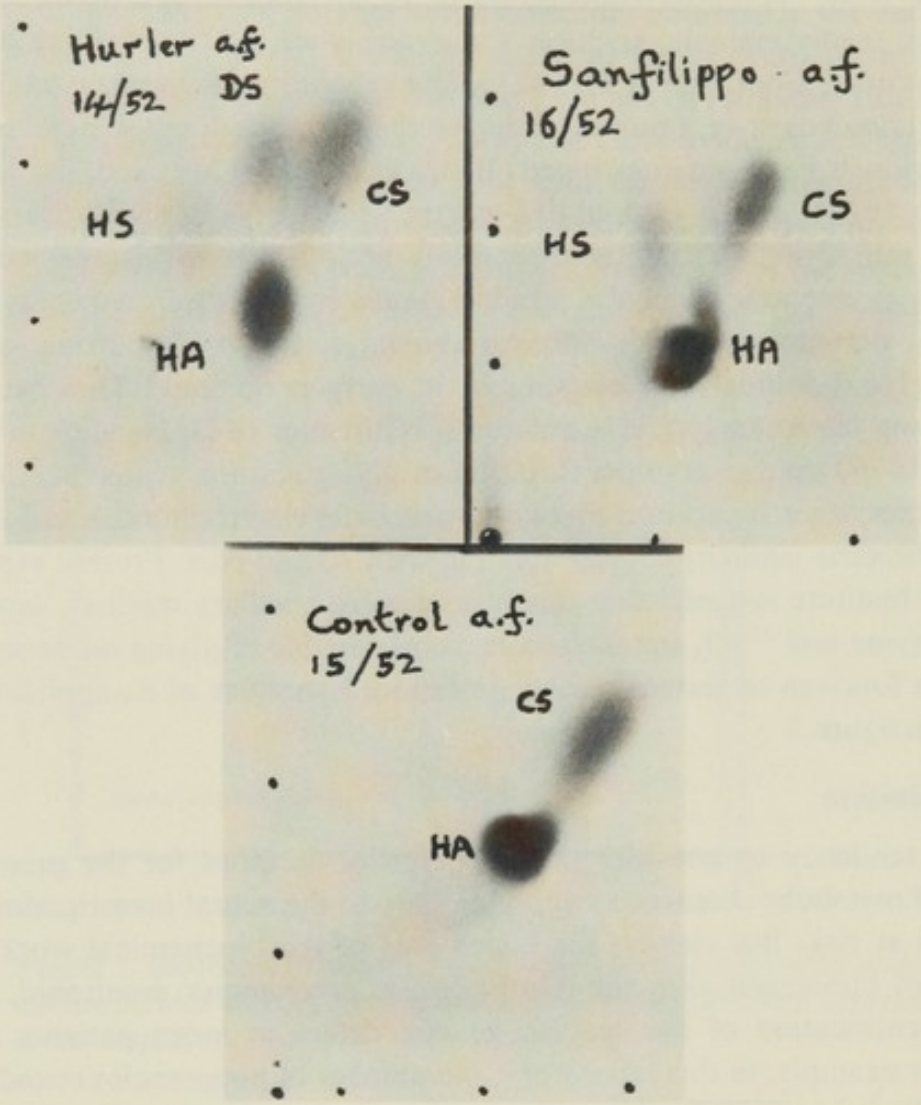

Figure 2. Two-dimensional electrophoresis of glycosaminoglycans isolated from Hurler (14 weeks), Sanfilippo (15 weeks) and normal (15 weeks) amniotic fluid. D S = dermatan sulphate; H S = heparan sulphate; C S = chondroitin sulphate; H A = hyaluronic acid (Courtesy of Dr P. Whiteman).

However, genetic metabolic disorders are generally too rare to require multiple centres for their diagnosis, and so some thought should also be given to the need for a policy of centralisation of particular tests at one or two laboratories, instead of the unsatisfactory replication that now exists. Only in this way can the necessary experience be acquired of such factors as the genetic heterogeneity of a disease, and the variation in enzymic activity of cultured cells, on which the precise indication for amniocentesis and the reliable interpretation of biochemical results depend.

A further aspect of organisation for prenatal diagnosis is that of communication between the various specialists involved. There is usually close cooperation between paediatrician and biochemist in the diagnosis of an index case, and parents should then receive prompt genetic counselling. But we find that there may be some breakdown of communication when a pregnancy eventually occurs, and this can result in failure or delay in taking the appropriate course of action. It seems to us that this difficulty might be mini-

mised if, in addition to the usual channels of transmitting records of family history, the mother was given a fully explanatory letter by the genetic counsellor. *This would be passed to the obstetrician attending the pregnancy*, and would stress the need to contact a named biochemical laboratory *well before the amniocentesis.*

Finally, there is the important need to maintain the obstetric and biochemical link in the event of selective abortion following a prediction of fetal abnormality. The method of termination will be decided by medical considerations, but the obstetrician should bear in mind the need for confirmation of the biochemical abnormality in the tissues of the aborted fetus; this provides an essential check on the reliability of the methods used in prenatal diagnosis. Provision of normal fetal tissues for comparison may present some difficulty, to which a possible solution might be the use of banked cultures of fetal tissue cells.

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THE MANAGEMENT

Mid-Trimester Termination of Pregnancy

The techniques of prenatal diagnosis can detect many congenital abnormalities in the mid-trimester. Most methods involve investigation of the amniotic fluid, which is obtained by transabdominal amniocentesis from the sixteenth week of gestation onwards. If investigations are of a biochemical nature results may be available in a few days, whereas if chromosome culture is involved they are generally not available for two to three weeks. By the time a prenatal diagnosis is made the pregnancy will have advanced to weeks 17–20 of gestation, and consequently any terminations will be performed at this late stage.

At the Western General Hospital, Edinburgh, over the three-year period 1974–76, twenty-one pregnancies were terminated following a prenatal diagnosis of a congenital abnormality. The prenatal diagnoses are shown in table 1. Twenty of the twenty-one terminations were carried out during weeks 17–21 of gestation; the remaining pregnancy was terminated during the fourteenth week. If the prenatal diagnosis was based on a raised liquor alpha-fetoprotein level, termination was carried out one to two weeks after the amniocentesis. whereas if chromosome culture was required then termination was carried out three to four weeks after amniocentesis. Nineteen of the pregnancies were terminated by intra-amniotic prostaglandin and the remaining two by hysterotomy.

Table 1. Mid-trimester terminations for genetic reasons. Western General Hospital, Edinburgh, 1974–76.

Neural tube defect	14
Male fetus	
Duchenne's muscular dystrophy	2
haemophilia	1
X-linked mental retardation	1
Trisomy 18	1
Hurler's syndrome	1
Cytomegalic inclusion disease	1
	21

The morbidity and mortality associated with therapeutic abortion increases markedly with advancing gestation and maternal age. In terminations carried out after the twelfth week of gestation the incidence of sepsis and haemorrhage is almost double that found in terminations carried out during the twelfth week and before. Similarly, the mortality rate in second trimester abortion is higher than in first. Berger *et al.* (1974) surveyed all the abortions carried out in a two-year period in the early 1970s in New York State and found that the mortality associated with abortion after the twelfth week was seven times higher than that before the twelfth week.

Mid-trimester abortion is not without risk both in terms of morbidity and mortality. The methods used to terminate mid-trimester pregnancies are summarised in table 2.

Table 2. Methods of terminating second trimester pregnancies.

Surgical evacuation	Hysterotomy
	Hysterectomy
Intra-uterine insertion of devices	Bougie
	Metreurynter
Intra-uterine injection of solutions	Intra-amniotic
	Extra-amniotic
Extra-uterine administration of drugs	Oral
	Vaginal
	Intravenous
	Intramuscular

Surgical Evacuation

During the first trimester, suction evacuation and dilatation and curettage are safe and simple methods of terminating pregnancy. These methods are generally not applied to pregnancies beyond fourteen weeks' gestation. Liu, Martin and Hudson (1974) reported a series of 812 pregnancies that were terminated vaginally at fourteen to eighteen weeks' gestation. They found that the complication rate in this group was twice as high as in those terminated before the fourteenth week. Although there were no deaths in this study, the Report of the Committee on the Working of the Abortion Act (1974) in Britain found that the relatively low mortality associated with terminating first-trimester pregnancies was increased by a factor of five when this method was applied to second-trimester pregnancies (table 3).

Hysterotomy was at one time a popular method for terminating mid-trimester pregnancies, but with the introduction of effective non-surgical methods its popularity has declined. In fact, in 1973 only twelve per cent of pregnancies terminated after the fourteenth week were terminated by this method. Hysterotomy is a major surgical procedure with all its attendant risks. In addition, there are the added risks of implantation endometriosis and a scarred uterus in any subsequent pregnancy. Nottage and Liston (1975) surveyed 700 hysterotomies carried out in Aberdeen between 1968 and 1972

Table 3. Deaths from legal abortions in England and Wales 1969–71: 10–29 age group, terminated by D & C/ vacuum aspiration (Report of the Committee on the Working of the Abortion Act 1974).

Gestation (weeks)	No. of cases	No. of deaths	Fatality rate per 100000 abortions
0–12	116580	3	2.6
13+	30939	4	12.9

and found that the immediate risks of thromboembolism and sepsis were high. The Report on Confidential Enquiries into Maternal Deaths in England and Wales 1970–72 showed that the fatality rate associated with hysterotomy was 31 per 100000, whereas Berger *et al.* (1974) found the fatality rate to be 271 per 100000 in New York State. These surgical methods are now rarely indicated as a primary method of mid-trimester abortion; they have been superseded by non-surgical methods.

Intra-Uterine Insertion of Devices

The insertion of inert devices through the cervix into the extra-ovular space causes uterine irritation resulting in contractions and fetal expulsion. These mechanical methods are popular in Japan. Two devices are in current use, the gum elastic bougie and the metreurynter. Both devices have the disadvantage of requiring mechanical dilation of the cervix before insertion, to number 12–16 Hegar in the case of the metreurynter. However, using the laminaria-metreurynter method of Manabe and Nakajima (1972) the cervix can be slowly dilated over the preceding 24 hours with laminaria tents. Using these devices, the induction-abortion interval is prolonged and supplemental syntocinon is generally required. For pregnancies at sixteen to twenty weeks' gestation Manabe (1969) successfully aborted seventeen out of twenty-one with the metreurynter and seven out of ten with bougies. The average induction-abortion interval was 35 hours. In over half the cases the fetus was live born. The only complication was fever, and the liberal use of antibiotics was recommended.

Extra-Amniotic Injection

The extra-amniotic injection of soft soaps was first used as a method of procuring abortion in Germany in the 1930s. Utus paste can be administered transcervically without an anaesthetic. The induction-abortion interval is generally prolonged and the incidence of complications high. Sood (1971), in a series of 83 cases, found induction-abortion intervals of up to four days, even with the use of supplemental syntocinon. Two of his abortions failed and required hysterotomy. There were three cases of septicaemia and one case of death following uterine perforation. Thomas, Galizia and Wensley (1975)

reported a patient who had a cardiac arrest and subsequently died following the injection of Utus paste. This was accompanied by intravascular haemolysis and consumptive coagulopathy, thought to be due to the Utus paste gaining entry to the vascular compartment. The Report on Confidential Enquiries into Maternal Deaths in England and Wales 1970–72 showed a mortality rate of 194 per 100000 for Utus paste. This is an unreliable and dangerous method of terminating second-trimester pregnancies and should be abandoned.

In Japan the extra-amniotic administration of 0.1% Rivanol solution (a derivative of acridine) is a popular method of terminating mid-trimester pregnancies. Nabriski *et al.* (1971) successfully terminated 52 pregnancies between the fourth and six month of gestation. 95% of the patients aborted in less than 24 hours, 90% were given supplemental syntocinon. Most of the fetuses were live born. In two patients the cervix was torn and required repair. There were no infections and this may be due to the antiseptic nature of the solution. Side-effects would not be expected if the solution gained access to the vascular compartment, as Rivanol has been used intravenously for the treatment of infections (Manabe 1972). This appears to be a safe and effective method of terminating mid-trimester pregnancies, but Rivanol is not marketed in the United Kingdom at present.

Intra-Amniotic Hypertonic Solutions

Intra-amniotic hypertonic saline was first used on a large scale for terminating mid-trimester pregnancies in Japan in the late 1940s and early 1950s. Wagatsuma (1965) surveyed the Japanese experience with intra-amniotic saline for the years 1946–52 and found that there had been twenty-five maternal deaths. This early experience led the Japanese to abandon intra-amniotic saline and develop other methods. Cases of maternal death have also been reported in the British literature; Cameron and Dyan (1966) reported two cases. Despite these reports, intra-amniotic saline became a popular method of terminating mid-trimester pregnancies. In fact, in the United States in 1974 intra-amniotic saline was used in 70.3% of all abortions carried out at sixteen weeks' gestation or beyond (United States Centre for Disease Control 1976).

Several large series of intra-amniotic saline abortions have been reported from the United States. Ballard and Ballard (1972) reported a series in which 3242 patients out of 3249 were successfully aborted, with a maximum of 200 ml of 20% saline. Intravenous syntocinon was only given if the abortion process did not commence within 48 hours. If it had not commenced within 72 hours a second intra-amniotic injection of saline was given. 12.2% of the patients required syntocinon and 1% required a second intra-amniotic injection. With this regimen the average induction-abortion interval was 35.2 hours.

Kerenyi, Mandelman and Sherman (1973) reported 5000 saline abortions in which supplemental intravenous syntocinon was given within four to six hours of saline instillation. The average induction-abortion time was 25

hours and all patients were aborted vaginally. Table 4 shows that most complications were common to both series, while cervical lacerations and uterine rupture were seen only in the syntocinon-augmented series. This raises the question of whether or not syntocinon should be administered routinely. There is no doubt that syntocinon augmentation will decrease the induction-abortion interval and consequently the incidence of fever, but it will increase the incidence of uterine trauma. Using intravenous syntocinon at a rate of up to 50 milliunits per minute Kerenyi, Mandelman and Sherman (1973) found three cervical lacerations in 1000 patients. Earlier in the series, when intravenous syntocinon had been used at a rate of up to 200 milliunits per minute, uterine trauma was more common and more extensive.

Table 4. Complications associated with intra-amniotic hypertonic saline abortion.

	% Complications	
Complication	Ballard & Ballard (syntocinon after 48 hrs)	Kerenyi *et al.* (syntocinon after 4.6 hrs)
Fever	4.8	2.3
Haemorrhage > 500 ml	1.7	2.3
Blood transfusion	0.9	0.5
Re-admission	2.7	0.5
Cervical lacerations	No	0.1
Uterine rupture	No	Yes
Coagulopathy	Yes	Yes
Hypernatraemia	No	No
Water intoxication	No	No

Berger, Edelman and Kerenyi (1975) showed that when intravenous syntocinon was used at a rate of 17–67 milliunits per minute, the rate of administration had no apparent effect on the induction-abortion interval. However, the time of commencing the infusion was significant. If the infusion was commenced within eight hours of the saline instillation the median induction-abortion time was significantly decreased, whereas after eight hours it was not. It would therefore seem prudent to use the lower infusion rate commencing within eight hours of the saline instillation.

Intra-amniotic saline is known to produce changes in the coagulation factors in the blood. The level of fibrinogen, factor V, factor VIII and platelets decrease, the thrombin clotting time is prolonged and fibrin degradation products appear at three hours. These changes are thought to be maximal between 10 and 24 hours after the saline instillation. Cohen and Ballard (1974) reported ten cases of coagulopathy associated with intra–amniotic saline. They found the incidence of coagulopathy to be one in 161 with syntocinon augmentation and one in 823 without. They postulated that the increased incidence of haemorrhage with syntocinon might be due to either increased release

of thromboplastin into the maternal circulation or to more patients being aborted early during the stage when the coagulation factors are most depressed.

Clinically evident hypernatraemia is a rare but potentially fatal complication of intra-amniotic saline. Inadvertent intravascular injection of saline is the most common cause, but this risk can be minimised by employing a Teflon sleeve needle. A sensation of warmth, flushing and nausea are experienced during the early stage of the intravascular injection. If the injection is discontinued at this stage, the more severe manifestations of hypernatraemia do not develop. For this reason it is important to have the patient fully conscious at the time of the injection. Signs of severe degrees of hypernatraemia include cardiac arrest, hypotension, bradycardia and alterations in the state of consciousness. The appearance of central nervous system signs is sinister, proving fatal in five out of eight such cases reviewed by Burnett, Wentz and King (1974).

Water intoxication is another rare complication associated with intra-amniotic saline. However, it has only been reported in conjunction with high-dosage syntocinon augmentation and it is a complication of the syntocinon rather than the saline. Live births occasionally occur in association with intra-amniotic saline. Stroh and Hinman (1976) quote an incidence of 1.7 live births per thousand saline abortions. They found that the mean gestation for the live-born fetuses, calculated from their birth weight, was six weeks more than the mother's estimated gestation. Therefore, most of these live births were associated with late abortion after 24 weeks' gestation. Intra-amniotic hypertonic saline is an effective, even if somewhat prolonged, method of producing mid-trimester abortion. If the solution is inadvertently injected intravascularly the serious complication of hypernatraemia results. In order to avoid this complication of intravascular injection, hypertonic solutions of glucose or urea have been employed.

There has been a degree of reluctance to use intra-amniotic hypertonic glucose, as the solution provides an ideal culture medium. In recent years Lewis, Smith and Speller (1969) and Droegemuller and Greer (1970) used 50% glucose solution with prophylactic antibiotics and did not encounter any serious infections. In both series the induction-abortion interval was prolonged, averaging 85 hours in Lewis's series. A method that requires prophylactic antibiotics and has a prolonged induction-abortion interval is far from ideal. Intra-amniotic hypertonic urea is free from the potentially serious side-effects of saline and glucose. Greenhalf and Diggory (1971), using hypertonic urea, found the induction-abortion interval to be prolonged, the average induction-abortion interval being 59 hours. In a small series of thirty patients given supplemental syntocinon Craft and Musa (1971) reduced the average induction-abortion interval to 26 hours. It is surprising that a large series of 508 patients with simultaneous administration of 400 milliunits syntocinon per minute produced an average induction-abortion interval of 43.4 hours, 14% of the patients taking longer than seven days to abort (Weinberg, Linman

and Linman 1975). In all these series the incidence of side-effects was low. Despite the apparent safety of intra-amniotic urea, the prolonged abortion-induction interval detracts from its acceptability.

Prostaglandins

The introduction of the prostaglandins as mid-trimester abortifacients during the present decade has called for a re-evaluation of the established methods of procuring mid-trimester abortion. The prostaglandins are a group of long-chain carboxylic fatty acids, and they can all be considered as being derived from prostanoic acid. The molecule contains twenty carbon atoms arranged as a cyclopentane ring with an alkyl and carboxylic acid side-chain (see figure 1). The cyclopentane ring can be one of four configurations and this decides to which group—E, F, A or B—the prostaglandin belongs. The subscript number describes the number of unsaturated bonds in the side-chains, hence PGE_2, for example, has two unsaturated bonds (see figure 2). There are thirteen known naturally occurring prostaglandins. They have a wide distribution throughout the body and most organs seem capable of synthesising and metabolising them. Their actions throughout the body are protean. Their half-life is very short, for over 90% of PGE and PGF compounds are metabolised in one circulation through the lungs and liver. The oxidation of the hydroxyl group at position 15 is the initial metabolic step. Substitution in the 15 and 16 positions protects the hydroxyl group from oxidation and produces longer acting analogues, e.g. 15-(S)-15-methyl-$PGF_{2\alpha}$ and 16-16-dimethyl-PGE_2. In all there are nearly 500 prostaglandin analogues. It is the prostaglandins of the E and F series that have been employed as mid-trimester abortifacients. The various routes by which prostaglandins have been administered are now considered.

Intravenous. In the initial studies on the abortifacient action of the

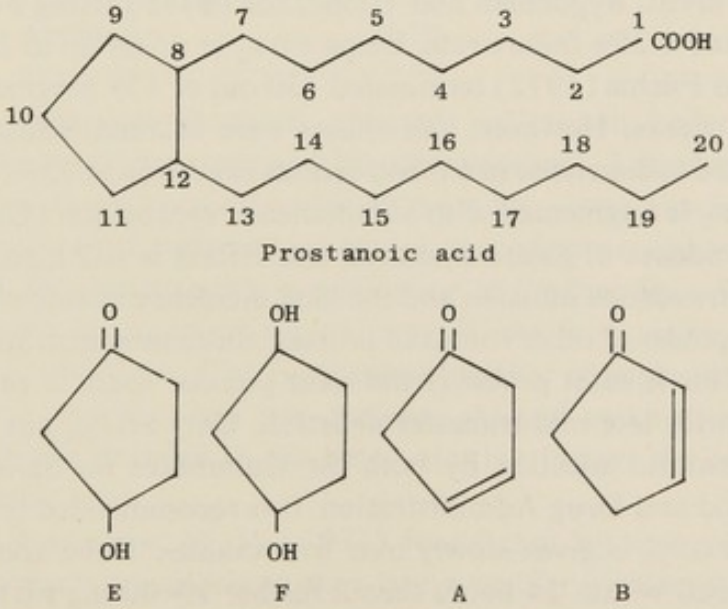

Figure 1. Nomenclature of prostaglandins: prostanoic acid, and differences in the cyclopentane ring.

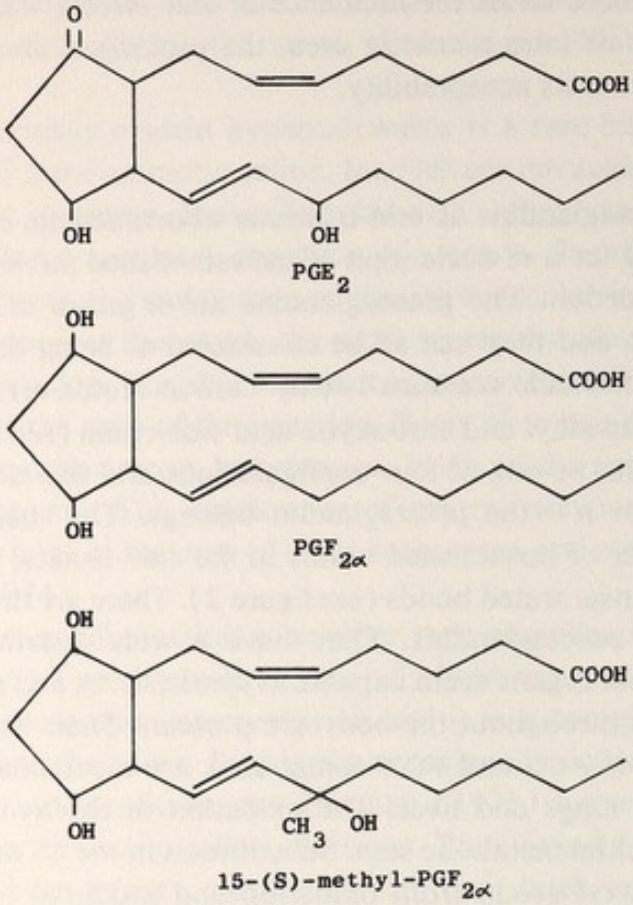

Figure 2. PGE_2, $PGF_{2\alpha}$, and 15-(S)-methyl-$PGF_{2\alpha}$.

prostaglandins, the intravenous route of administration was employed. Now the intravenous administration of both $PGF_{2\alpha}$ and PGE_2 has been approved. PGE_2 can be used in a dose up to 10 μg per minute and $PGF_{2\alpha}$ in a dose up to 100 μg per minute. Wiqvist, Bygdeman and Toppozada (1971), using $PGF_{2\alpha}$ in a maximum dose, had a 25% failure rate. Using PGE_2 in doses up to 20 μg per minute, Karim and Filshie (1972) terminated 130 out of 139 pregnancies at gestations of 5–26 weeks. However, side-effects were marked, nausea or vomiting occurring in 36%, diarrhoea in 6% and venous erythema in 22%. Even when intravenous PGE_2 is augmented with simultaneous syntocinon (Coltart and Coe 1975) the incidence of gastro-intestinal side-effects is still high. The inconvenience of an intravenous infusion and the high incidence of side-effects encouraged the development of other routes of prostaglandin administration.

Intra-amniotic. This is most probably the most popular route of prostaglandin administration for late mid-trimester abortion. Only $PGF_{2\alpha}$ has been approved for intra-amniotic injection by both the Committee on Safety of Medicines and the Food and Drug Administration. It is recommended that an initial dose of 40 mg $PGF_{2\alpha}$ is given slowly over five minutes. If the abortion process is not established within 24 hours then a further 10–40 mg $PGF_{2\alpha}$ is given. The intra-amniotic injection can be given through a spinal needle or an epidural catheter inserted through a Tuohy needle. The epidural catheter can

be left in place, thus avoiding the need to repeat amniocentesis if a second injection is required.

Anderson and Steege (1975), following the recommended regimen in 600 cases, found an average induction-abortion interval of 25.9 hours. In a smaller series of 122 patients, Duenhoelter and Gant (1975) found a mean induction-abortion interval of 25.9 hours for multigravid patients and 29.5 hours of primigravid patients. In the former series, 33% of patients required a second intra-amniotic injection, while in the latter the corresponding figure was only 15.6%. In Anderson and Steege's series, 55% of the patients were given intravenous syntocinon. In both series the incidence of minor side-effects was high (see table 5). The gastro-intestinal side-effects can be alleviated to some extent by the prophylactic administration of anti-emetic and anti-diarrhoeal drugs. In Anderson and Steege's series 21.7% of patients received antibiotics, but most received them prophylactically after curettage, rather than for any specific infection. In both series there were four cases of cervical laceration, all occurring in primigravida. Cervical lacerations following $PGF_{2\alpha}$ seem to occur solely in young primigravida. Karim and Ratnam (1974) collected thirteen such cases from the world literature. These cervical lacerations are typically situated posterior to the external os, which is intact. The lacerations are cervical fenestrations through which the fetus has been delivered.

Table 5. Side-effects with intra-amniotic prostaglandin $F_{2\alpha}$ (Anderson & Steege 1975).

Nausea and vomiting	41%
Diarrhoea	16.8%
Haemorrhage > 50 ml	5.3%
Blood transfusion	2.6%
Cervical laceration	0.67%
Uterine rupture	—
Antibiotics	21.7%

Other complications, although not seen in Anderson and Steege's large series, have been reported by other workers. Lauersen and Wilson (1974a) aborted twenty patients with 40 mg $PGF_{2\alpha}$ intra-amniotically, and found that all patients complained of breast engorgement and lactation. There are no other references to this complication in the literature. Shearman *et al.* (1975) reported six cases of major convulsions in a series of 555 intra-amniotic $PGF_{2\alpha}$ abortions. Electroencephalographic changes were noted in five out of sixteen patients, and two patients showed spike discharges. There are no significant changes in the blood clotting factors during abortion with intra-amniotic $PGF_{2\alpha}$.

Kochenour *et al.* (1972) found that by using 10–15 mg $PGF_{2\alpha}$ and simultaneous syntocinon they had a smaller incidence of side-effects. Globus (1974) found that the insertion of laminaria tents immediately before the intra-amniotic injection of $PGF_{2\alpha}$ decreased the induction-abortion interval

from 23.3 hours to 15.8 hours. There was also less need for pethidine analgesia and a decrease in the incidence of gastro-intestinal side-effects.

Duenhoelter, Gant and Jimenez (1976), using the combined method, found no cervical lacerations among 62 young primigravid patients, although if prostaglandins alone had been used three or four lacerations would have been expected. It was postulated that the tents protect the cervix by gently dilating it before the active phase of prostaglandin dilatation starts. Intra-amniotic urea will augment intra-amniotic $PGF_{2\alpha}$ and allow a smaller dose of prostaglandin to be used. King *et al.* (1974), using 80 g of urea and 20 mg $PGF_{2\alpha}$, found an average induction-abortion interval of 16.3 hours. Despite the lower dose of prostaglandin gastro-intestinal side-effects were troublesome, and 71% of patients experienced vomiting. One patient sustained a cervical laceration.

Although the intra-amniotic administration of PGE_2 has not yet been approved by the Committee on Safety of Medicines or the Food and Drug Administration, there are numerous research publications on the subject. Fraser and Brash (1974), employing 10 mg PGE_2, successfully aborted fifteen out of sixteen patients with an average induction-abortion interval of 14.2 hours. The remaining patient was successfully aborted in 41.7 hours with additional syntocinon. MacKenzie, Embrey and Hillier (1974), using 10 mg PGE_2, found a mean induction-abortion interval of 25 hours. By administering a second dose of 10 mg PGE_2 intra-amniotically six hours after the first they reduced the mean induction-abortion interval to 13 hours. They also demonstrated that the simultaneous administration of intravenous syntocinon at a rate of 80 milliunits per minute did not significantly reduce the induction-abortion interval. The incidence of gastro-intestinal side-effects in both series was similar to that experienced with $PGF_{2\alpha}$. Cervical lacerations (Fraser 1974) and major convulsions (Brash 1976) have also been reported. Craft (1975), employing 2.5 mg PGE_2 and 80 g urea, successfully aborted 110 mid-trimester pregnancies with an average induction-abortion interval of 10.7 hours. Vomiting occurred in 41% and cervical lacerations in 4.5% of the patients. Although such lacerations are easily repaired surgically, the incidence of cervical incompetence in a subsequent pregnancy remains to be established.

Analogues of both $PGF_{2\alpha}$ and PGE_2 have been used intra-amniotically. Wiqvist, Bygdeman and Toppozada (1973), using 15-(S)-15-methyl-$PGF_{2\alpha}$, found 2.5 mg to be a satisfactory dose. They successfully aborted 49 out of 50 patients within 48 hours with a mean induction-abortion interval of 18.8 hours. The incidence of gastro-intestinal side-effects was less than encountered with the recommended dose of $PGF_{2\alpha}$. In a small series 15-(S)-15-methyl-PGE_2 has been shown to be superior to its parent compound in terms of shortened induction-abortion interval and reduced incidence of side-effects (Amy, Karim and Sivasamboo 1973). The place of prostaglandin analogues still has to be determined by large clinical trials.

Extra-amniotic. Prostaglandins can be introduced into the extra-amniotic

space by way of a transcervical Foley catheter. Embrey, Hillier and Mahendran (1972) aborted 61 patients with $PGF_{2\alpha}$ using a dose of 750 μg at one- to two-hourly intervals. The average induction-abortion interval was 24.1 hours. Using PGE_2 at a dose of 200 μg one- to two-hourly, they aborted 33 patients with a mean induction-abortion interval of 19.5 hours. They found no significant difference in the mean induction-abortion interval between the hourly and two-hourly regimen with either PGE_2 or $PGF_{2\alpha}$. Similarly, they found no significant difference between the mean induction-abortion intervals for multigravid and primigravid patients. In a series of 103 patients, using extra-amniotic PGE_2, Fraser and Brash (1974) found a mean induction-abortion interval of 18.6 hours. Minor side-effects were common to both series (see table 6). Cervical lacerations were encountered in Embrey's series and bronchospasm in Fraser's series. The simultaneous infusion of syntocinon will shorten the mean induction-abortion interval. Morewood (1973) found a mean induction-abortion interval of 13.7 hours using PGE_2 augmented by intravenous syntocinon.

Table 6. Side-effects with extra-amniotic prostaglandin.

	PGE_2 and $PGF_{2\alpha}$[1]	PGE_2 only[2]
Nausea and vomiting	31%	57%
Diarrhoea	2%	3%
Pyrexia	5%	29%
Cervical laceration	1%	—
Bronchospasm	—	2%

[1] Embrey *et al.* 1972.
[2] Fraser & Brash 1974.

Lauersen and Wilson (1974b) administered $PGF_{2\alpha}$ extra-amniotically by a continuous infusion pump at a rate of 4 mg per hour, and achieved an average induction-abortion interval of 16.2 hours. 9% of their patients developed endometritis, which was treated with intravenous antibiotics. In cases of endometritis the induction-abortion interval was longer than average. The development of endometritis was thought to be due to the prolonged presence of the Foley catheter.

The main disadvantage of the extra-amniotic administration of prostaglandin is that it requires multiple instillations. If, however, the prostaglandin is administered in a thick viscous gel, its action is prolonged and most patients can be aborted with a single injection, enabling early removal of the Foley catheter and a consequent reduction in the incidence of sepsis. Using PGE_2 in a thick viscous paste, MacKenzie and Embrey (1976) found a single dose of 1.5 mg to be an effective abortifacient. The average induction-abortion interval was 16.3 hours. The incidence of gastro-intestinal side-effects was dose-dependent, although the mean induction-abortion interval was not. $PGF_{2\alpha}$ 10 mg in a viscous gel was also found to be effective in a single instillation.

16-16-dimethyl-PGE_2 may yet prove to be even more efficient.

Extra-amniotic prostaglandin appears to be a safe and effective means of terminating early second trimester pregnancies, but once the sixteenth week has been reached and amniocentesis is relatively easy, the intra-amniotic route is generally preferred.

Oral. Neither the natural prostaglandins nor their analogues are successful oral abortifacients, for in the dose required the gastro-intestinal side-effects are too severe.

Intramuscular. The natural prostaglandins are unsuitable for intramuscular injection because of their short half-life and local irritant nature. In contrast, the analogues, with their longer half-life and non-irritant nature, are suited to intramuscular administration. Lauersen and Wilson (1976) successfully aborted 117 out of 120 second-trimester patients with two hourly injections of 15-(S)-15-methyl-$PGF_{2\alpha}$. The average induction-abortion interval was 14.1 hours. Although all patients were prophylactically given anti-emetics and anti-diarrhoeals, 54% had nausea or vomiting and 7% had diarrhoea. Similar results were obtained by Lauersen, Secher and Wilson (1975) with intramuscular 15-(S)-15-methyl-PGE_2. They aborted 29 out of 30 patients within 24 hours with a mean induction-abortion interval of 9.5 hours. The incidence of gastro-intestinal side-effects was much lower than with the $PGF_{2\alpha}$ analogue, but 29 out of the 30 patients experienced a temperature rise of 2 deg F or more, and five patients complained of shaking and chills. Both analogues would appear to be good second trimester abortifacients, especially for patients in the twelve to sixteen weeks' gestation range.

Vaginal. The uterine stimulatory effect of vaginally administered prostaglandins is largely dependent on their absorption into the systemic circulation, and there is therefore an associated high frequency of gastro-intestinal side-effects. Bolognese and Corson (1974), using 20 mg triglyceride PGE_2 vaginal pessaries administered every two to four hours, successfully terminated 40 out of 40 late mid-trimester pregnancies with a mean induction-abortion interval of 12.7 hours. 75% of the patients experienced gastro-intestinal side-effects. The 15-methyl prostaglandin analogues have similar efficacy and side-effects to their parent compounds. A single dose method for 15-(S)-15-methyl-$PGF_{2\alpha}$ is being developed by Lauersen and Wilson (Anonymous 1976). A silicone rubber disc impregnated with the prostaglandin analogue is inserted high into the vagina and the prostaglandin slowly released over the following 48 hours. Abortion was successfully induced in 54 out of 60 patients with an average induction-abortion interval of 15.3 hours. Only gastro-intestinal side-effects were experienced.

Third Stage

Retention of the placenta after delivery of the fetus is commonly seen with all medical methods of inducing mid-trimester abortion. There is no universally recognised time interval after which the placenta is said to be retained. Kerenyi, Mandelman and Sherman (1973) took an interval of four hours,

whilst Stim (1972) took an interval of one hour. Oxytocics seem to be employed rarely. Only Fraser and Brash (1974) mention routine intramuscular ergometrine following delivery of the fetus. Anderson and Steege (1975) suggest that fundal pressure and cord traction immediately following fetal delivery will decrease the incidence of retained placenta.

Most authors recommend routine post-abortion curettage. Ballard and Ballard (1972) found that without routine post-abortion curettage, 3.4% of their patients returned with complications, whereas this number was decreased to 2% following routine curettage. Kerenyi, Mandelman and Sherman (1973) felt that routine post-abortion curettage is not indicated as it results in greater average blood loss, increased anaesthetic complications and perhaps the development of intrauterine synechiae.

Long-Term Effects of Mid-Trimester Abortion

The longer-term effects of therapeutic abortion are now well documented. Trichopoulos *et al.* (1976) have demonstrated the high risk of secondary infertility after therapeutic abortion. It is generally agreed that there is also an increased incidence of spontaneous second-trimester abortions in subsequent pregnancies (Richardson and Dixon 1976; Wright, Campbell and Beazley 1972). Richardson and Dixon (1976) also found that there was increased incidence of premature labour in subsequent pregnancies but Wright, Campbell and Beazley found no such increase. The studies so far mentioned have included terminations at all gestations but mainly the first trimester. Embrey (1975) reported the outcome of 53 pregnancies that had been preceded by prostaglandin mid-trimester abortion. He was unable to demonstrate any marked increase in spontaneous mid-trimester abortions or premature labour. The true significance of long-term effects of mid-trimester abortion will become apparent after long-term follow-up of these patients.

Conclusion

Mid-trimester abortion has a significant associated morbidity and occasional fatalities. The surgical methods with their high risk of morbidity and mortality should be abandoned. There seems no justification for the continued use of Utus paste. The extra-amniotic administration of Rivanol appears to be an effective mid-trimester abortifacient with a low incidence of side-effects. Unfortunately it is not available in the United Kingdom. The bougie and metreurynter methods have a prolonged induction-abortion interval and a high incidence of sepsis. Their acceptability is questionable. Although the early Japanese experience with intra-amniotic hypertonic saline included a high maternal mortality, recent American experience has shown the method to be relatively safe and effective. Hypertonic urea and glucose appear to be inferior abortifacients to saline.

The introduction of prostaglandins into clinical practice in 1970 challenged the position of intra-amniotic saline as the mid-trimester abortifacient of choice. Nevertheless, in the United States in 1974, intra-amniotic saline

was used in over seventy per cent of terminations carried out at the sixteenth week or beyond. However, it would be expected that intra-amniotic $PGF_{2\alpha}$ will supersede intra-amniotic saline as it has several distinct advantages. Intra-amniotic saline causes an increase in the intravascular volume, an added sodium load and a decrease in the circulating clotting factors. These changes do not occur with $PGF_{2\alpha}$. There is also a risk of hypernatraemia from inadvertent intravascular injection of saline. Intravascular injection of $PGF_{2\alpha}$ does not appear to be as serious as the compound is rapidly metabolised. Deaths have been reported following intra-amniotic saline, but to date there have been no reports of death following intra-amniotic $PGF_{2\alpha}$. The induction-abortion interval for $PGF_{2\alpha}$ is significantly shorter than for saline. $PGF_{2\alpha}$ does, however, have some disadvantages. A second intra-amniotic injection is required in up to a third of patients. The incidence of gastro-intestinal side-effects is much higher. $PGF_{2\alpha}$ is more expensive. However, intra-amniotic $PGF_{2\alpha}$ would appear to be superior to intra-amniotic saline as a mid-trimester abortifacient both in terms of efficacy and safety.

The prostaglandins are now established as mid-trimester abortifacients. Their main disadvantage seems to be the high incidence of gastro-intestinal side-effects. So far only the natural prostaglandins and a small number of their 500 analogues have been tried as mid-trimester abortifacients. It is to be hoped that in the future an analogue with uterine stimulatory action, but devoid of gastro-intestinal stimulatory action, will be found. Intra-amniotic administration is likely to remain the route of choice for the administration of prostaglandin, although the extra-amniotic, intramuscular and vaginal routes seem to be offering a serious challenge.

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Contraception Following Mid-Trimester Abortion

The contraceptive requirements of a couple undergo a series of changes during their reproductive life-time. Modifications in their family planning methods are conditioned by the success or otherwise in achieving their ambitions of producing healthy children which would make up their desired family unit. Fertility regulation, and indeed expression of their sexual relationship, can have particular problems for the couple who have been unfortunate enough to have these aims thwarted by the conception of a fetus whose potential of being malformed is such that a mid-trimester abortion has been carried out. In these situations genetic counselling, giving a clear indication of the relative risks of recurrence of the abnormality as far as is possible, is essential to enable this couple to make the all-important decision on their future reproductive career and help them come to terms with this limitation thereof. In this circumstance, empathic discussion is of more value than dogmatic ground rules.

In this paper the subject of contraception following mid-trimester abortion for congenital deformity is discussed by firstly reviewing the relative merits or otherwise of the methods available to such a couple. Those situations in which the couple are likely to find themselves are identified and an outline of the reproductive options open to them are reviewed. Guidelines for contraceptive usage are also defined.

Choice of Contraceptive

The choice of contraceptive method depends upon the particular indication for the mid-trimester abortion, the chance of recurrence of the condition for which the pregnancy was terminated, the impact that this event has had on the couple and their relationship, and how they see their future reproductive ambitions. In addition, the way in which such a couple will regulate their fertility, if indeed they do so at all, will be determined by their tolerance and acceptance of the method chosen, its degree of effectiveness, as well as the length of time for which they will wish to prevent conception. The various contraceptive methods available to the couple need to be reviewed so that the options open to them might be examined. These then need to be related to

their individual requirements during the counselling process.

The couple are highly likely to have had previous experience of contraceptive methods and may prefer to continue their previous practice. The methods available might be categorised into four major groups:

Mechanically Occlusive Methods

These methods, which depend upon the arrest of sperm migration in the genital tract, require maximal effort on behalf of the user but have the reassurance of no long-term interference with reproductive function or potential. Indeed the couple can be assured that side-effects are almost unknown, apart from the occasional hypersensitivity to the spermicide or the latex; even in these circumstances alternatives in material used or brands can overcome this problem. In fact, in Britain the condom remains the most common method used by couples, especially those married before 1965 (Bone 1973). It also carries the bonus of providing some protection against sexually transmitted diseases, perhaps not of high relevance in the context of this subject. Its ready availability, ease of use and acceptability has resulted in its increasing use in many developing countries. Improvements in design and marketing techniques may be contributory factors. In selected couples where adjunct compatible spermicides are used, the sheath is a reliable method provided that constant care and attention is maintained each time the method is used (Potts and McDevitt 1975).

For the couples who prefer the woman to use a mechanically occlusive method the cap or diaphragm may have a high degree of acceptability and reliability (Vessey and Wiggins 1974). Vessey and his co-workers (1976) state there are 'no material side effects associated with the use of the diaphragm apart from the risk of pregnancy and that there may be some unintended benefits' such as a diminution of risk of sexually transmitted diseases. In their long-term detailed study they found that users of the diaphragm tended to persist more with the method, and that a high percentage continued with it.

Nevertheless for many couples the constraints of these mechanically occlusive methods may be too restrictive for satisfactory sexual relationships. In some instances where short-term spacing may be applicable, the use of spermicidal foam alone might be appropriate provided that the significant failure rate is acceptable or the couple have a relatively low sexual drive or they have demonstrated evidence of, or are likely to have, a below average fertility potential.

Hormonal Contraceptives

Hormonal methods, in the form of the combined oestrogen-progestogen oral pill, are the most efficient, readily acceptable and convenient; other hormonal contraceptives include the different varieties of sequential formulations, the low dose oral and the injectable progestogens. The place of the intrauterine delivery system of progesterone will be discussed with other pharmacologically active IUDs. The concept of the sequential formulations attempts to mimic the endogenous ovarian hormonal cyclic pattern; they have no

ancillary genital tract contraceptive action and are rarely prescribed because the dose of oestrogen exceeds the now recommended level. Regimens with a variable dosage of oestrogen and progestogen with a major oestrogenic effect in the first part of the course, yet retaining some measure of local sperm-blocking effect on the cervical mucus, and a major progestogenic effect in the second half of the cycle, have been found to be effective and acceptable (Brosens, Robertson and Van Assche 1974). Varying dose combined formulations of ethinyl oestradiol and norgestrel are likely to be an interesting hormonal contraceptive development in the near future.

The low-dose progestogens produce a superimposed endocrine influence and are alleged to cause little interference with the woman's endogenous hormonal state. However, a variable degree of alteration in pituitary-ovarian function has been demonstrated in addition to the changes in the local genital tract, which is the site of the primary contraceptive action (Elstein *et al.* 1976). Although they have a high incidence of disturbed menstrual function with poor cycle control and a significant pregnancy rate, they have some application for the group of women who might wish to use a hormonal method, but might be intolerant of oestrogenic side-effects and yet accept the shortcomings of these preparations. Similarly with the injectable progestogens there is poor cycle control, but greater effectiveness. The place of these formulations in contraceptive practice is still being debated; they are particularly useful for the couple considering sterilisation (Elstein 1975).

The combined oestrogen-progestogen oral contraceptive pill is convenient, acceptable and the most efficient method available. The original pills contained far more steroid than was necessary to prevent conception. During recent years the trend has been to reduce the dose of steroid in the formulations in order to minimise side-effects and yet ensure continued effectiveness. It is because the climate of opinion was against abortion while the oral contraceptives were being evaluated and developed that the basic pharmacological principle, of achieving the desired therapeutic effect by starting with the lowest possible amount of agent and gradually increasing the dose, was reversed.

The combined formulations can be divided into three groups depending upon the type of progestogen and its biological effect. Of the two synthetic oestrogens, ethinyl oestradiol is the more potent and now more frequently used. The primarily secretory progestogens derived from 17-hydroxy-progesterone are no longer used, mainly because the Beagle dog developed breast nodules and large doses affected carbohydrate metabolism in the species. All progestogens used in combined oral contraceptives are derived from the 19-norsteroids. Those preparations, containing norethisterone or its acetate, range from a highly anti-oestrogenic and progestogenic balance towards a low-dose formulation with an oestrogenic bias (table 1). The gonadotrophin-inhibiting properties of these lower-dose combined pills are not as severe as the earlier higher dose contraceptives. As a result, on some occasions pronounced endogenous ovarian steroidogenesis occurred, and often significant oestrogen levels were seen in women taking tablets containing 50 μg mestranol and

Table 1. Oral contraceptives containing norethisterone, ranked in order of hormonal patency.

Progestogenic ↑ / Anti-oestrogenic on Genital Tract ↑ / Slightly oestrogenic ↓

Product	mg	Oestrogen	mg
Anovlar 21	4.0	ethinyl oestradiol	0.05
Gynovlar 21	3.0	ethinyl oestradiol	0.05
Norlestrin 21	2.5	ethinyl oestradiol	0.05
Ortho-Novin 2	2.0	mestranol	0.10
Loestrin 20	1.0	ethinyl oestradiol	0.02
Norinyl 1			
Norinyl 1/28	1.0	mestranol	0.05
Ortho-Novin 1/50			
Minovlar (ED)			
Orlest 21 (28)	1.0	ethinyl oestradiol	0.05
Con-Fer			
Ortho-Novin 1/80	1.0	mestranol	0.08
Ovysmen	0.5	ethinyl oestradiol	0.035
Brevinor			

1 mg norethisterone. This suggested that the margin of contraceptive effectiveness of those lower-dose formulations was less than that of the earlier preparations (Elstein *et al.* 1974). An anti-oestrogenic effect refers to a counteraction of the effects of oestrogen rather than competition with oestrogen for receptor sites in the cell as would occur with an oestrogen antagonist. In particular the anti-oestrogenic action of norethisterone is directed towards the genital tract, resulting in a diminution of withdrawal bleeding and a drying of secretions of the vagina. Hence these pills are especially appropriate for women with heavier periods and those who lubricate well. However, there are systemic and often undesirable oestrogenic effects.

There is then a group of combined preparations containing progestogens of mixed effect, in which some oestrogenic and slight androgenic properties are present in addition to those above (table 2). These effects are very much dose- and species-dependent and rarely show any adverse effect to the women. Indeed lynoestrenol exhibits potent synergism with oestrogen in their inhibition of ovulation (Johansson 1975). This synergistic action of the progestogen

Table 2. Oral contraceptives containing progestogens with mixed action, ranked in order of hormonal patency.

Progestogenic ↑ / Anti-oestrogenic ↑ / Oestrogenic ↓

Product	Progestogen	mg	Oestrogen	mg
Lyndiol 2.5	lynoestrenol	2.5	mestranol	0.075
Minilyn	lynoestrenol	2.5	ethinyl oestradiol	0.05
Ovulen 50	ethynodiol diacetate	1.0	ethinyl oestradiol	0.05
Ovulen 1 mg	ethynodiol diacetate	1.0	mestranol	0.10
Demulen 50	ethynodiol diacetate	0.5	ethinyl oestradiol	0.05
Demulen	ethynodiol diacetate	0.5	mestranol	0.10
Conovid	norethynodrel	5.0	mestranol	0.075
Conovid E	norethynodrel	2.5	mestranol	0.10

and doses of oestrogen below the recommended 50 μg are of great importance in ensuring an adequate margin of contraceptive effectiveness with the currently favoured low-dose formulations. This is particularly relevant to the norgestrel-containing formulations, which have only 30 μg of ethinyl oestradiol (table 3). Preparations containing variable amounts of norgestrel are more acceptable and are widely used. A major factor is that this progestogen seems to have a greater anti-oestrogenic systemic action rather than a pronounced genital tract effect (Briggs 1974). In spite of the low oestrogen content of the norgestrel oral contraceptives, excellent cycle control is achieved (Bye and Elstein 1973). Indeed, the combined mode of action of these low oestrogen formulations—suppression of the pituitary-ovarian axis and a hostile cervical mucus—has been demonstrated (Elstein *et al.* 1974). Furthermore there was rapid recovery of FSH secretion on withdrawal of the contraceptive.

The advantages of these potent steroids are self-evident. In addition to spontaneity and convenience, there is the opportunity for the woman to be in control of her menstrual function—she can menstruate when convenient socially. With the lower-dose formulations the incidence of side-effects is exceedingly low provided that due care in prescription and after care is taken (Elstein 1975). Additionally there are other benefits such as diminution of menstrual loss, control of premenstrual molimina and mood changes and the decrease in incidence of benign tumours of the breast (Vessey, Doll and Sutton 1972). However, there is a price to pay for their convenience. The inhibition of pituitary-ovarian function can be prolonged. Indeed there is clear evidence of some temporary impairment of fertility after discontinuing oral contraception (Vessey *et al.* 1976). Even after excluding women with a past history, there remains an increased, albeit small, risk of thromboembolism. In particular the hazards of myocardial infarction, especially in the predisposed group of women, increase dramatically in those over 35 (Mann and Inman 1975). The combined oestrogen-progestogen pills are dangerous in women who have demonstrated a sensitivity to oestrogen with respect to hepatic function and vascular responses. Finally there are many women who are unable to tolerate these contraceptives for a variety of poorly defined symptoms which include breast discomfort, feelings of bloatedness, headaches,

Table 3. Oral contraceptives containing norgestrel, ranked in order of hormonal patency.

	Product		mg	Oestrogen	mg
Systemically anti-oestrogenic and progestogenic ↑	Eugynon 30	d1-Ng	0.5	ethinyl oestradiol	0.03
	Ovran 30	4-Ng	0.250	ethinyl oestradiol	0.03
	Ovran Eugynon 50	41-Ng	0.5	ethinyl oestradiol	0.05
	Microgynon 30 Ovranette	d-Ng	0.15	ethinyl oestradiol	0.03

general malaise, and whose incidence remains unaltered during administration of a placebo. The finer margin of effectiveness with the lower dose formulation becomes particularly relevant for those women who tend to be unreliable in the regular taking of their tablets, and this can be especially applicable in this group of women who might be poorly motivated when they are experiencing conflicts about their reproduction.

Intrauterine Device

The intrauterine device, be it inert or pharmacologically active, has convenience as a major advantage. However, its great convenience—spontaneity and freedom from any involvement of the acceptor apart from the occasional need for medical check-up—encourages these women to minimise its very real drawbacks. The loop and double coil remain most useful devices, whose action depends on their inert foreign body presence in the uterine cavity. Side-effects, in particular abnormal bleeding, pain, expulsion and inadvertent pregnancy, result in a progressive decrease in the number of women who continue to use this as a satisfactory method of contraception (Tietze and Lewit 1970). There were advances in design in an attempt to improve its retention and coverage of the endometrial surface in the form of the shield, but when evaluated objectively the earlier extravagant claims were refuted (Jones, Parker and Elstein 1973). Furthermore, for the shield there was a 'sting in its tail', since the allegation that its multi-filament thread was a vehicle for the ascent of organisms leading to mid-trimester abortions and septicaemia, resulted in its withdrawal (Tatum *et al.* 1975). Indeed the cervical appendage in all IUDs has long been considered to have this potential (Elstein 1967) and tubal damage has been demonstrated in those women who developed symptoms of pelvic inflammation in association with IUD usage (Elstein 1969).

In studies currently in progress we have found the uterine cavity to be sterile. When there is an IUD *in situ*, organisms have been recovered in most instances except in those cases where the UID had no cervical appendage (Sparks *et al.* 1977).

The pharmacologically active devices were introduced to overcome the problems of bleeding and expulsion. With the copper-releasing IUD, although the amount of bleeding has decreased, this and expulsion continue to be drawbacks to the method. A further limiting factor has been the need for its replacement, the precise duration of two or three years or longer being the subject of current debate. The recently introduced progesterone-releasing IUD is associated with less pain and a lesser degree of blood loss, although the number of days of bleeding is not reduced and it has the further drawback of being very much more expensive and requiring annual replacement (Brenner, Cooper and Mishell 1975). Nevertheless, for these women in particular who may require short term contraception while they are sorting out their reproductive problems in the counselling situation and while they are undergoing emotional readjustments in the light of the need for a mid-trimester termination of pregnancy, the IUD may prove particularly useful and appropriate, especially as their motivation may not be of the highest order. For the woman

whose needs are short-term and whose periods are a little heavier, a pharmacologically active IUD may be extremely useful.

Sterilisation

The other and final option open to these couples is sterilisation, which should be regarded as a permanent method when another pregnancy would be unacceptable in any circumstance. Having decided upon sterilisation (and this decision should be made only after detailed investigations have been completed and due counselling has taken place) careful consideration should be made as to which member of the couple should undergo the procedure. The couple need to be assessed as a marital unit. It makes practical sense to sterilise the individual whose genes are responsible for the potential transmission of the anomaly. Yet to perform a sterilisation procedure on such a person might compound their feelings of guilt and inadequacy even further. Sensitive handling under these circumstances by an empathic gynaecologist/counsellor team is essential.

In the case of the male one of the many techniques of vasectomy might be performed. Because of the risk of recanalisation, many surgeons advocate the removal of a segment of the vas, while others recommend that the divided ends are folded back on themselves so that the cut and ligated ends point in opposite directions. The latter technique would seem to have particular application where male sterilisation is performed purely for family planning reasons, and hence there is always the possibility of a request for an attempt at reversal operation. Indeed patency of the vas can often be achieved, although restoration of fertility does not necessarily and invariably follow. The former method should be reserved for those men who themselves carry the offending gene.

The fact that there are so many methods of female sterilisation indicates that there is no generally accepted ideal technique (Jordan 1977). As in the case of the male destructive operations, removing all chance of restoration of fertility potential, should in this context be reserved for those women who have a high risk of recurrence of the genetic defect, whoever their partner might be. Sterilisation procedures that involve minimal damage to the tube should certainly be preferred for those younger women who request a permanent method of contraception, especially when they have just recovered from such an emotionally charged situation. There are a number of simple occluding methods, involving minimal trauma, which are currently being developed for application by the laparoscope, and these have particular application here. If the procedure is to be done by laparotomy a simple ligation should be performed, either with removal of a small loop of tube (Pomeroy) or without (Madlener). The more extensive cautery procedures via the laparoscope—there are many who advocate division as well—should only be done in those cases where the gynaecologist does not consider a request for restoration of fertility likely. Although the more complex procedures, in which the uterine end of the tube is either folded back on itself (Irving) or buried into the broad ligament (Uchida), were designed to prevent spontaneous recanalisation, it is

possible to mobilise both ends of the ligated tube for reanastomosis, but in view of the damage inflicted the results are not likely to be as good as in the case where anatomy was less distorted. Indeed, still hotly debated is the suggestion that all these procedures, and especially laparoscopic cautery, can bring an aftermath of gynaecological ill-health, especially abnormal bleeding (Neil *et al.* 1975). For those women who carry the genetic risk, or whose reproductive days are over, more destructive methods with no chance of restoration of fertility are in order. Indeed for the woman with heavier periods, or fibroids, or even some degree of genital prolapse, hysterectomy by the most appropriate route is the operation of choice (Elstein 1970).

Family Planning Counselling

The couples who have had a mid-trimester abortion in order to prevent the birth of a deformed child fall into several groups, and the method of contraception for the couple in these situations in particular depends upon the indication for the termination of the pregnancy and how the couple see their reproductive future.

Non-Recurrent Fetal Defect

Those couples where there is a non-repetitive cause of fetal defect, e.g. rubella, accidental exposure to X-rays, etc., can be encouraged to try for another pregnancy should they so desire. All that is required is support to help the couple weather the storm of such an event and to give assurance that their chances in another pregnancy of having a normal child are as good as any other couple's. They may need some advice as to a short-term method such as spermicide alone, or in this case even a non-method like coitus interruptus may suffice.

Low Risk of Recurrence

These are the couples who have a significant risk, approximately one in twenty, of recurrence of an abnormality, such as in the case where neural tube defect had been diagnosed following screening for maternal serum alpha-fetoprotein or by ultrasound. By judicious counselling the couple may accept the challenge of trying for another pregnancy fairly soon after the event and will only require interim short-term contraceptive advice while they are getting over its emotional trauma. They may well find that they need a spell of contraception, during which time they might come to terms with the implications of the midtrimester abortion. They clearly need reassurance concerning their next pregnancy and in particular that the appropriate screening and amniocentesis will be carried out. The methods chosen depend primarily on their previous contraceptive experience and how long they intend waiting before trying to conceive. In most cases a mechanically occlusive method will be the method of choice, although the oral contraceptive pill may be more acceptable. An IUD should only be used in exceptional circumstances.

High Risk of Recurrence

For many couples the recent mid-trimester abortion will have been the second reproductive disaster, since their risk of fetal abnormality (and indica-

tion for amniocentesis and antenatal diagnosis) will have been recognised by the previous birth of an abnormal infant who may still be alive and severely handicapped. These are usually young couples, and there is a high risk of recurrence of the defect which might once again be diagnosed antenatally, the order of risk being one in ten or more, grouped according to transmission of the anomaly:

1. Parents of two offspring with neural tube defects with a risk of recurrence of about one in eight.
2. Couples where one partner is the carrier of a balanced chromosome anomaly (a translocation or inversion) with a risk of recurrence of about one in ten (e.g. translocation Down's syndrome and other unbalanced karyotypes). This carrier condition is usually identified by a reproductive catastrophe or a strong family history.
3. Couples where a woman is a carrier of a mutant X-linked recessive gene where the recurrence risk is for fifty per cent of male offspring (e.g. haemophilia, Duchenne type of muscular dystrophy).
4. Couples where both partners are heterozygotes for the same mutant autosomal recessive gene where the recurrence risk is one in four (e.g. Tay Sachs', Hurler's).

The decision on whether to proceed with a further pregnancy, knowing the high risk of having to face another mid-trimester abortion because of fetal abnormality, will be a difficult one for these couples and much will depend upon emotional, social and family factors. The stability of the marriage, the emotional effect of the recent mid-trimester abortion, and the present family pattern with normal or abnormal living children, will all play a part in the eventual decision.

Should they decide that they can accept the high risk of recurrence then relatively short-term contraception will be requested. As pregnancy is desired at some time, 100% contraceptive reliability is not the first priority and the method most acceptable to the couple can be advised.

If the couple feel they cannot accept the high risk of having to face a further mid-trimester abortion, long-term reliable contraception must be offered, and oral contraception with 100% contraceptive reliability and social acceptability is the method of choice. The problem with all other methods is the small, but appreciable, failure rate, and this must be discussed with the woman who finds 'the pill' unacceptable. An accidental pregnancy will place this couple in exactly the same situation they have decided they cannot face.

The place of sterilisation must be considered in the context of each individual couple's risk. If the couple is young and the family already stressed by a handicapped child, marital breakdown may occur, or either partner could die, and in these circumstances each partner may wish to have his/her reproductive potential unimpaired. Where the high risk only pertains to the couple as a marital unit, i.e. when both partners are the carriers of the same autosomal recessive gene, then either partner with any other spouse could expect unaffected offspring. Where one or other partner is the transmitter of the

abnormality (such as the translocation carrier) then he or she continues to carry the high risk of an abnormal fetus with any other spouse. The unaffected partner of course carries no added risk.

In the autosomal recessive carrier situation, or where the male is the translocation carrier, artificial insemination by donor (AID) might be discussed. Where the marriage is stable and the desire for normal children is high this alternative may be more acceptable than the high risk of mid-trimester abortion. The need for manœuvrability and maintenance of the woman's fertility is of major importance so that full consideration and discussion can be given to this alternative.

With the young female X-linked carrier future advances must first be discussed—such as the possibility of accurate antenatal diagnosis of an affected male fetus with Duchenne type muscular dystrophy or haemophilia becoming available—but the very high risk of having to face a further mid-trimester abortion would remain.

In the case of the family with two offspring with neural tube defect, either partner will carry a high risk of recurrence with another spouse. Either of these parents, translocation carriers and X-linked carrier women may present a stronger case for sterilisation, but in all instances there should be due regard to the possibility that given a changed situation or a change in marital status, the previously unacceptable risk of mid-trimester abortion may become acceptable, and reversal of the sterilisation may be requested.

Persuading a carrier parent to be sterilised soon after a mid-trimester abortion may appear to be adding further punishment in an already guilt-ridden situation, and a period of adjustment using an acceptable contraceptive method should be advised. Indeed the contraceptive needs of these unfortunate couples will alter with change of circumstances whether this be a further pregnancy leading to the birth of a normal offspring or a further mid-trimester abortion, the breakdown of the marriage or natural ageing. Hence the contraceptive method advised following the mid-trimester abortion will need to be reviewed continually.

The Older Couple

The situation may be different for the older couple, who may already have family, and where the fetal chromosomal defect for which the mid-trimester abortion was performed was identified by amniotic fetal cell culture performed because the maternal age was raised over 38 years. In these circumstances, even if the risk of recurrence is not great, they may decide not to embark upon a further pregnancy, particularly as the maternal hazard is also increased, and thus may opt for sterilisation of either partner. On the other hand, if there is no issue of their marriage, they may go ahead and try again as soon as possible for these very same reasons.

Conclusion

The contraceptive needs of couples who have faced a mid-trimester abortion vary, depending upon the factors underlying the indication for the termination

of the pregnancy, their reaction to this reproductive disaster, the risk of recurrence of this fetal defect and the individual couple's reproductive ambitions. All these factors need to be considered in relation to the advantages and disadvantages of the contraceptive options available to them.

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THE COST

Cost-Effectiveness of Antenatal Screening for Chromosome Abnormalities

The development of antenatal diagnosis for chromosome anomalies has made the prevention of chromosome aberrations possible. Theoretically it would be possible to eradicate chromosome aberrations if all pregnancies were examined by amniocentesis, culturing of amniotic fluid cells, chromosome analysis and finally selective abortion of abnormal fetuses. The procedure, initially used sporadically in certain high-risk situations, is now moving towards a public health measure for systematic screening of certain groups of pregnant women.

In countries where public health programmes are planned or introduced, the cost-effectiveness of such programmes has been considered (Stein, Susser and Guterman 1973; Nielsen, Mikkelsen and Wamberg 1974; Glass 1975; Hagard and Carter 1976). Economic considerations play a part in determining whether such screening programmes are carried out, but just as or more important are ethical, political and medical problems. Social benefits are compared with social cost, but social benefits cannot be measured in monetary terms only. However, it is the aim of this paper to concentrate only on the economic considerations.

Development of Early Antenatal Diagnosis in Denmark

Prenatal diagnosis was introduced in Denmark in 1970. As in all other countries where the method was introduced, the number of cases has been more than doubling every year. In 1976 in Denmark, with a population of about five million, more than 1000 amniocenteses were carried out for prevention of chromosomal disorders. When the question of economics in prenatal diagnosis came up in Denmark, and the possibility of a screening programme for pregnant women of certain age groups was discussed, we decided to evaluate the costs and savings of such a programme (Nielsen, Mikkelsen and Wamberg 1974; Mikkelsen and Nielsen 1976).

Compared to other genetic diseases chromosome aberrations are frequent events. They occur in 0.5% of the general population (Hamerton *et al.* 1975). All chromosome aberrations diagnosable by antenatal screening were

considered. However, compared to Down's syndrome other autosomal syndromes are rare. They are not compatible with longer survival but the exact time of hospital care necessary would be difficult to calculate. For sex chromosome aberrations in males, other problems occur. Data on the natural history of the aberrations are unknown. Morbidity may be higher than in the normal population, but mortality seems to be the same as in the normal population. The problems of sex chromosome aberrations in females are even more complicated. We therefore confined ourselves to the evaluation of the cost-effectiveness of prenatal screening for Down's syndrome in different maternal age groups. High paternal age, which was recently shown to influence the incidence of Down's syndrome (Stene *et al.* 1977), was not included in the study; the number of fathers over the age of 55 was considered insignificant in Denmark. There may exist a discrepancy between population incidences of chromosomal aberrations and incidences found by prenatal diagnosis for the highest maternal age groups (Ferguson-Smith 1976), and this is discussed below. However, there is an acceptable correlation between population incidences of Down's syndrome and incidences found by antenatal diagnosis (table 1). For economic consideration, therefore, the population data for Down's syndrome were used.

Table 1. Incidence of trisomy 21 (%).

	Maternal age		
Authors	35–39	40–44	45+
Highest population incidence			
Lindsjö 1974	0.4	1.5	6.2
Lowry *et al.* 1976	0.4	1.5	3.9
Mikkelsen *et al.* 1976	0.4	2.2	3.3
Incidence diagnosed antenatally			
Ferguson-Smith 1976	0.7	4.9	?[1]
Polani *et al.* 1976	0.4	2.3	4.1
Simpson *et al.* 1976	0.3	2.8	9.5
Mikkelsen *et al.* 1977	0.4	3.7	20.0[2]

[1] Only 10 pregnancies, 0 trisomy 21.
[2] Only 5 pregnancies, 1 trisomy 21.

Material

The study was based on recent Danish Down's syndrome data (Mikkelsen *et al.* 1976) and the maternal age distribution in Denmark in 1973 (table 2). The population studied consists of those born during the period 1 January 1960 to 31 December 1971 in the Copenhagen Metropolitan Area. About 1.2 million people, or about one quarter of the Danish population, live in this area, which has many medical and cytogenetic services. During the period 1960–71, 204 771 births were recorded.

The sample consists of all 235 patients with Down's syndrome referred from the Metropolitan Area. Both living and deceased patients are included. The records of all patients diagnosed as having Down's syndrome or suspected of Down's syndrome and referred to the Service were examined. Referral to the Service is compulsory by law in Denmark.

Table 2. The expected number of patients with Down's syndrome, calculated from the incidence in Copenhagen 1969–71 and the maternal age distribution in Denmark 1973.

Mother's age	Incidence (per 1000)	Total births (Denmark 1973)	Calculated no. of Down's syndrome
15–19	0.69	4 702	3.2
20–24	0.91	24 510	22.3
25–29	0.64	28 200	18.0
30–34	1.83	10 273	18.8
35–39	4.34	2 982	12.9
40–44	14.62	520	8.0[1]
45+	?	30	—
Total		71 217	83.2

Data were also obtained from death certificates, records from paediatric departments, obstetrical departments, maternity clinics, midwife notifications, the cytogenetic central register and the central register of the Government's Service for the Mentally Retarded. All the information was cross-checked. Of the patients 177 were examined cytogenetically (75%). In five cases the parents refused a chromosome analysis of their child. In 53 cases (23%) the patients died before chromosome analysis could be carried out.

All estimates were based on the price levels in Denmark in 1974–75, i.e. constant prices. A discount rate of five per cent over the lifetime of the affected individuals was used. A five per cent discount rate is recommended by the Danish Economic Authorities for cost-benefit analysis of social expenditure projects, and means that the value of a cost or a benefit decays at a rate of five per cent per year at constant prices: the longer the cost or the benefits are delayed the lower their value. The analysis was limited to public expenditure. The present-day value for future cost and future benefit at the time of the birth of the affected cohort was discounted back to the time of the individual births. The study also supposed a 100% success rate and a 100% participation, although neither of the two assumptions is true. The failure rate depends greatly on the experience of the operator and is, in the specialised ultrasound laboratory working with us, about one per cent. The culture failure rate in our laboratory, initially five per cent, is now less than one per cent. It is more difficult to foresee the degree of participation in such a programme. It will apparently differ from country to country and may even differ in different regions in the same country. In evaluating the economic benefits

the replacement situation (the woman becomes pregnant again after termination and delivers a normal child) was not considered.

Expenses

The expenses consist of the cost of amniocentesis, laboratory examination and of interruption of pregnancy. The cost of an amniocentesis was calculated by Dr Bang, St Joseph Hospital, Copenhagen, as 650 DKr.

The laboratory costs, based on the figures for the same period from the John F. Kennedy Institute, were, per annum: (a) working expenses = 903 240 DKr; (b) estimated expenses for maintenance of building = 23 885 DKr; (c) interest to be paid and expenditure for depreciation (estimated period for the physical plant, 20 years) = 21 275 DKr; and (d) interest to be paid and expenditure for depreciation of equipment (estimated period 15 years) = 19 111 DKr. The total estimated expenditure was thus 967 511 DKr. With a capacity of about 500 examinations a year, the cost for one chromosome study of amniotic fluid cells was 1935 DKr.

The cost of interruption of pregnancy was calculated as the cost of three days' admittance in a general hospital, plus the cost of an abortion. The average daily expenses per person for general hospitals in Denmark in 1974–75 were 728 DKr. An abortion was calculated to cost 2184 DKr.

It was much more difficult to calculate the expected cost for the indi-

Table 3. Life expectancy for Down's syndrome patients.

Age (years)	No. of surviving patients	Average lifetime for patients 0–5 years old
Birth	83.2	102 days
1	64.7	1 year + 108.8 „
2	61.9	2 years + 108.8 „
3	61.1	3 years + 108.8 „
4	60.3	4 years + 108.8 „
5	59.5	5 years + 108.8 „
10	54.6	
15	53.8	
20	52.4	
25	50.1	
30	48.4	
35	47.0	
40	45.6	
45	43.1	
50	37.6	
55	33.7	
60	25.3	
65	12.7	
70	3.6	
75	0.7	

Average expected lifetime for five-year-old patient is 50.2 years.

Table 4. Percentage of institutionalised patients in different age groups.

Age (years)	% institutionalised	Age (years)	% institutionalised
0	10[1]	35–39	59
1–4	20	40–44	67
5–9	26	45–49	72
10–14	36	50–54	77
15–19	48	55–59	87
20–24	59	60–64	78
25–29	53	65+	100
30–34	58		

[1] Plus 22.2% in hospital.

viduals with Down's syndrome because (a) the life expectancy for patients with Down's syndrome is shortened considerably, and (b) the expenses for care vary and depend on the support given by the authorities to the parents or person with Down's syndrome in different periods of life.

The mortality rate was calculated for patients aged zero to five years from the Copenhagen Regional Center I (Mikkelsen and Nielsen 1976). For individuals older than five years, the data used concerned 524 patients examined in the late 1940s and re-examined in 1960 and 1972 (Øster, Mikkelsen and Nielsen 1975; see table 3). To calculate the costs for institutionalised individuals, material from Øster and van den Temple (1973) was used to estimate the percentage of patients over the age of twenty. For younger individuals, the material from Center I was used (Mikkelsen and Nielsen 1976; see table 4). The expense for the care of surviving persons with Down's syndrome was calculated according to the regulations of the Danish Government Service for the Mentally Retarded. For the institutionalised patients the average cost of a Danish institution was calculated. For the others, the costs were calculated depending on their ages. The economic consequences of the actual service given to the individuals in the different age groups were estimated. Kindergarten costs for the two- to six-year-old children and school expenses for the seven- to sixteen-year-old children were calculated. From seventeen to twenty years of age the cost of attending a youth school was calculated, and after twenty-one years a place in one of the sheltered workshops was assumed. The ordinary child allowance and disability pension were included in the estimation. The total sum was then estimated for every age group depending on how many of the individuals alive belonged to the different groups. The expenses for the different types of measures were multiplied by the number of patients in the different age groups. The calculations were carried out for all ages until the age of 77. After that age the number of persons with Down's syndrome was so small as to be insignificant.

Table 5 shows the economic considerations assuming that all pregnant women are examined, and also the individual data for different age groups.

Table 5. The cost-benefit analysis of the prevention of Down's syndrome. (Costs in thousands of DKr.)

Maternal age group	15–29	30–34	35–39	40–49	15–49
No. of births	57412	10273	2982	550	71217
Expected no. of cases	43.5	18.8	12.9	8.0	83.2
Cost of prevention	152191	27250	7922	1470	188833
Expenditure without prevention	28067	12130	8323	5162	53682
Cost *minus* expenditure	−124124	−15120	401	3692	−135151

The prevention of Down's syndrome for the age groups over 35 gives a surplus, and the greatest gain is found in the age group over 40. Overall, if all women were to be examined, a deficit of 135.151 million DKr was calculated.

Discussion

Cost-benefit analysis was considered by Stein, Susser and Guterman (1973) and by Milunsky (1973), who claimed that a screening programme for Down's syndromé would pay for itself for mothers over 35 years old. Their analyses were attacked by Glass (1975), who, in an extensive study confined to the U.K., using the treasury-recommended discount rate of ten per cent over the life time of the affected cohort, found that amniocentesis pays for the age group over 40, but gives a deficit if the age group 35–39 is included. In a recent study confined to Scotland, based on the data from Ferguson-Smith (1976), Hagard and Carter (1976) found that the potential economic benefit would be greater than the cost for women over 40, probably equal for those aged 35 and over, but of less economic benefit if the service were extended to women below 35.

In our first cost-benefit analysis (Nielsen, Mikkelsen and Wamberg 1974) it was found that prevention of Down's syndrome for the age groups over 35 was a net benefit to society. The greatest gain was found in the age groups over 40. The critical age, where prenatal diagnosis paid, was found to lie between 38 and 40 years of age. The greater benefit found in the present study compared to the previous one can be explained by three factors. The incidence of Down's syndrome is higher than previously calculated, especially in the age groups 35 and above (Lindsjö 1974, Lowry *et al.* 1976, Mikkelsen *et al.* 1976). The cost for prevention is relatively lower because of rationalisation of puncture and culturing techniques. The expenses for the surviving patients are relatively higher in 1974–75 because of the constant rapid rise in costs for the institutionalised cases.

It is interesting that two cost-benefit analyses, one on Scottish data and ours on Danish data, are so much in agreement, showing that prenatal diagnosis pays for the maternal age group over 35 and gives a considerable economic benefit for the age group over 40. In high-risk groups, such as translocation families, the economic benefit is certainly much greater, as a much

smaller number of women at risk have to be examined to find an affected individual.

Only about a quarter of Down's syndrome cases are born to mothers over 35. It would be of immense importance to find families at risk in the general population. Intensive research should be conducted into this direction.

As mentioned at the beginning of this paper, it may be discussed whether calculations should be based on incidences found by antenatal screening or on population incidences. Conley and Milunsky (1975) studied the cost-effectiveness of antenatal screening based on the data provided by Milunsky's laboratory. They claimed an obvious discrepancy between Down's syndrome population incidence based on liveborn infants and on the incidence found prenatally. Population data may be too low because of incomplete ascertainment. Very ill newborns may be transferred to special intensive care units before chromosome examination is carried out, giving an underestimate also in newborn incidence studies. A number of trisomic fetuses may end as late abortions. When considering these factors no real differences exist (table 1), especially when only age indication for prenatal diagnosis is considered.

But one cannot ignore the fact that more women carrying an affected fetus approach the obstetrician for prenatal diagnosis than women carrying a normal fetus, possibly because of a family history of Down's syndrome or malformations. Screening programmes covering the whole population of women of a certain age group will be able to show if the incidence is the same as in the present situation, where prenatal diagnosis is offered on demand to high-risk groups. Down's syndrome accounts for about one- to two-thirds of the chromosomal aberrations diagnosed antenatally because of advanced maternal age (Simpson *et al.* 1976; Ferguson-Smith 1976; Mikkelsen, unpublished observations). The detection of a number of fetuses with trisomy 13, trisomy 18 or other unbalanced karyotypes add a considerable gain to the benefits shown when Down's syndrome only is considered.

Summary

To evaluate the costs and the economic benefits of prenatal diagnosis the cost-benefit aspects of prevention of age dependent trisomy 21 (Down's syndrome) in Denmark were studied. Trisomy 21 was chosen as it is the only chromosomal aberration numerous enough and well enough studied to provide the necessary data.

The evaluation was based on Danish population data, data for birth and life expectancy of children with Down's syndrome, and Danish experiences with institutionalisation of these patients. The costs of prenatal screening were calculated for amniocentesis, laboratory expenses and therapeutic abortion. The savings were calculated from the budget of the Danish Government Service for the Mentally Retarded. The economic consequences were estimated for the service provided for the different age groups according to the percentage of surviving patients and degree of institutionalisation. The benefits were calculated with a discount rate of five per cent per annum. All esti-

mates were based on the price levels in Denmark for 1974–75, i.e. constant prices. The analyses were limited to the consequences for public expenditure.

By antenatal screening of all pregnant mothers aged 35 and above, 21 cases of Down's syndrome could be prevented in Denmark every year. For the age group above forty a large economic benefit was found. A small surplus was calculated for the age group thirty-five to forty.

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Cost-Effectiveness of Screening for Neural Tube Defects

The paper sets out the results of a D.H.S.S. study into the economic effects of screening programmes for neural tube defects. The cost of mounting a programme is compared with the financial impact for the public sector of a reduction in the incidence of spina bifida. The former appears to be quite low in relation to the latter. It is stressed that considerations such as these can only be one element in a decision as to whether to have a screening programme and how it should be organised.

Introduction

The discovery of an association between the presence of neural tube defects in the fetus and raised levels of alpha-fetoprotein in the amniotic fluid in mid-trimester has led to the possibility of preventing the birth of affected infants by means of a therapeutic abortion. Obviously an amniocentesis could only be offered to mothers at 'high risk' (for example those with a previous history of neural tube defects) but the further discovery that a new 'high-risk' group might be defined on the basis of the analysis of alpha-fetoprotein levels in maternal serum opened up the possibility of a comprehensive 'screening' programme (Brock, Bolton and Monaghan 1973; Brock, this volume).

In these circumstances it is clearly of interest to know the financial cost to the public sector of possible alternative 'screening' programmes, the likely yield of such alternatives in terms of the number of cases averted and the implications of a reduction in the incidence of neural tube defects for the health and education services, social security payments and so on. This is not, of course, to say that such considerations are the only, or even the principal, ones relevant to a decision to launch such a programme but simply acknowledges the fact that a screening programme of this kind may compete with other worthwhile endeavours for a limited budget. Information on the extent to which it will pre-empt resources and on the number of cases prevented as a result of the programme is therefore highly relevant.

A study in Glasgow (Hagard, Carter and Milne 1976) suggested that 'on economic grounds, screening [of maternal serum] may only be worth-

while in populations in which the incidence of spina bifida is high', by which the authors appeared to mean that for the general population the cost of detecting cases of neural tube defects by examining maternal serum exceeded the 'savings' from a reduction in the incidence of such malformations. Leaving to one side the question of whether the phrase 'on economic grounds' has any meaning in this connection, it was felt that there were a number of questions that remained to be answered and that new information, particularly concerning laboratory costs, might alter the results radically. Accordingly, the Economic Advisers' Office of the D.H.S.S. were asked to undertake a study of this question. This paper summarises the results of a much longer study which is available on request.

Eligible Population

The first parameter to be determined is the number of women who are likely to be eligible for the maternal serum test. This depends on the number of likely births (assumed to be 650000 per annum) and upon the 'cut-off' date of gestation beyond which no test would be carried out because it would imply an abortion delayed beyond some acceptable limit. The later this 'cut-off' date the greater the proportion of pregnant women likely to have presented themselves at their booking clinic. There is therefore a trade-off between a greater yield and the risks of late abortions.

Table 1. Antenatal clinic attendance rates for gestational age sixteen to twenty weeks.

Gestational age (weeks)	St Thomas's (1973) n=2619	U.C.H. (1975) n=1000	St Mary's (1975) n=4337
16 or less	60.0%	72.4%	67.7%
17 „ „	65.0	79.0	
18 „ „	69.0	84.9	
19 „ „	72.0	87.6	
20 „ „	75.0	90.3	78.2

Data were gathered from three hospitals in order to determine how many women were likely to present themselves by sixteen weeks, eighteen weeks and twenty weeks. These data are presented in table 1. It was estimated that about two-thirds of pregnant women would appear by sixteen weeks, a further 10% by eighteen weeks and a further 5% by twenty weeks. A detailed analysis did not suggest that the yield would be greatly affected by the tendency of lower social-class women to turn up at the booking clinic later in their pregnancy (table 2) because the number of births in social class v formed a relatively small proportion of the total (table 3).

Sensitivity

In order to determine how many cases will be detected among the eligible women it is necessary to know (a) the overall incidence of anencephaly and

Table 2. Results of the chi-squared test used to test the hypothesis that 'late arrivers' (i.e. after 20 weeks) include a disproportionate number of women in lower social classes (St Thomas's Hospital). The differences between observed and expected values gave a chi-squared value of 48.87, which is highly significant at the 5% significance level.

Social class	Arrivals after 20 weeks observed	expected	Total in class
1	134	147	639
2	197	225	974
3	1438	1533	6651
4	1083	1041	4516
5	335	241	1047

spina bifida, (b) the proportion of relatively closed lesions, since these are undetectable, and (c) the sensitivity of the test. The national incidence of anencephaly was estimated at 1.8 to 1.9 per thousand live births and of spina bifida at 2.3–2.4 per thousand (Weatherall 1976). It is estimated that 14% of all 'spina bifida' births are closed lesions. The calculations were carried out using two test sensitivities for spina bifida: a low one, 45%, based on the results of retrospective studies, and a high one, 75%, based on preliminary results from prospective studies. These sensitivities refer to open lesions. The sensitivity of the test to anencephaly appears to be 90% or more.

The calculations also assumed that those women who had a previous history of neural tube defects would be offered an immediate amniocentesis. These women, estimated to account for 10% of neural tube defect births, were therefore excluded from the calculations of the implications of the maternal serum test programme.

Using all of these assumptions table 4 sets out the number of all spina bifida births (both live and still, open and closed) detected for two test sensitivities and for three cut-off dates. It was further assumed that 25% of all open spina bifida births are stillborn. These stillbirths need to be deducted later in estimating the impact on hospital and other services.

Table 3. Distribution of births by social class of husband.
(From Social Trends No. 6 1975. Tables 1.1 and 2.3,
Social Classes IIIn and IIIm aggregated to S.C.3.)

Social class of husband	Est. no. of births (thousands)	% births	% in social class
1	51	6.8	5.2
2	116	15.5	17.8
3	404	54.0	50.9
4	130	17.3	17.8
5	48	6.4	8.3
Total	749		

Table 4. Numbers of all spina bifida births detected by maternal serum test.

Sensitivity	Time limit for test 16 weeks	18 weeks	20 weeks
45%	362 (23.7%)	415 (27.1%)	442 (28.9%)
75%	602 (39.3%)	692 (45.2%)	736 (48.1%)

Costs

In order to estimate the costs of the various possible programmes it is necessary to know the specificity of the maternal serum test, since this will determine the number of repeat tests and amniocenteses. It was estimated that the above sensitivities would be achieved with 5% of all eligible women having a second blood test and 3% having an amniocentesis. This implies that about five out of six women would have 'unnecessary' amniocenteses.

Alternative assumptions were made about the number of extra blood samples that would need to be taken as a result of the screening programme, which varied according to the proportion of women needing to be recalled specially during the sixteen to twenty week period.

The major cost of the screening programme would be the laboratory costs. Manual, semi-automated and automated radio-immunoassay methods were examined. Estimates were obtained of the cost of three types of scheme: (a) a single central automated laboratory; (b) two automated laboratories; (c) five or six supraregional semi-automated laboratories (Bagshawe 1976, Ward 1976).

From this it appeared that a programme based on a single laboratory was the least costly and one based on five or six laboratories the most costly. Depending on the number of samples taken the total cost of the screening programme would be about £400000 using a single laboratory, £500000 with a two-centre scheme and £550000 for a supra-regional design (table 5).

Table 5. Annual cost of screening programme under various assumptions (£).

Type of Programme	Cut-off date 16 weeks	18 weeks	20 weeks
One centre automated	380600	415400	432900
Two centre automated	460400	501800	522500
Multi-centre semi-automated	530500	554500	572700

On the other hand, the automated method is comparatively unproven in this particular application and a single central laboratory might give rise to logistical problems. In particular, given the need to act quickly on positive results and to maintain contact with clinicians, a decentralised scheme might have advantages outweighing its higher costs.

Cost and Yield

If we assume a single laboratory, a test sensitivity of 75% and a 'cut-off' date of eighteen weeks, the cost per live open spina bifida birth detected is about £800. With a sensitivity of 45% it is £1350.

If an ultrasound investigation were given to all women 'uncertain of their dates' it is estimated that the cost per extra live spina bifida case discovered as a result of the more exact dating would be about £80000.

With 40% of women estimated to be uncertain of their dates, ultrasound might be expected to improve 'dating' in 11% of the 40%, i.e. 4% of all women (see table 6). An improvement in 'dating' would only be of importance for half of these women (those who erred in the wrong direction) and in only half these cases were AFP readings not expected to be sufficiently high to make precise dating more or less irrelevant (Brock *et al.* 1975). Thus at most six extra cases of spina bifida would be discovered at a cost of between £400000 and £500000. This calculation does not, however, take into account other benefits from a policy of general ultrasonography.

Table 6. Relative accuracy of the predicted dates of delivery from ultrasonic cephalometry and Naegle's calculation in patients with certain and suspect dates (Campbell 1974).

Method of maturity prediction	Spontaneous labour < 14 days	< 10 days	Total
Ultrasound EDD	292 (93%)	251 (80%)	313
Impeccable dates EDD	163 (89%)	140 (76%)	184
Suspect dates EDD	98 (82%)	82 (68%)	120

If a chromosome analysis were carried out on all samples of amniotic fluid yielded by the programme, the cost per case of mongolism discovered would be about £35000, assuming no interaction between raised maternal serum AFP and chromosomal disorders. This takes no account of rarer chromosomal disorders that might be detected at the same time. A screening programme confined to 'high-risk' mothers with a previous history of neural tube defects, who would receive an immediate amniocentesis, would have a cost per case discovered of £550–£750 depending on the laboratory method.

Savings

A reduction in the incidence of live spina bifida births would have a large impact on hospital resources, although the size of the impact would depend on the surgical policy currently being pursued with respect to spina bifida. In the longer run there would be a reduction in the need for special education provision and for certain types of social security outlay.

Estimates of survival and disability were based upon a study of a selectively-operated cohort in Edinburgh (Stark and Drummond 1973). Hospitalisation data was obtained from the Hospital In-patient Enquiry and

costs from a special study of children's surgical units. Data on educational requirements were obtained from *ad hoc* studies. Family allowances, attendance allowances and mobility allowances were estimated.

The screening programme would prevent the birth of a group of spina bifida infants. Estimates were made of the costs such a group would have imposed in the ten years after their birth if their survival and disability were similar to those in the Edinburgh study.

If the test has a sensitivity of 75% it is estimated that the public sector costs of such a group would exceed the public sector costs of a screening programme within a year (table 7).

Table 7. Costs and savings of various screening programmes (£ thousands).

Sensitivity of test		Size of programme 16 weeks	18 weeks	20 weeks
45%	10-year saving	1218	1407	1492
	Cost	530	554	572
75%	10-year saving	2031	2345	2487
	Cost	530	554	572

With a test sensitivity of 45% the costs of the group exceeded the costs of the screening programme within, at most, four years.

Conclusion

It should, perhaps, be stressed again—since the point is often misunderstood—that the results presented here do not imply that a screening programme for neural tube defects would be either 'desirable' or 'undesirable' in 'economic' or any other terms. They are only one element in any decision, but they are important because they illustrate the monetary price to the public sector of such a programme. The D.H.S.S. has commissioned a study in South Wales that will examine many of the operational problems of a screening programme.

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THE IMPLICATIONS

Legal Implications of Antenatal Screening

Law, unlike medicine, is not an international science. Different countries have, unfortunately, different laws. Considerations of time and space make it impossible to deal with the legal implications of antenatal screening in all countries. In this paper emphasis will be placed on the position under English law and Scots law, with the hope that the points mentioned will at least give food for thought to those working under other legal systems. Four situations will be studied: (a) injury to the mother; (b) death of the fetus; (c) non-fatal injury to the fetus; and (d) failure to diagnose a defective fetus.

Injury to the Mother

This can be dealt with briefly. The applicable principles seem to be the same as for any other medical procedure. One theoretical danger is prosecution, or a civil action for damages, for assault. This danger will materialise only if the mother does not give 'informed consent' [1] to the procedure or if she denies giving such consent and there is no way of proving she did. It is doubtful if this is a serious danger in practice. Practitioners in this area will usually make very sure that the mother *has* given informed consent and that an adequate record is kept of it. A consent form can be useful in this context.

A second danger, if injury to the mother occurs, is a claim for damages because of professional negligence. The obstetrician may have negligently failed to use a sterile procedure. He may have failed to take reasonable precautions against Rhesus factor complications. A claim for damages in such a case would usually, in the United Kingdom, be based on what is called delict in Scotland and tort in England [2]. There is no special legal difficulty about such a claim. The principles are the same as for other medical or surgical procedures and the main difficulty in practice is the difficulty of proof. At present, in the United Kingdom, it is possible to contract out of liability for negligence [3], so it would be *legally* possible (whatever the ethical considerations might be [4]) for an obstetrician to get the mother to absolve him from all liability for injury however caused, including injury caused by professional negligence. The law on this point will be changed if a Bill before Parliament at the moment goes through. It is called the Avoidance of Liability (England

and Wales) Bill 1976, which is a highly misleading title because it is in fact proposed to extend its provisions to Scotland. The Bill prohibits contracting out of liability for death or personal injury resulting from negligence. It applies only to what is called 'business liability' but it provides that 'business' includes a profession. So contracting out of liability for death or personal injury resulting from professional negligence may soon be impossible in Great Britain.

Death of the Fetus

The crucial point here is that under the laws of the United Kingdom the fetus is not a legal person. Legally it is merely a part of the mother. Because the fetus does not yet have any independent legal existence, causing its death is not murder or manslaughter or culpable homicide. The only criminal law liability in Scotland would be for abortion, and accidentally or negligently causing fetal death in the course of antenatal screening would not be abortion, because the necessary criminal intent would be absent. In England there could also be liability under the Infant Life Preservation Act of 1929 for wilfully destroying a fetus capable of being born alive, which normally means a fetus over 28 weeks old. Again, however, this would not cover accidentally or negligently causing fetal death. The Act does not apply to Scotland.

What about *civil* liability for causing the death of a fetus? In the United Kingdom, it is recognised that a woman can claim damages from someone who negligently causes her to have a miscarriage [5]. Most cases have arisen out of road accidents, but the rule could clearly be relevant to antenatal screening. The mother would need to prove negligence on the part of the obstetrician *and* a causal link between the negligence and the miscarriage. The obstetrician would not be liable if the miscarriage would have happened anyway, quite apart from his negligence. Clearly the problems of proof in this area are formidable. I would guess that it would often be *extremely* difficult for a mother to prove that an abortion was caused by negligence in performing an amniocentesis or other fetal test. Moreover the damages recoverable would probably not be very great. The claim is not, technically, for the death of a child but only for the shock and distress caused to the mother. These would obviously be much less in the screening situation (where the mother is contemplating termination of the pregnancy in certain eventualities anyway and where she is under medical supervision) than in, say, a road accident situation. Does the *father* of the child have any claim if an abortion is induced in the course of antenatal screening? In the United Kingdom, the answer is generally 'No'. The legal identity of mother and fetus means that no injury is done to the father. His consent is not legally necessary for screening operations, although no doubt usually desirable for other reasons.

Other legal problems arise if it is decided deliberately to terminate the pregnancy. Here the law on abortion comes into play. In the United Kingdom an abortion by a registered medical practitioner is lawful if *two* registered medical practitioners are of the opinion formed in good faith: (a) that the con-

tinuance of the pregnancy would involve risk to the life or health of the pregnant woman, or any existing children of her family, greater than if the pregnancy were terminated; *or* (b) that there is a substantial risk that if the child were born it would suffer from such physical or mental abnormalities as to be seriously handicapped [6].

In England, because of the Infant Life Preservation Act 1929, the abortion must be carried out before the end of the 28th week. In Scotland that Act does not apply and there is legally no time limit, except that it would be homicide to kill a child once it had been born alive. Under the Abortion Amendment Bill presently before Parliament it will not normally be lawful, in England *or Scotland*, to terminate a pregnancy after the 20th week, but this is extended to 24 weeks if the ground for termination is a substantial risk that the child would suffer from such abnormalities as to be seriously handicapped and there is no time limit if termination is necessary to save the mother's life or 'prevent grave *permanent* injury to her physical or *mental* health'. The provisions of this Bill would clearly cut down the period available for legal intervention. It now seems, however, that the Bill is unlikely to become law, at least in the current Parliamentary session.

Injury to the Fetus

Background to the Present Law

The problem here concerns the possibility of liability for damages if the child is born disabled in some way as a result of antenatal screening procedures. It seems that the risk of injuring the fetus without killing it is very slight, but nonetheless it could happen. If it did, could an action be raised on behalf of the child claiming damages for the child? This of course, was the problem raised by the thalidomide cases, and those cases reveal the practical difficulty of proving both negligence and the causal link between the negligence and the disability. There is also a theoretical difficulty in an action on behalf of the child based on antenatal injuries. How can the child claim damages for injuries suffered when it was not a legal person? The courts have got round this in various ways [7]. Sometimes, they have relied on the rule or fiction found in a number of legal systems that a child *in utero* is regarded as already born when that is to its direct advantage (the so-called *nasciturus* doctrine). This rule was developed in relation to property and succession but has been extended in some jurisdictions [8] to cover damages for injury. Sometimes, as in several American jurisdictions [9], the courts have simply recognised that for purposes of reparation legal personality begins before birth, at conception for example, or when the unborn child is viable in the sense of being capable of life apart from the mother. The disadvantages with this approach are (a) that it means giving a right of action to various 'relatives' of the unborn child—the father, for example—even if the child is never born alive, and (b) that on the 'viability' view it adds a further difficult question of proof, namely 'was the child viable when the injury occurred?'. Some American courts have simply recognised that a person has a right to be born

free of defects caused by another's fault. This is a straightforward approach which can be objected to only, if at all, on the ground that it smacks of judicial legislation. Sometimes, and this was the approach generally favoured by English legal writers before the matter was resolved by legislation, it has been argued that the damage actually dates from birth [10]. So there is no problem. The child says 'Here I am, a living plaintiff claiming reparation for damage that I suffered just as I was coming into existence'. The artificiality of this argument is obvious: everyone knows that the damage was really suffered earlier.

The position when this question was considered by the English and Scottish Law Commissions a few years ago was, therefore, that in various countries children born alive had been allowed, by various legal arguments, to recover damages caused by negligence affecting them while still *in utero*. There was, however, no decision directly in point in England or Scotland. The thalidomide cases had been, or were in the course of being, settled without any resolution of the question of liability.

The approach of the two Law Commissions was very different. The Scottish Law Commission thought that the existing law was adequate and that legislation was unnecessary. They thought the courts *would* recognise that the child born disabled or deformed as a result of antenatal injury was entitled to damages either on the basis of the *nasciturus* doctrine (a child *in utero* is deemed to be already born when that is to its direct advantage) or on the (unconvincing) reasoning that the 'injury is sustained and damage is suffered when (the child) is born alive suffering from a defect imputable to the fault of another' [11]. The result is that there is no legislation dealing with the problem of antenatal injuries in Scotland. The position in England is entirely different. The English Law Commission was not content to leave the courts to sort things out on the basis of ancient fictions and doubtful logic. They preferred, rightly in my view, to deal with the problems by legislation.

English Law

The present English law turns on the Congenital Disabilities (Civil Liability) Act 1976, which resulted from the Law Commission's recommendations and which applies to England and Wales and Northern Ireland, but *not* to Scotland. The Act makes it clear that a child born disabled as a result of antenatal injury has a right to claim damages from anyone whose negligence caused the injury. I would like to comment briefly on the more important features of the Act.

First, it applies only to the child born alive. It confers no rights on the fetus as such [12].

Secondly, the Act replaces any liability that might exist at common law [13]. The practical effect is important. The lawyer advising on liability to a child for antenatal injury under English law knows that if there is no liability under the 1976 Act there is none at all.

Thirdly, the principle of the Act is to make liability to the child depend on a sort of fictional liability to the parent. The Act says, in effect, that a

person is answerable to the child if he was liable in tort to the mother or would have been liable if actionable injury had occurred [14]. In simpler language this means that the obstetrician who is negligent in relation to the pregnant woman at the time of antenatal screening is liable to the child under the Act even if no injury was caused to the woman herself [15]. Liability is, however, subject to the later provisions of the Act. Of these the most important for our purposes is unquestionably section 1 (5). This provides that:

> The defendant is not answerable to the child, for anything he did or omitted to do when responsible in a professional capacity for treating or advising the parent, if he took reasonable care having due regard to the then received professional opinion applicable to the particular class of case; but this does not mean he is answerable only because he departed from received opinion.

This provision was expressly 'designed to protect doctors from the risk of a multiplicity of actions by children' [16] but it probably does no more than declare the existing law. Even without the subsection there would be no liability in tort to the mother if the doctor took reasonable care. The last words of the subsection, saying the doctor or professional adviser is not answerable *only* because he departed from received opinion, were added to cover the case of the doctor using new or experimental techniques [17]. He is not liable merely because he breaks new ground, but in this sort of situation he would no doubt be expected to be particularly careful.

The Act makes it clear that in English law a mother can by contract sign away her child's rights to claim damages for antenatal injury. Indeed, if she signs away her own rights in a contract this will be taken as signing away the child's rights too [18]. That is the present law, but it will be changed by the Avoidance of Liability (England and Wales) Bill 1976. The Congenital Disabilities (Civil Liability) Act also deals with the problem of contributory negligence on the parent's part. If, for example, a pregnant woman ignores the instructions given to her clearly by her pharmacist and takes twice as many pills of a certain kind as she was told, then even if it was negligent to give her these particular pills and the child is born disabled as a result, damages would be reduced to take account of the mother's partial responsibility [19].

So the Act gives the child a statutory right to sue for damages for antenatal injury and deals expressly with the questions of contracting out of liability and contributory negligence.

Scots Law

The Scottish Law Commission, as we have seen, thought that a child would be able to recover damages for antenatal injury under the existing law and that legislation was unnecessary, so the Congenital Disabilities Act does not apply to Scotland. On principle, the rights of the unborn child would not in Scotland be affected by any contracts entered into or risks voluntarily accepted by the mother [20]. Similarly her contributory negligence would not affect the child's claim, but would merely make her jointly liable along with the other person, say an obstetrician, responsible for the child's injury [21].

In these respects the obstetrician is less well protected under Scots law than under the new English law. In my view it would have been better to extend the Congenital Disabilities (Civil Liability) Act to Scotland. That would have clarified a doubtful area of the law and introduced a welcome uniformity throughout the United Kingdom in an area of the law where it is difficult to see any justification for national differences.

Negligent Failure to Diagnose a Defective Fetus

Suppose there is no injury to the mother and no injury to the fetus but the obstetrician fails to diagnose a defective fetus. The parents made it quite clear that they would ask for the pregnancy to be terminated if the fetus were abnormal. Is there any risk of legal liability in this situation? The question hardly arises if there is no negligence on the part of the obstetrician. Let us suppose that he exercised all due care and skill, but that the defect was simply one that could and did remain undetected. In this situation there would be no legal liability unless the obstetrician had been foolish enough to guarantee that all defects would be discovered, in which case there could be liability for breach of contract. But no obstetrician in his right mind would enter into such a contract. Quite the reverse. From a small survey of obstetricians carried out recently it is clear that the usual practice would be to explain carefully to the parents what could and could not be detected and to make it absolutely clear that the tests being carried out would not diagnose all fetal abnormalities. So we can ignore the possibility of liability in the absence of negligence.

Liability to the Mother

Negligence in carrying out tests. Much more interesting legally is the possibility of liability to the mother for *negligently* failing to detect an abnormality. Suppose, to take an extreme and no doubt unlikely case, the obstetrician is proved to have been so drunk or preoccupied that the tests carried out were a mere travesty of what they should have been. Could the mother claim damages in tort or delict for negligence? The traditional approach would be to ask three questions: Was there a duty to take care? Was there a breach of that duty? Was there resulting damage which is not too remote? At first sight there can be little doubt that there is a duty to the mother to take due care in carrying out tests of this nature [22]. Clearly also there would be a breach of that duty in the sort of extreme case we are envisaging. The difficulty arises with the next question: was there resulting damage which is not too remote? The mother does not suffer any physical damage. But it is foreseeable that she might suffer distress and psychological damage, and it is foreseeable that in certain circumstances she might also suffer financial damage through having to support the child. Would this be regarded as too remote? There are types of damage for which reparation cannot be recovered. For example, one person cannot normally recover damages for financial loss suffered by him as a result of physical injury to another person or another person's property [23]. So an employer cannot recover damages for loss suffered by him as a result of injury to his employee. Sometimes this is ex-

plained by saying there is no duty of care to the employer, sometimes by saying the damage is too remote. In reality, as so often in this area of the law, the decision is a policy one disguised as a legal one. The courts have simply not wished to enable employers to sue in this situation: that would be extending the law too far. Policy considerations clearly enter into the case of the disabled child and it is impossible to predict how the courts would decide.

The courts *could* say that a parent has no legally recognised right to be spared the birth of a severely disabled child, that this is not a right protected by the law. But the mother could argue that she has a right not to be caused foreseeable and avoidable emotional distress and financial loss though the fault of another [24]. The courts *could* draw an analogy from the employer's situation and hold that there is no right to recover damages for financial loss caused by physical injury to another. But the mother could argue that there was no physical injury to the child, who was not even legally in existence at the time of the test and that, while it may be acceptable to say that someone running down an employee has no duty of care to the employer it is hardly acceptable to say that an obstetrician owes no duty of care to his patient. The courts could try to say that there is a break in the chain of causation: that the mother cannot prove that the pregnancy would have been terminated if the abnormality had been diagnosed. But the mother could argue that this *is* a matter susceptible of proof and that to deny liability on this ground is rather like denying liability for failure to diagnose a readily removable malignant tumour merely because the patient might have refused consent to have it removed. The courts *could* say that even if there is a duty to the mother and a breach of that duty there is no damage: that a child cannot be regarded as a 'harm' for which reparation is due. But the mother could say that the distress and the financial burdens of having a severely disabled child do constitute a harm, and that, even if it is difficult for a disabled child to argue in an action for wrongful life at his own instance that his existence is a harm for which reparation is due, there is no such difficulty in an action by a mother, who can point to a straight comparison between her life as it is and her life as it would have been had the defender exercised due care. The courts *could* say that even if all other hurdles are cleared the financial loss is likely to be minimal. This, however, would depend on the facts.

In Scotland, the father of a legitimate child is primarily liable for the child's support. The mother would be liable only if the father had no means or was dead. The liability, however, can continue throughout the child's life if he or she is incapable of self support [25]. So it is potentially a very heavy liability. State benefits for the disabled child would need to be taken into account, but not the possibility of charitable help. Clearly the process of quantification would be a difficult one, involving a great deal of prediction and indeed guesswork, but that does not prevent the courts awarding damages in other types of case where the process is equally difficult.

On the whole issue the most that can be confidently said is that, while the law is as yet undeveloped there is a possibility that the courts would recog-

nise that the obstetrician who negligently fails to diagnose a defective fetus is liable in delict or tort to the mother.

If there is a *contract* between the mother and the obstetrician (as in the case of a private patient) the mother would have a very much stronger claim, for it would normally be an implied term of such a contract that the obstetrician would exercise due care. And if he failed to do so there would be every possibility of a claim for damages succeeding, unless the courts were to take the view that the birth of a disabled child is simply not a harm for which damages are due. It is interesting to note that in a West German case the court awarded damages against a pharmacist who negligently supplied laxative instead of contraceptive pills. He was found liable to the mother for the cost of maintaining the child, but his liability was reduced by half because of the mother's contributory negligence [26].

Negligence in interpreting results. So far, emphasis has been on liability for negligence in actually carrying out the tests, but the obstetrician's potential liability for negligently failing to diagnose a defective fetus could arise not only if he carries out the tests negligently but also if he interprets or communicates the results negligently. After earlier hesitations it is now accepted in the United Kingdom that there can be liability for negligent statements causing financial loss [27]. Such liability can arise if there is some special relationship between the parties, and the person making the statement has special knowledge or expertise on which the other party is likely to rely. These conditions would be fulfilled in the type of case we are considering.

Failure to offer diagnostic tests. If it is once accepted that there can be liability for negligently failing to diagnose a defective fetus (and this is a jump that has not yet been made) further questions arise. Could there be liability for failing to carry out an amniocentesis where all the indications were for it? This depends on the relationship in question and on the accepted sound medical practice. There can be no question of liability to unknown women at large for failing to screen. But if a pregnant woman places herself under the charge of a doctor, so that he becomes responsible for the medical management of her pregnancy, there could be liability for failure to come up to the expected standard of care and competence in discharging this responsibility. Once the special relationship of doctor and patient under his charge is created the distinction between omissions and commissions becomes irrelevant: a doctor can be liable in delict or tort for omitting to take certain steps. Just what is the required standard of care in managing a pregnancy will depend on many factors, including the state of the science, the currently accepted sound practice, the availability of facilities for tests and so on. If it is, or becomes, the recognised proper practice in certain cases (e.g., women over a certain age, or known to have a certain type of genetic background) to refer for amniocentesis or some other antenatal test, then the doctor who negligently fails to live up to the required standard will be guilty of negligence. In this, as in other areas, the medical profession sets its own standards, which change with developments in knowledge and techniques.

Liability of genetic counsellors and technicians. If the courts take the step of recognising that damages can be recovered for negligent failure to detect an abnormal fetus then people other than obstetricians and general practitioners may be at risk. The genetic counsellor who negligently gives a false assurance that there is no risk would seem to come squarely within the category of people with special skills giving advice that they know is going to be relied on. He could be liable for a negligent statement causing financial loss. In certain cases there might also be liability based on breach of contract. The technician who is negligent in carrying out the actual tests could also be liable, although normally any action would be raised against the hospital authorities as being vicariously liable for the negligence of their employees.

Liability to the Father

What about liability to the father? Here the law is even more uncertain. I think that the courts in the United Kingdom would hesitate before saying that in carrying out an operation an obstetrician owed a duty of care to the husband of his patient. The courts would be tempted, I think, to say that the duty was only to the patient. On the other hand, there *is* a special relationship between the obstetrician interpreting and explaining his tests and the father who is relying on his expert advice, and it *is* conceivable that this type of situation could be brought under the heading of negligent statements causing financial loss. Certainly the father will often be primarily liable for the support of the child and this may be a very severe burden.

Liability to the Child

What about liability *to the child* who is born disabled but who would not have been born at all had the obstetrician exercised due care and skill? This is the famous problem of damages for wrongful life which arose in the American case of *Glectman* v. *Cosgrave* [28]. A woman contracted German measles while one month pregnant. She consulted her doctor, who advised her that there was no danger to the child. The child was born severely handicapped. An action was raised on behalf of the child claiming that if the mother had been given proper advice she would have sought an abortion. The Supreme Court of New Jersey held that it was impossible to award damages to the child for having been born. That would have involved comparing the value of the child's life, taking into account his disabilities, with 'the utter void of non-existence'. Probably a court in Scotland would take the same view. In England the Congenital Disabilities (Civil Liability) Act 1976 seems to rule out the possibility of any claim for damages for wrongful life in respect of births after 22nd July 1976 [29]. It would be different, of course, if the negligence was failing to diagnose a condition that could have been treated at an early stage. Then the child could say that but for the neglect he would have been healthy.

Summary of Legal Risks

The legal risks involved in antenatal screening may be summarised and assessed as follows:

1. The risk of injury to the mother raises no special legal problems.

2. The legal risks arising out of negligently causing the death of a healthy fetus *are* real. The mother could claim damages for having been caused to have an abortion. But although the risk is there, it is not a very important one. The mother would face formidable difficulties of proof, and damages would probably not be very great.

3. The legal risks arising out of negligent non-fatal injury to the fetus are potentially serious. Fortunately such cases seem to be extremely rare, and again there can be no doubt that the child would have a formidable burden of proof.

4. The legal risks arising out of *negligently* failing to diagnose a defective or abnormal fetus are very difficult to assess. The law is as yet undeveloped. There is, however, a possibility of liability to the mother, and perhaps even the father, being recognised in the United Kingdom. The damages claimed could include the cost of maintaining the child over a long period. There is, however, no risk in the United Kingdom of a successful action *by the child* for damages for wrongful life.

Consent Forms

Can anything be done to guard against the legal risks? What is the utility of consent forms? It appears that the use of a specific consent form is not widespread in relation to amniocentesis. Usually, only a verbal explanation of amniocentesis is given. In one hospital, however, a form on the following lines is used:

> We the undersigned, have requested that amniocentesis should be carried out. The risks and limitations of this procedure have been explained to us by ..... and we appreciate that the procedure may have to be repeated. We understand that the birth of a normal child cannot be guaranteed from the results of studies on amniotic fluid and its contained cells.
>
> Signed .....
>
> Date .....
>
> Hospital .....
>
> Case No. .....

This type of form has two advantages. The first is that it helps to make it clear that informed consent has been given, thus minimising the risk of criminal or civil proceedings for assault. A mother would find it difficult, in the face of such a form, to argue that a long needle had been stuck into her without her consent. She could, in theory, argue that the explanation given by the named obstetrician had been sketchy and inadequate and that for this reason her consent was not properly informed. In a case like this evidence of what was actually said would be necessary. This risk, which is probably slight in practice, could be further minimised if the consent form were expanded to include a check list of particular points which had been explained to, or discussed with, the patient, the form making it clear that the list was not meant to be exhaustive of the points discussed.

The second advantage of a form of the above kind is in relation to a claim based on breach of contract. In a private consultation, for example, a mother might argue 'I entered into a contract with you whereby you expressly or impliedly undertook to detect such and such an abnormality. I paid you a high fee. You failed to detect the abnormality. Therefore, you are in breach of contract and I am claiming damages.' The form would make it clear that success had not been guaranteed and would minimise the risk of an action based on breach of contract. Again it would probably be better, from this point of view, if the form included a non-exhaustive list of particular points that had been discussed and perhaps a complete list of the actual conditions being tested for. This would prevent the mother arguing later that the fee had been for a wider range of tests than had in fact been carried out.

What such a form does *not* protect against is an action based on negligence in carrying out or interpreting the tests. It would, at the moment, be possible in the United Kingdom to go further and to include in the form a waiver of all rights to claim damages for negligence. But, as stated above, this whole question of clauses exempting from liability for negligence will be profoundly affected by the Avoidance of Liability (England and Wales) Bill presently before Parliament. Such clauses may soon be legally ineffective.

In one sense there is no legal *need* for consent forms in relation to amniocentesis and similar operations. The fact that consent has been given, the nature of that consent, and the nature of any contract with a patient can be proved by other means. On the other hand consent forms may make proof much easier (particularly if personnel have moved away) and may prevent disputes even getting off the ground. They may also have non-legal advantages in helping to bring home to the parents exactly what is involved.

Preventative Law

The law is not concerned only with damages or prosecution after the event. A great deal of modern law is preventative law, designed by means of regulations and licences to stop damage occurring. This is the real contribution of the twentieth century to legal science. The traditional view was that the licence for breeding was the marriage certificate, and there have been suggestions that some screening should be carried out at that stage. I quote from the Report of the Kilbrandon Committee on the Marriage Law of Scotland in 1969;

> It was represented to us from three sources that both parties to a marriage in Scotland should be required to undergo a medical examination before marriage. It was suggested by one witness that the examination should be exhaustive, should include an X-ray and tests for conditions linked with various hereditary diseases . . . and that the results of the tests and medical examinations should be made known to both parties . . . . Bearing in mind that 80000 people marry in Scotland each year, we do not think it would be practicable to make such examinations compulsory, at least at the present time. We are advised that, unless a major

examination under hospital conditions were made, many latent conditions would not be discovered. We realise, too, that the public might reasonably object, on the ground that compulsory medical examinations would be an unwarrantable infringement of the freedom of the individual [30].

The Committee, therefore, did not recommend the introduction of compulsory pre-marital medical examinations. That is as near as we have got to preventative law in this area.

Notes and References

1. See Skegg, 'Informed Consent to Medical Procedures' 15 Medicine, Science and the Law p.124 (1975).
2. If there is a contractual relationship between doctor and patient the same negligent act may be both a breach of contract and a delict or tort. Cf. *Chesworth* v. *Farrar* [1967] 1 Q.B.407. The position is the same in German law. In French law, however, if there is a contractual relationship the doctor is liable only for breach of contract. *Cour de Cassation* Civ.20.5.1936, D.P.1936.1.88. For further comparative references, see Giesen, Civil Liability of Physicians with regard to New Methods of Treatment and Experiments (Gieseking, Bielefeld, 1975) p.48.
3. See the Second Report of the Law Commission and the Scottish Law Commission on Exemption Clauses (Law Com. No.69, Scot. Law Com. No.39, 1975) for a review of the present law and proposals for change.
4. Cf. Giesen, *vide supra* n.2, p.73.
5. Cf. *Bourhill* v. *Young* 1941 S.C.395 and 1942 S.C.(H.L.)78, where however, the pursuer failed on other grounds.
6. Abortion Act 1967 s.1.
7. See the very valuable comparative study in the Appendix to the Scottish Law Commission's Report on Liability for Antenatal Injury (Scot. Law Com. No.30, 1973).
8. E.g. Quebec and South Africa. *Ibid.*, pp.25-7.
9. *Ibid.*, p.22. The American decisions are reviewed in *White* v. *Yup* (1969) 458, p.2d 617.
10. See Street, *Torts* (5th ed.) p.109; Winfield and Jolowicz on Tort (9th ed.) p.611. See also the Australian case of *Watt* v. *Rama* [1972] V.R.353.
11. Scot. Law Com. No.30, p.6. The Commission's reasoning is criticised by Rodger in 1974 Juridical Review pp.83–90.
12. This is clear from ss.1(1) and 4(2).
13. This is the effect of s.4(5).
14. S.1(3).
15. He is not, however, liable to the child for 'wrongful life', e.g. for failing to diagnose an abnormality which, if diagnosed, would have led to an abortion. This is discussed later.
16. Lord Stair Hill in moving the second reading of the Bill. Parl. Deb. H.L. Vol. 370 col.363.
17. *Ibid.* cols.378–382.
18. S.1(6).
19. S.1(7).
20. Scot. Law Com. No.30 p.14.
21. *Ibid.*
22. It could be argued, however, (a) that the problem of duty has to be approached with reference to the particular kind of damage resulting, (b) that while there is

clearly a duty to avoid injuring the mother as a patient, there is no duty to avoid injuring her in her capacity as a potential parent, and (c) that with regard to damage sustained as parent of a disabled child the mother is in the same position as the father.

23. *Reavis* v. *Clan Line Steamers* 1925 S.C.725; *Weller* v. *Foot and Mouth Disease Research Institute* [1966] 1 Q.B.569; *Dynamco* v. *Holland and Hannens and Cubitts* 1972 S.L.T.38.
24. At one time it might have been argued that financial or economic loss was not recoverable as such—that reparation could be claimed only for damage to person or property—but this argument was dealt a death blow by *Hedley Byrne & Co. Ltd* v. *Heller & Partners Ltd* [1964] A.C.465. See, in particular, the observations by Lord Devlin at p.517 where he puts the case of the doctor negligently advising a patient that he cannot safely pursue his occupation when in fact he can. Lord Devlin said it was 'nonsense' to say that the doctor was not liable in the absence of a contract for the man's financial loss in that situation.
25. Cf. *Beaton* v. *Beaton's Trs.* 1935 S.C.187 ('child' aged 62).
26. Landgericht Itzehoe, *Familienrechzeitung* 1969, 90.
27. *Hedley Byrne & Co. Ltd* v. *Heller & Partners Ltd* [1964] A.C.465 (an action against a bank for having negligently given a reference as to the standing of one of its customers).
28. 49 N.J.22; 227 A.2d.689(1967).
29. See s.1(1) (child 'born with disabilities which would not otherwise have been present') and s.4(5).
30. Cmnd.4011 paras 43 and 44.

Medical Implications of Antenatal Screening

Coming as I do to speak in the penultimate stages of this meeting, with a title indicating that I should talk on medical implications of antenatal screening, you are no doubt wondering—as I did—what on earth I can say that has not already been adequately said by the talented speakers who have preceded me in the past two days.

I have interpreted my role as the link man, making the bridge between the discussions on the technical arguments supporting the introduction of the various screening procedures and techniques, and discussions and arguments that are going to be advanced to justify the financing and organisation of screening programmes throughout the country. I aim to present the views from a medical standpoint in the broadest sense—not talking just as an obstetrician, but as one who has experienced many, indeed most, of the implications to which I refer.

We who are attending this meeting could in many instances be classed as enthusiasts—people who for one reason or another feel committed to the view that in the case of fetal malformations prevention *is* better than cure, particularly as in the majority of instances, at least for the present, *there is no cure*. In our enthusiasm, however, we must recognise the implications of our crusades.

Antenatal screening activities commonly commence as research type projects, funded from university, N.H.S. or private research fund sources. This research produces methodology and evidence that calls for expansion of investigations to confirm the original proposition. This expanded activity is commonly collaborative between centres in various parts of the country but still on a research basis as far as funding is concerned, though by now thoughts of a service commitment are usually beginning to emerge. The next phase involves publicising, primarily to the medical profession, the techniques and potential benefits. At this point a variety of things begin to happen and pressures begin to build up. The public becomes interested and requests for the service begin to be made through GPs. Around the same time the Joint Standing Sub-committee on Screening in Medical Care at D.H.S.S. level may be looking at the evidence in respect of the particular screening possibilities

and may then recommend a policy to the Standing Medical Advisory Committee (S.M.A.C.) who may then pass on the advice, modified as they think fit, to D.H.S.S. as represented by the Secretary of State for Social Services or to the equivalents in Scotland and Wales. This advice may then be issued by D.H.S.S. as recommended policy for implementation within the Health Service. By now, however, real problems over financing have generally emerged and I am sure you are all familiar with the difficulties in the changeover from research to service commitment. However, let me leave the financial overtones for the moment and return to some of the other implications of antenatal screening programmes.

In order to indicate the wide-ranging implications of the introduction of new antenatal screening techniques, I've tried to construct an outline diagram (figure 1). I'm looking at a few of the considerations and the groups of people they affect. As you can see, I'm interpreting medical implications in the widest sense.

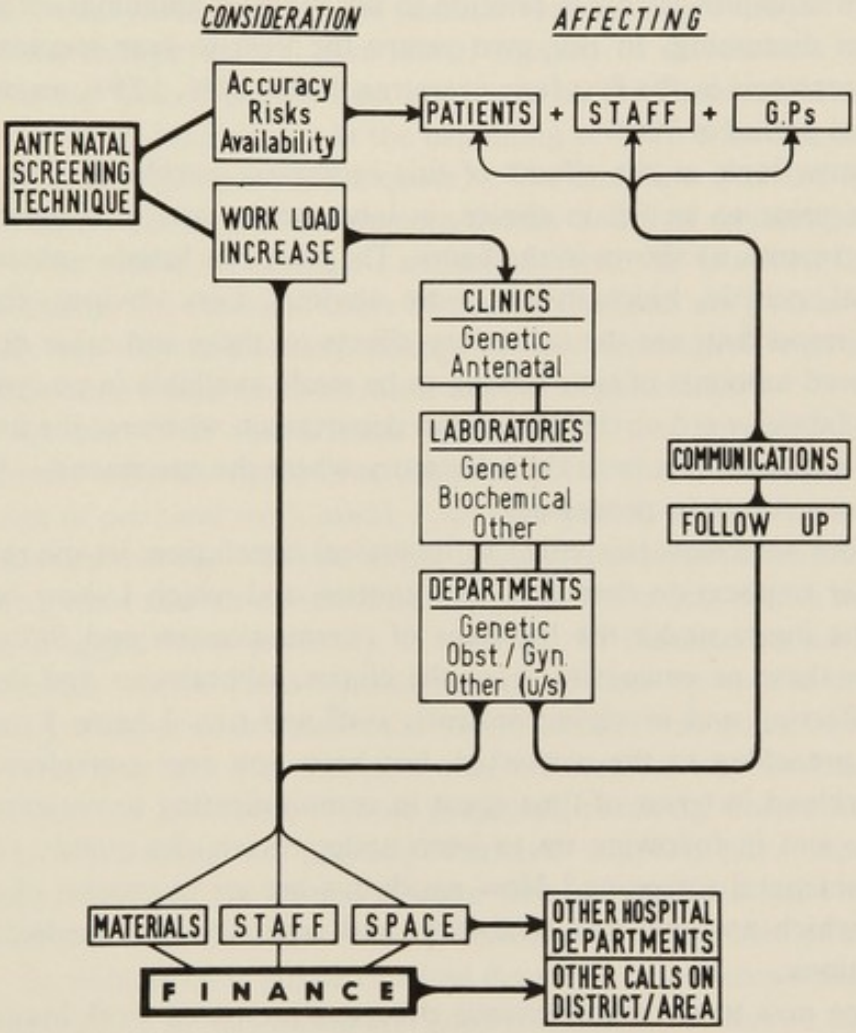

Figure 1. Flow diagram of considerations and effects.

Looking at the first considerations—*accuracy* and *risk*—these have been more than adequately dealt with by the speakers over the past two days as far as the technicalities are concerned. Clearly those aspects are of primary

concern to the patients, but of course also have implications for staff and GPs involved in advising patients. For all of us dealing with these problems, there is the implication that we must be well enough informed about current practice and experience to give reliable counsel and advice.

The consideration of *availability* of the screening test is also highly relevant to patient, staff and GP, and pressures upon medical personnel to find access to centres performing the newer tests must be recognised. The implications of the requirement of patients to travel often quite long distances to reach a suitable centre must also be mentioned.

The next consideration really gets down to the nitty-gritty, namely the predictable and insidious increase in demand for the test at the centres offering what is now interpreted as a service. Note the change: patients or specimens are now being sent, not to assist in research programmes, but to have a service investigation as part of a newly accepted routine. This inevitably produces a dramatic *increase in work load* and I do not have to put up any figures to give examples of this in relation to the types of antenatal screening we have been discussing. In our own centre the year-to-year increases in genetic amniocentesis in the first four years ran 100%, 50%, 175%, an overall increase in that period of 700%.

Let us now look at the effects of this increased workload. The main pressures are going to be felt in clinics, in laboratories and in a variety of hospital departments, as shown in the figure. The ones I've listed — obstetric/gynaecological, genetic, biochemistry — are obvious. Less obvious, though none the less important, are the secondary effects on these and other departments: increased amounts of time having to be made available in counselling clinics, in the labour ward or the ultrasound department, wherever the amniocentesis is to be performed, or in the laboratory where the specimens — blood or liquor — are going to be processed.

Just before we follow this effect to its logical conclusion, let me remind you of another implication that may be forgotten and which I show on the sidearm of the figure under the headings of *communication* and *follow-up*. Again I show these as emanating from the clinics, laboratories and departments, but affecting and involving patients, staff and GPs. I know I may in the main be preaching to the converted, but have you ever considered the increased workload in terms of time spent in communicating to patients and to colleagues, and in following up to keep under review the quality of our activities in antenatal screening? How assiduous are we in pursuit of these two factors, which are vital and necessary, and certainly have medical and other implications.

Returning now to the more obvious effects of increased work loads and the results of the pressures on clinics, laboratories and departments, basically these can be expressed in terms of requirements for increased *materials*, increased *staff* or staff time and ultimately possible increased *space*. All this adds up to *finance*, but more of this anon.

The implications to the individual departments concerned are obvious,

at least to those in the departments, but unfortunately apparently not so obvious to those outside, and sometimes even our colleagues do not seem to understand. In the present climate of financial stringencies and cutbacks, what hope is there for the introduction of new techniques or tests on a service basis? In the case of our own antenatal screening services, I began to indicate to the N.H.S. authorities over five years ago that there was a need for financial commitment to take over what was then a cost being borne by the university and by research grants. Some two years later the matter was still under discussion at local and regional level without any financial provision forthcoming, and by now the name and address of our laboratories and clinics had appeared on official regional publications as centres providing a service. By 1975 the workload built-up was such that an impassioned plea was made to our Regional Medical Officer for established financial support for what they at Region were publicising as a Regional service. At hospital level I had now managed to negotiate a small token payment to help provide half a technician's salary. And so into the familiar routine: *Region* says 'this is a matter for Area, though we will give *moral* support to discussions.' *Area* sends a letter to say 'your problem must be viewed by the District Management Team as part of their budgeting.' At the beginning of 1976 a letter from our District Organisation Manager (and I quote): 'The situation over the next few years is that there will be no development money and that any increased costs will have to be met from savings. I understand that the Obstetric and Paediatric Departments are the main users of this service, and am wondering if you can suggest any way of effecting savings in your department in order to increase the allowance which could be made to your departments.'

By mid-1976 our District informed us that it would be responsible financially only for those tests originating from its own clinics (about thirty per cent of our total work load). I am sure you do not want me to go on relating this tale of woe—as you might imagine, the situation is still unresolved in the long term and we continue to stagger on from one month to the next with funds dragged from a variety of sources. In the interim we have lost two technicians unable (and reasonably so) to face up to the uncertainties of knowing whether or not their next month's salary was going to be provided.

You may say that I'm now way off medical implications of antenatal screening, but just think—these pressures, resulting from a service demand that is not resting on properly financed support, have repercussions on patients, staff and the public at large that *are* medical, quite apart from the unthinkable effects on our blood pressures and coronaries as we try to penetrate the bureaucratic jungle to avoid denying our patients what they consider is their right to an up-to-date and good service. We do have a responsibility to justify and argue for any new methods that we introduce and this, in the case of antenatal screening, I think we can honestly claim to have fulfilled. It is quite wrong, however, to leave the solution of these problems at a local level if the problem is a national one. We should not have to be forced into the situation I reached some months ago when I had to give notice of withdrawal

of service unless supporting N.H.S. funds were forthcoming. Such action, if it had to be carried through, would be damaging not only to our departments, but also to the hospital, the Area and the Region, and of course inevitably to the patients. It is important, therefore, that our colleagues in other parts of the Region know the problems we face and why, for instance, some of us have to make what appear to be rigid decisions regarding the cases or specimens we accept, e.g. not advising amniocentesis for the under 40s if age is the only indication.

Looking again at my chart, you will see I indicate the pressures exerted by space and finance on other departments in our own establishments and on the other calls on our District or Area finances. We are in competition with many others for an increasing share of an ever decreasing kitty.

If we contemplate a national antenatal screening service, it seems reasonable to conclude that the most efficient way of organising this is on a Regional basis for the majority of the investigations, probably two or three centres at most per Region being set up and geared to deal with the service workload. For more specialised tests, where the demand is small scale, the centres would be limited to one or two for the whole country—national centres in the true sense.

Although I am now probably stretching the privilege of this platform, I feel bound to advance what I feel is the only reasonable approach to the financing of this type of service. I've shown that this whole superstructure about which we've been talking comes down to and rests on this base of *finance*. If the base is inadequate or shaky, the whole structure will topple. Figure 2 shows a possible sound base. In the long run all financing comes directly or indirectly from National Budget from Health Service Funds, illustrated here as D.H.S.S. One small pillar of support rises direct to the local level in respect of the small number of national centres to which I have already alluded. The greatest portion of funding flows through to the Region and

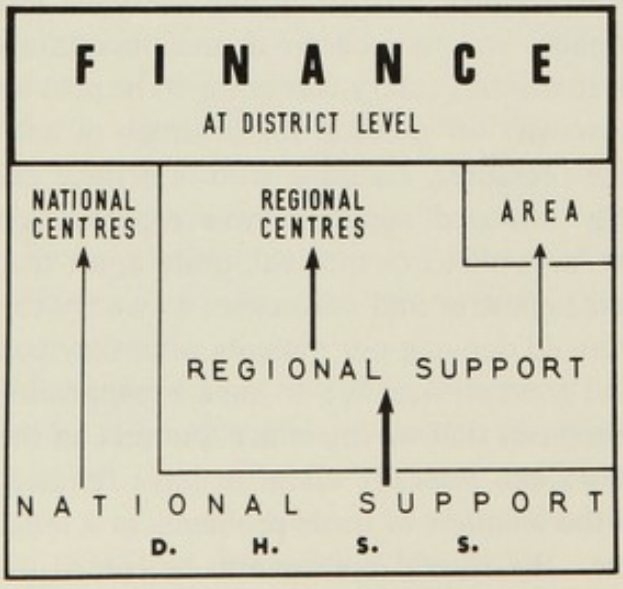

Figure 2. Suggested finance support.

thence directly to take the main weight of the structure at local level, representing the Regional centres throughout the country, and finally a small pillar props up and balances the structure from Area finances.

Some of you may be lucky enough to have already achieved something approaching this type of foundation, but I know of many Regions, like mine, where there is still refusal to allocate funds on a direct basis. We have now reached the stage where some positive direction must be given. If we can't afford to introduce the new techniques, the public must be told why and we must stop pretending that we can go on absorbing new measures into an already overstretched local, departmental or university budget. Mikkelsen and Glass, in the preceding papers, confirm the cost-effectiveness of what we are proposing, and if this is the case we must not be fobbed off by continued wrangling about showing savings before we can introduce the measures. The savings are few in the short term but mainly in the long term—the planners and the budgeteers have to realise that the investment has to be made now if our efforts of the past years are not to be wasted.

Let me now look more generally at the implications of antenatal screening. Currently, antenatal diagnosis is still largely restricted to high-risk pregnancies and is therefore applied particularly at the level of individual families, either retrospectively, where an affected child has already been born, or prospectively where one of the parents is at risk of having affected children because of some previous family history. In addition to particular families there may be specific groups in the population at greater risk, e.g. mothers over 40 years.

Now, however, extension of screening tests to all pregnancies is being suggested, serum AFP for instance. Extension to population screening is on the cards, e.g. sickle cell trait in negroes, thalassaemia screening in Greek Cypriots, so that couples found to be at risk could request antenatal diagnosis. New instrumentation in the form of fetoscopy is facilitating the possibility of even greater advances. Are we approaching the time when we will be able virtually to eliminate certain diseases by a combination of antenatal diagnosis and selective abortion?

We must ponder very carefully the implications of our progress and the pressures which are going to be made on us. Some have already emerged, e.g. requests for antenatal sexing of the fetus where there is no consideration of X-linked disease. I personally feel that this is *not* a valid indication for amniocentesis.

We in our respective professional roles have a responsibility to cushion our patients from the effects of these technical advances. We have to constantly evaluate our techniques for safety, accuracy and applicability. We must ensure that only those who can properly benefit from the screening tests are subjected to them and that all who are tested understand the limitations and dangers of any of our procedures. On the other hand, it is also our responsibility to see to it that screening tests of proven value are made available throughout the country and that individual departments taking service re-

sponsibilities for such tests are properly supported.

Professor Passarge in his paper reminds us of the ethical problems we face, and the need to protect consumer interests. Considerations such as whether we should do chromosome culture on liquors taken primarily for AFP, and vice versa. Are there ethical issues? Can we afford this, or is it a luxury? The field of antenatal screening is, par excellence, an example of the need for real team work — a potentially large team including our patients as well as the many disciplines mentioned. The pitch on which we play continues to have its boundaries extended and the rules of the game are sometimes not too clear. The supporters are on the side lines urging us forward, but if our hard-pressed teams are not to be forced to retire exhausted, some referee must sound the whistle for half-time, and hopefully we may look to our respective English, Welsh and Scottish D.H.S.S. pavilions for the financial oranges to refresh us and enable us to continue the game.

I trust that some of the considerations that I have voiced may enable a better understanding of the wider issues with which we must contend in the medical world when we move into new fields.

Implications of Antenatal Screening for the Health Service

I believe it is worth emphasising that the movement from clinical care to screening is not just a shift of gear, it is really a movement into a different dimension. For example, when we have a woman come to us aged 40, or thereabouts, and say to us, as obstetricians, 'please look after me, I am pregnant', this is a simple relationship that we are very familiar with; in this circumstance we assume a responsibility for her welfare and that of her unborn child, we deploy our skills, our knowledge of obstetric science and so on to do the best we can for her. But if on the other hand we go to her as a result of a population screening procedure, as opposed to individual antenatal care, and say 'Madam, I have reason to think that your fetus may be a mongol child and I think that perhaps you should consider having this pregnancy terminated', then we have moved into a different world with a changed set of relationships.

There is equally a responsibility upon the individual clinician and on the Health Service to be absolutely certain in this kind of screening situation that our tests are valid, that they are effective—in terms of their freedom from false positives in particular—that they are acceptable, that we have an effective treatment to offer when we do impose this psychological and physical stress on our patients, and that the treatment we have to offer is also acceptable. Fortunately for us in the United Kingdom we have had some of the best thinking on the social and epidemiological implications of screening, and I instance the outstanding contribution made by McKeown *et al.* (1968). Our policies have been shaped with the help of the Joint Standing Sub-Committee on Screening in Medical Care. Thanks to this Sub-Committee we have looked at many possible screening procedures in the United Kingdom, and have avoided errors that come from plunging into screening policies before they are properly evaluated. We know that this Sub-Committee has been looking at antenatal screening; we will look at its recommendations with the greatest care and I will make brief reference again to this later.

There is no doubt that tremendous progress has been made in recent years in techniques for screening for congenital malformations, and the

interest with which this facility has been received by clinicians is growing steadily. Figure 1 is information from Ferguson-Smith *et al.* (1976), showing the trend over a five-year period of referrals to the pilot study being conducted by his Unit, and it demonstrates evidence of substantial increase in demand. Most of the activity relates to screening offered because of maternal age or previous history of neural tube defect. But I understand that Professor Ferguson-Smith would say that his total of 500 cases in 1975 represents only the fringe of potential demand in the West of Scotland, a large region for which he is providing a research linked service to the limits of his capacity at the present time.

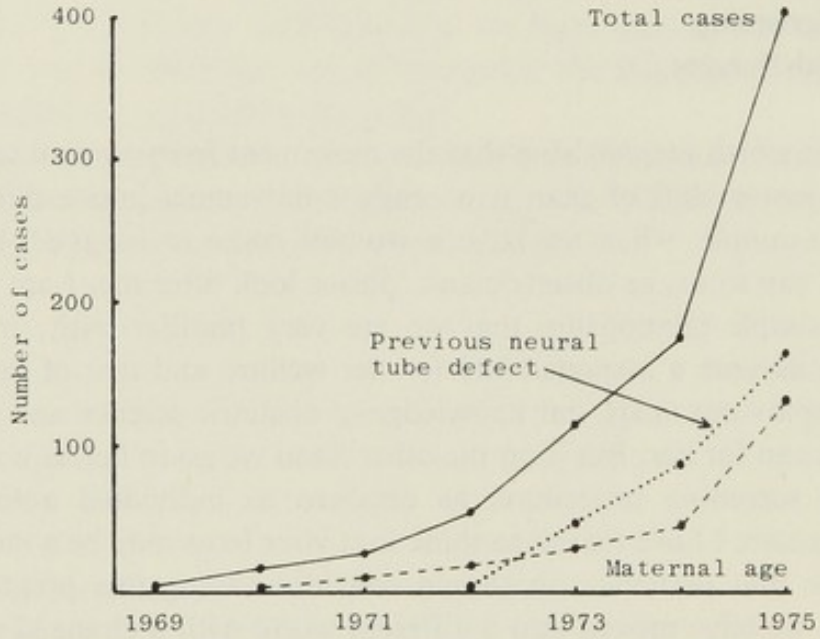

Figure 1. Annual number of prenatal diagnoses in the West of Scotland 1969–75 (Ferguson-Smith *et al.* 1976).

Again, support for the proposition that the new-found diagnostic capability is gaining ground is provided by the data shown in table 1. Here we have taken data from our Scottish Abortion Statistics, selecting terminations at eighteen weeks and over and extracting the figure for those where the grounds for termination include the so-called Ground 4, that is to say where there is a substantial risk that the child if born would have suffered from such physical or mental abnormalities as to be seriously handicapped. If we look at the overall trend of abortions we see first of all a reduction in the numbers of week 18 + abortions, reflecting appreciation of the advantages of intervention at an earlier stage of pregnancy. We are seeing again some increase in the later abortions, but the interesting feature is the proportion of these late abortions where the reason for termination is given as risk to the fetus. One in eight of these is now attributed in that way. It is reasonable to assume that the explanation for this sudden increase is related to an increased detection of affected fetuses by means of antenatal screening.

What could we hope to achieve if we were to set out to maximise the

Table 1. Abortions at 18+ weeks carried out for statutory grounds that include ground 4 (risk to the fetus) as a proportion of all abortions at 18+ weeks (Scottish Abortion Statistics).

Year	Abortions including ground 4	Total abortions	Proportion (%)
1969	9	330	2.7
1970	17	362	4.7
1971	11	372	2.9
1972	10	344	2.9
1973	20	316	6.3
1974	13	250	5.2
1975	36	291	12.4

benefits of screening for fetal malformation? To help answer that question we should first examine the implications of a non-interventionist policy, measuring how much perinatal loss is attributable to this course and how much handicap affects those who survive. There is another aspect to be taken into consideration—one that is highlighted by Mikkelsen *et al.* (this volume)—and that is the influence of a child handicapped by congenital anomaly on the rest of the family. Clearly the amount of stress and anguish such an event generates is immeasurable. But I want to dwell on it first for a moment, since it stimulates a train of thought that in a world in which we encourage family planning and restriction of family size we are in some ways taking aboard a responsibility in relation to the quality of that smaller family. At any rate one can surmise that, in this world of fewer children and smaller families, there is going to be a growing sense amongst those who have the families that they want their children as far as possible to be fully fit physically and mentally. In the long term, that is perhaps the biggest pressure which may come upon us.

Table 2 shows, over a twenty-five-year period, the shifts in the proportions as well as the absolute rates of deaths from different causes of stillbirth as between social classes. The particular point that I want to emphasise is the fact that although the rates for all classes have fallen the disproportion between social class I and II and social class IV and V, in rates for stillbirths due to congenital anomalies, has substantially increased.

Figure 2 illustrates the extent of perinatal loss, and it shows two separate things. It shows the fall in total perinatal mortality rates in Scotland in the last decade, and the fall has been substantial and fortunately continues. It shows also the percentage of these perinatal deaths that are attributable to congenital abnormalities and specially to neural tube defects.

Figure 3, presenting the data taken out of the last but one line of table 2, brings out more clearly the changes in the stillbirth rate from congenital anomalies, and emphasises how it is now considerably more than twice as great in social class IV and V compared with social class I and II. Later in this

Table 2. Legitimate stillbirth rates per 1000 legitimate total births by social class and selected cause, Scotland 1950–75 (Registrar-General).

Cause of stillbirth	1950–1952			1960–1962			1970–1972			1973–1975		
	I and II	III	IV and V	I and II	III	IV and V	I and II	III	IV and V	I and II	III	IV and V
Chronic and other maternal conditions unrelated to pregnancy	0.7	0.9	1.1	0.5	0.5	0.8	0.3	0.2	0.3	0.1	0.1	0.2
Toxaemias	2.1	2.5	2.4	1.4	1.8	1.9	0.8	1.0	1.2	0.8	0.8	1.0
Difficult labour	3.1	3.2	3.7	1.0	1.1	1.5	0.2	0.4	0.5	0.2	0.2	0.3
Other complications of pregnancy and childbirth	3.0	3.2	4.2	2.1	3.0	3.7	0.3	0.6	0.7	0.2	0.3	0.4
Placental and cord conditions	4.9	5.0	6.1	3.5	4.0	4.6	2.9	4.2	4.9	2.7	3.6	4.3
Other conditions of fetus and newborn	4.0	5.4	6.3	3.9	4.4	5.3	3.2	3.3	3.5	2.2	2.8	3.2
Congenital anomalies	3.7	5.2	5.7	2.9	4.8	6.0	2.5	3.3	4.2	1.7	3.2	4.1
All causes	21.6	25.4	29.5	15.3	19.8	23.9	10.1	12.9	15.3	8.0	11.1	13.5

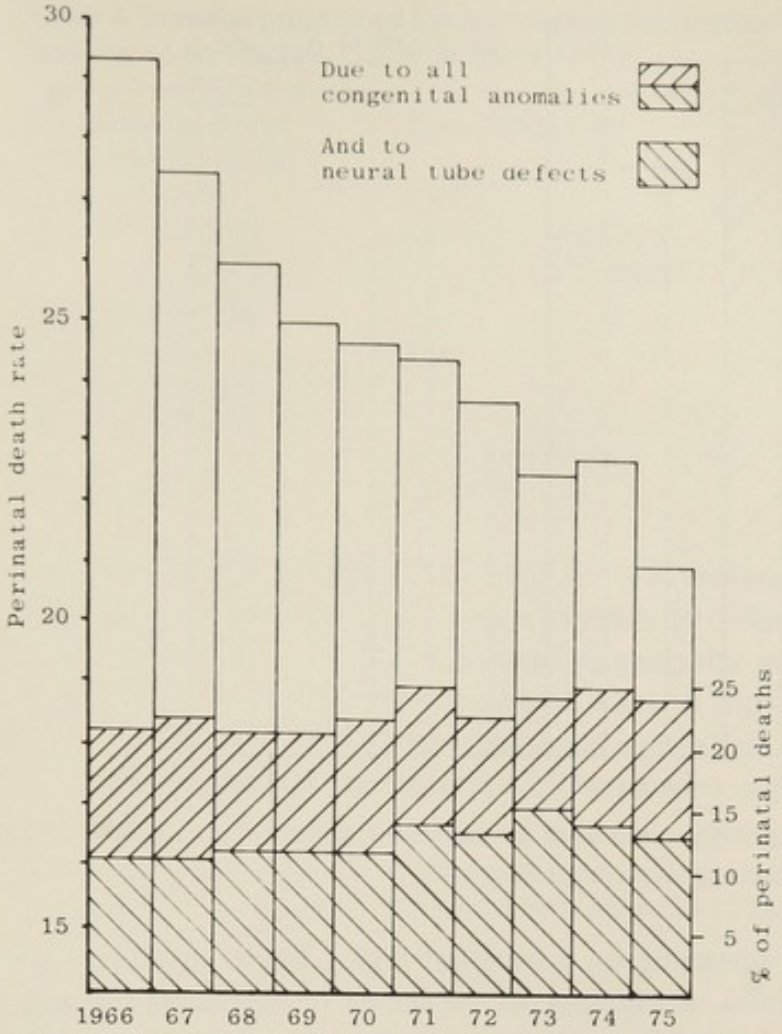

Figure 2. Perinatal death rates, and percentages of perinatal deaths, in Scotland 1966–75 (Registrar-General).

paper I shall speculate very briefly on this point as a challenge in terms of prevention.

A practical issue likely to emerge in presenting a screening programme is the difficulty of gathering into the net all the patients who should be screened. We see in table 3 the proportion of late antenatal booking by social class in Scotland, and it is obvious that there is a substantially greater proportion of presentation late at antenatal clinics, that is to say after more than twenty weeks, in social class v as compared with social class i and ii. Screening is, to date, what one might call a middle-class kind of activity. It appeals to groups in the community that have a sense of time and future and a sense of programme; it is much less appealing to social groups that lack that kind of programme mentality. We have seen, for example, in the screening of cancer of the cervix that those who have the problem do not come and get it done, and those who come and get screened tend to be from upper social groups, which by and large do not have the problem. Equally, in the context of prenatal screening, there is a risk that high-risk groups may elude us unless our screening programmes are very carefully designed to capture them.

Let me continue my review of the size of the problem we face if we con-

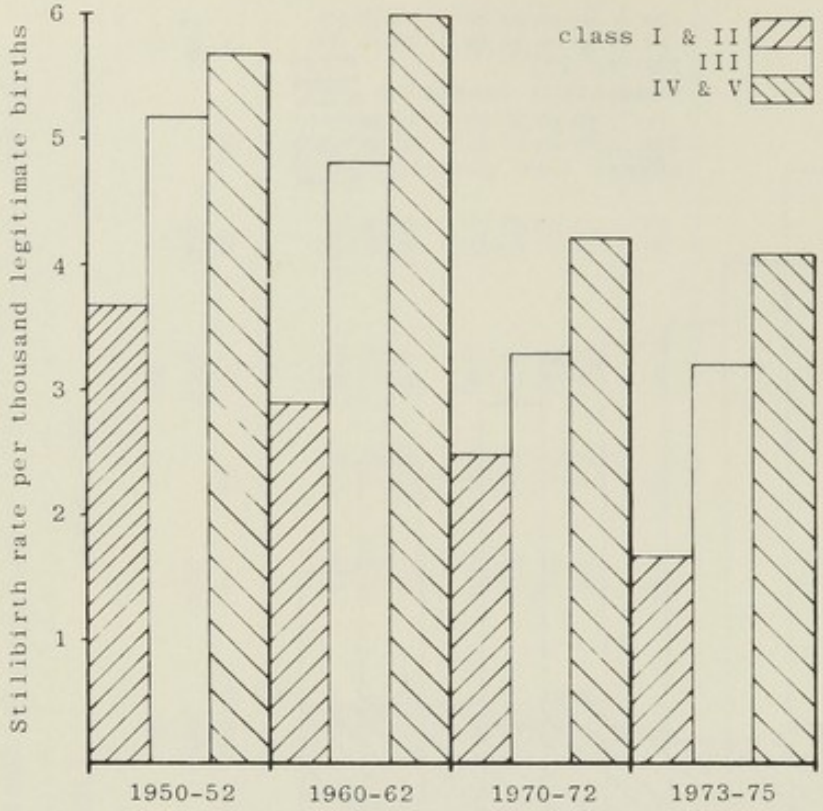

Figure 3. Congenital anomalies as causes of stillbirth in Scotland (Registrar-General).

template antenatal screening on a national policy basis. In table 4 we have taken our 1975 births, attributing an incidence of anencephalus and spina bifida in Scotland derived from the rates used by Hagard, Carter and Milne (1976). A total of 344 children would be affected, half of these would be anencephalics and would either be stillborn or die soon after birth. Birth survivors of spina bifida would amount to 141 and at one year 77 would still be alive. At 5 years those surviving would number 69, of whom nine would have a meningocele and sixty a myelocele. The great majority of meningocele patients have little handicap, many coming from the group of 'closed' lesions, which account for about fifteen per cent of all neural tube defects. Of the estimated sixty children with myelocele surviving at age 5 the degree of handicap would be as shown in table 5, and it is claimed that fifteen per cent of all

Table 3. Percentage of married women in each social class making an antenatal booking after more than 20 weeks' gestation (excluding women whose date of LMP is uncertain), Scotland 1971–73.

Class	1971	1972	1973
I	28.4	27.2	27.0
II	35.3	32.3	29.8
III	36.3	33.4	30.6
IV	39.3	37.8	35.3
V	47.1	44.2	40.5

Table 4. Survival projections for anencephalus and spina bifida cases in Scotland (based on Hagard, Carter & Milne 1976).

Estimated incidence	5 per 1000 births
1975 Scottish births (provisional)	68 708
Numbers affected	344 (172 anencephalus, 172 spina bifida)
Spina bifida births	
82% liveborn	141
45% surviving at 1 year	77
40% surviving at age 5	69 (9 meningocele, 60 myelocele)

survivors will be in permanent care at age 30—all seriously handicapped.

The incidence of Down's syndrome varies with different localities. While an overall incidence of 1.67 per thousand is generally accepted, Glasgow is said to have an incidence of 2 per thousand. Table 6 shows the distribution of prevalence of Down's syndrome by maternal age; again we have taken provisional birth figures for Scotland for 1975 to give a prediction of actual

Table 5. Breakdown of 60 children with myelocele surviving at age 6 by degree of handicap. Estimated numbers born in Scotland in 1975 (based on Hagard, Carter & Milne 1976).

Moderate physical	15 (25%)
Severe physical, normal intellect	33 (55%)
Severe physical, moderate mental retardation	8 (13%)
Severe physical, severe mental retardation	4 (7%)

numbers likely to be affected employing rates used by Hagard and Carter (1976). From 67 708 live births a total of 82 children would be born with Down's syndrome. 17% of these would be children of mothers aged 40 upwards, 31.6% to mothers aged 35 upwards.

Perinatal loss as a result of Down's syndrome is not remarkable and the survival rate shown in table 7 is probably a conservative one. (As an example of the increasing life expectancy of sufferers from Down's syndrome we

Table 6. Estimated numbers of liveborn children with Down's syndrome by 5-year maternal age groups, Scotland 1975 (based on Hagard & Carter 1976).

Maternal age	19–	20–24	25–29	30–34	35–39	40–44	45+	Totals
Birth prevalence, 1 in	1685	1352	1133	687	267	67	16	
All live births	8267	22700	23579	9169	3237	762	55	67770
Down's syndrome	5	17	21	13	12	11	3	82
% of total Down's	6.0	20.7	25.6	15.8	14.6	13.4	3.6	99.7

Table 7. Prospective survivors and mental handicap rating of 82 liveborn children with Down's syndrome (based on Hagard & Carter 1976).

76% survival rate at 1 year	= 62
69% survival rate at 5 years	= 57
27% survival rate at 45 years	= 22
IQ range 50–69	16 (20%)
IQ range 20–49	62 (75%)
IQ below 20	4 (5%)

recently noted for the first time that a patient so affected in one of our mental deficiency hospitals had had a prostatectomy.) Based on our predictions for 1975 births, we could expect to have 57 of the affected children surviving to 5 years by which time four, or seven per cent, would be in permanent care. It has not proved possible to arrive at an accurate figure for the actual numbers of survivors of Down's syndrome in Scotland at present. However, it is estimated that twelve per cent of the 7000 patients in mental deficiency hospitals in Scotland have this disorder and probably rather more than an equal number are cared for at home, so that there are likely to be more than 2000 affected individuals in the country.

We have heard good evidence of satisfactory cost-benefit analyses relating to Down's syndrome if antenatal screening is offered routinely to, and accepted by, women aged 40 and over. If the age were to be reduced to 35 we are told that the benefit-cost ratio is less striking but might still be economically justified. Initially at least the over 40s should be encouraged to take part in the programme. The cut-off point might subsequently be lowered in a national programme as our knowledge of the epidemiology and likely cost-benefits improved and as resources permitted. Even if it were decided that 35 be the cut-off point we could still be failing to detect over two-thirds of all children born with this condition. The trend towards couples completing their families at an earlier age could add to this proportion. Dr Mikkelsen (this volume) makes the point that it would be of immense importance to identify women with risk factors other than age group, for example, and to encourage intensive research in this direction.

I have given examples of the kind of burden we have to carry if there is no intervention. The costs of non-intervention are considerable in economic terms as well as in human misery. Past attempts at early surgical intervention for neural tube defects raised the survival rate but the later quality of life in survivors cast doubts on the wisdom of such procedures unless applied employing strict case selection.

How then do we in the Health Service see ourselves responding to this problem? I think I can say that despite all the other discouraging elements in the situation, which Professor Fairweather (this volume) quite rightly refers to, there are a few signs that might give you encouragement about prospects of support in the future. Screening can be said to be a component of preventive medicine and having in mind the emphasis now being placed on preven-

tion in such publications as 'Prevention and Health—Everybody's Business' (D.H.S.S. 1976a), 'The Way Ahead' (S.H.H.D. 1976) and 'Priorities for Health and Personal Social Services in England' (D.H.S.S. 1976b), we can expect viable screening programmes to attract reasonable support. 'Prevention and Health' devoted a section to antenatal screening for congenital disorders, and among the first of the follow-up papers will be one focussing on services relating to pregnancy and childbirth and setting out the possible problems as well as the advantages of a genetic counselling and prenatal diagnostic service.

We have also to consider the possibilities emerging from the Amniocentesis Report prepared by the Sub-Committee on Screening in Medical Care, which is being circulated amongst all the various consultative groups of the Health Service at the present time. If this report says that antenatal screening should become policy on some basis such as for women over the age of 40, for women with a previous child with Down's syndrome, for women with a previous child with neural tube defect, then I think the health services are bound to look at it very carefully. But let us remember the very substantial gap that exists at the present time between the resources that a national screening policy would need and what we have actually got on the ground. If resources are made available, we ought to think of these in the first instance as providing opportunities for building upon our existing capacities to do the job. It is vitally important to us not to underestimate the difference that exists between a research-based procedure and a routine service procedure. There is a very substantial psychological difference, which we know about from experience where we have made this move in other services. The research worker can only be expected to carry a systematic service load for a certain period of time, after which his interests and his personality take him on to other things and he wants to disengage from a service commitment. One of the things that we have got to learn to do is how to pass over this responsibility from a research group to a service group. We must also take great care in doing this that we do not overwhelm our resources, not just in terms of money, but in terms of the technical skills to do the work accurately. In other situations we have had experience of moving into a mass service too quickly and getting into all sorts of trouble with false positives, false negatives and so on, because we have not given our skills time to build up.

There has been reference in discussion to the substantial decline in the birthrate that we have experienced in the past few years and the conclusions that may be drawn from this, including the expectations of economies. If you find that these conclusions are mistaken, if you feel that there is some new service that needs to be given, and that fewer births afford the opportunity to provide greater quality of service, then you have got to make this point. Your comrades-in-arms in the ranks of obstetrics who function in the central consultative process are already beginning to respond to this challenge.

Let me stress that it is important we should be thinking of this problem in relation to primary prevention as well as secondary prevention. Figure 3 indicates some kind of environmental influences at work, which we need to

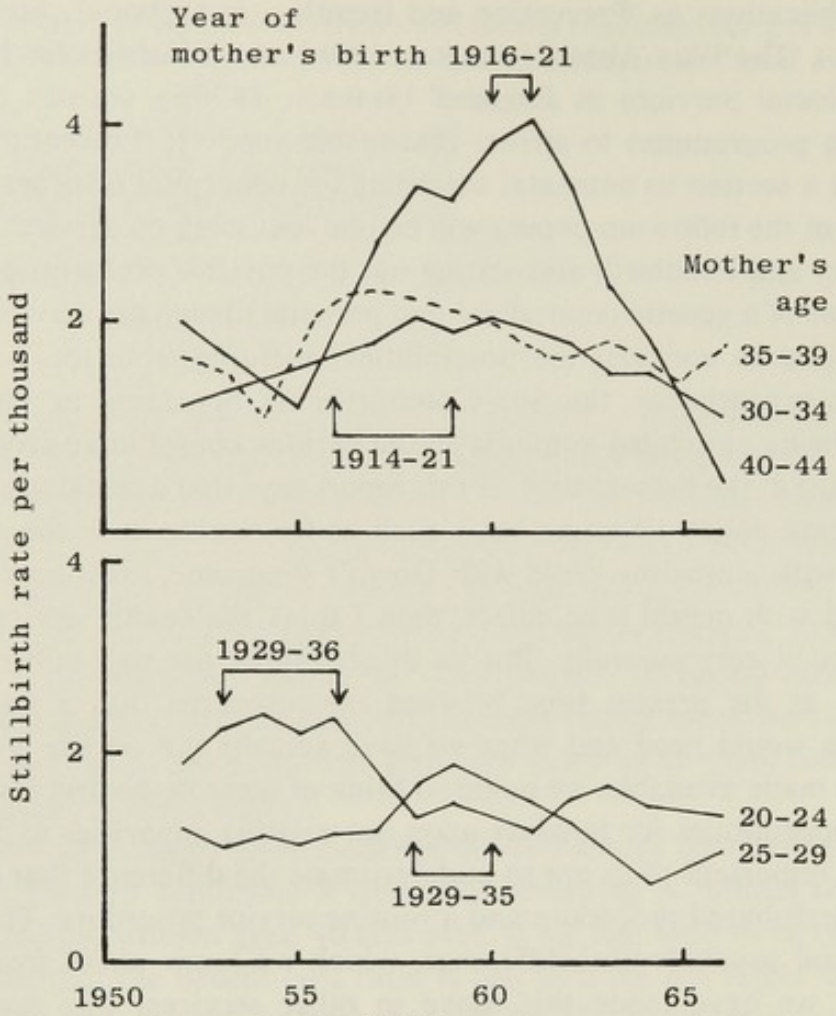

Figure 4. Changes in the stillbirth rate from anencephalus in social classes I and II by age of mother, Scotland 1952–66 (Baird 1974).

know more about, which we need to study so that we can look at the possibilities of diminishing the whole incidence of congenital anomaly. Figure 4 comes straight from Baird (1974) and is an example of his interesting work, relating periods of higher incidence of anencephaly to the date of birth of the mother, and showing that there seems to be some sort of congruence between the environment of upbringing of mothers and their later reproductive efficiency. Mothers who grew up in environments of deprivation, for example, during the great depression of the late 1920s and '30s show reduced reproductive efficiency. I cannot do more than leave you with the thought that this is an important field for us to think about, not only because of the traumatic processes that are involved in a policy of secondary prevention, but also because an understanding of environmental influences might enable us to diminish the total prevalence of these problems.

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THE FUTURE

Future Developments in Antenatal Screening and Diagnosis

In considering the future developments in screening for, and prenatal diagnosis of, fetal malformation or abnormality in relation to the prevention of the birth of seriously affected infants I shall look at two broad sets of issues. First, the scientific and technical side, which revolves around the perfecting of present techniques and the development of new ones, and secondly the many closely interwoven practical socio-ethical issues, which are both present and future issues.

Our present state in respect to prenatal diagnosis and genetic screening stems from a joining of two currents that coincided in flow, direction and timeliness in a rather remarkable way: a quite sophisticated level of technical achievement and a social change directed, in essence, at a variety of procreation changes and controls, including abortion. The result of the fusion of these two currents is selective abortion generally based on at risk concepts and, because of their predictive value, often related to high-detriment and incurable genetic or part-genetic conditions. Thus society, through our understanding of some of the basic biological facts, has begun to realise its power of control over some birth defects. The motivation has been to diminish the burden of unhappiness of individual families, a process that may be partly related also to the pressures in Western societies to limit family size. Organised society, namely the State and Government, has taken a more materialistic view by working out the balance sheet between the expense involved in applying the technology and the benefits derived, viewed, at least in great part, as a reduction of expense to society as a whole; a cost-benefit analysis. Admittedly other factors enter the balance equation, but their computation in the analysis formula and their weighting are extremely difficult because that weighting is the weighting of individual families and their very individual appreciations of risk, sorrow, anguish and their counterparts, relief, tranquillity and pleasure.

Looking at the future of prenatal diagnosis and genetic screening directed at the recognition of the malformed fetus, one might be tempted to take a long view, far into the future, and to work on the assumption that all is possible once it is thought desirable (Medawar 1963). The problem of gazing into the future would thus reduce to an exercise in forecasting what will be

considered desirable. Our present state on prenatal diagnosis is likely to be a transitory phase of the way we handle our genetic problems. To speculate on this theme we would have to look quite far into the future, as, for example, Haldane (1963) did. However, to quote Medawar, 'by what conceivable process can we predict what people are going to think desirable, even in fifty years' time'. So all in all it is far better to look at the near future, at the interface between now and then.

Consider first some of the many technical problems related to prenatal diagnosis, whose solution seems particularly important in practice. A first question that could be posed is: which way will things go in respect to diagnosing chromosome disorders prenatally? It seems that we shall want quicker methods of growing amniotic cells. At present one cannot foresee that fetal lymphocytes will be used, except on rather rare occasions. Speeding up of culture time is important for many reasons, for example to relieve patient anxiety. It could also be needed if there were an increase of the legal constraints on the timing of abortion. However, the difficulty is to persuade amniotic cells to divide rapidly though there may be ways of speeding them on in the future. Perhaps one could try to exploit differently the amniotic cells by selecting for quick culture those that are about to divide—the remaining cells would be cultured conventionally, just in case—and pay more attention to the details of handling the amniotic fluid immediately after withdrawal. However any speeding up of the chromosome culture procedures would be unacceptable if it were to lead to a loss, however small, of the present standards of accuracy and of present checks.

As part of the speeding up of the cytogenetic procedures it is probable that we shall want quicker methods of chromosome diagnosis. There are many different ways that could be considered, some more conventional, some less conventional. Among the former there is computer analysis based on pattern recognition or computer-aided analysis based on interaction between man and machine. Among the latter, one possible way could be chemical or chemico-cytological, which could be based on nucleic acid hybridisation techniques. Purely as an example one can take the human Y chromosome, but it must be stressed that the example is trivial because there are far simpler methods for recognising the Y. Nevertheless the method may have general applicability. It has been shown that the human Y chromosome contains a sizeable segment of a distinctive kind of DNA that accounts for about seventy per cent of the chromosome, according to Cooke (1976). The segment consists of several thousand copies of a simple DNA sequence. RNA copies can be made of the specific Y-chromosomal DNA and this complementary RNA anneals specifically to the sequences of the special Y-chromosomal DNA of the male. It is clear that the complementary RNA can be used for *in situ* hybridisation to reveal the presence of the Y chromosome. What is important is that it need not be necessary to culture the cells if this chemical method could be made to work on uncultured cell nuclei rather than on chromosomes. Or there may be more purely chemical approaches aimed at extracting the

nuclear DNA and showing up the special DNA component of the Y chromosome.

In essence, similar methods could be used for detecting and quantifying other multiple copy genes such as the ribosomal genes. As chromosome 21 is one of the chromosomes that carries a set of ribosomal genes, the hybridisation method may be sensitive enough to spot trisomy of chromosome 21, or its monosomy, by a chemical test. The situation is similar in respect to the other acrocentric autosomes of the human set. Other DNA segments may be used as chemical chromosome markers and even genes usually present in single copy, though there are many technical problems to be solved. Nevertheless, some of the basic molecular biology techniques are becoming available and would allow identification of a selected gene and thus of, say trisomy or deletion of a specific chromosome or chromosome segment that carried the selected gene. In the case of the globin genes deletion of a gene has been detected already (Ottolenghi *et al.* 1974), even in amniotic cells (Kan, Golbus and Dozy 1976). So in this special and restricted case a chemical approach would seem feasible, and, by an extension of these methods, in the future it may become possible to identify extra chromosomes, or parts of them, by a chemical dosage test of their genes and DNA stretches.

There are yet other suggestions about chemical tests for the detection of chromosome imbalance (see below), but, more conventionally, in chromosome studies, technical advances in the processing of chromosomes should aim at the recognition of more minute deletions than is possible at present. These may well underlie a number of serious diseases with dominant inheritance, posing as diseases caused by autosomal or X-linked dominant point mutations.

Turning to neural tube defects, the present situation concerning prenatal diagnosis in high risk pregnancies is well known. New developments, and perhaps new strategies will depend on the application of serum alpha-fetoprotein studies in pregnancy (Brock, this volume). The results of the U.K. collaborative study (1977), based on an investigation of many pregnancies in many centres, are now available. The study gives quite clear indications of the usefulness of pregnancy serum alpha-fetoprotein measurements as a screening test. Advantages and disadvantages in sheer terms of diagnoses made, especially of spina bifida, would require careful socio-ethical and practical consideration (see below; Brotherston, this volume; Glass, this volume). If the results of the U.K. study were to give support to schemes of pregnancy screening it is unlikely that population screening would *automatically* follow or indeed could be achieved immediately even if screening were accepted as desirable (Brock, this volume).

In the field of biochemical prenatal diagnosis the main emphasis is already toward true miniaturisation of the tests (Galjaard, this volume; Patrick, this volume) without loss of accuracy. One of the developments is going to be towards the detection of those difficult biochemical conditions that, at the moment, escape direct enzymological detection. Some of the

methods will be cytological in the first place. Of this type, for example, are the tests used, or to be used, in the assessment of sister chromatid exchanges for Bloom's disease, and of chromosome breaks and gamma-radiation sensitivity for Fanconi's anaemia and some of the ataxia telangectasias respectively. Mention should also be made of the possibility of using cellular electron-microscopical markers of genetic disease. And as an example of future developments one might consider the as yet unconfirmed observation (Wyatt and Cox 1977) that cultured fibroblasts in Duchenne's muscular dystrophy show characteristic electron-dense inclusion bodies when studied by transmission electron-microscopy. Such a morphological method, if applicable to amniotic cells, may well be applied to specific diagnosis but is likely to be an interim feature, and may be replaced by chemical tests on fetal blood (Galjaard, this volume). One of the developments that might be useful would be a battery of tests directed at the rapid, screening-like, simultaneous, group detection of a number of inborn errors. The tests, of course, would be rather non-specific. The idea is similar in concept to the alkaline phosphatase test, which Hösli (1976) claims could be used for the detection of a whole group of lysosomal mutants. Indeed, he has claimed that such a non-specific chemical test could be perfected to predict the presence of many chromosome anomalies with imbalance.

A difficult problem is posed by dominant conditions in general. Linkage, autosomal and X-chromosomal respectively, may be one approach in dealing with these 'dominant' disorders. As the gene map of man grows this method might well become useful compared with its negligible usefulness at present (Schrott, Karp and Omenn 1973; Smith 1973). However, in addition to the limitation that linkage tests can be useful only in the few families with opportune segregation, there is the problem that the marker gene must be expressed in fetal life and in the amniotic fluid or cells.

The detection of polymorphic situations in man when a mutant gene is frequent in the population could be particularly relevant. At the moment less than a hundred polymorphisms are known. If man is indeed polymorphic at about one-third of his gene loci (Harris 1975), there are perhaps some 10000 or more (Motulsky 1970) polymorphisms awaiting discovery. If the average individual is heterozygous at seven per cent of his gene loci, including those polymorphic, there is a fair chance that the detection of dominant detrimentals, through their linkage to genes with useful, i.e. high population, frequencies, might become a practical proposition until the time when direct prenatal tests for these disorders become available.

Naturally the greatest bonus would be the ability to detect in cultured amniotic cells the products from alleles that are ordinarily not expressed in these cells. The problem has an even wider interest because its solution would be a very important step forward, both practical and theoretical. This achievement is probably some way off but attempts at finding some either general or *ad hoc* solutions to the problem are being pursued. Galjaard (this volume) has touched on a general approach through cell hybridisation (see also

Darlington and Ruddle 1975). A different, *ad hoc* approach has made it possible to find the dose in amniotic cells of the alpha-globin structural genes usually expressed only in the erythron. By this means the prenatal diagnosis of homozygous alpha-thalassaemia has been confidently excluded in a high-risk pregnancy and the diagnosis of haemoglobin-H disease has been made in another (Kan, Golbus and Dozy 1976; Valenti, this volume).

The present state in respect to the haemoglobinopathies, especially those that reach polymorphic frequencies in some populations, for example sickle-cell anaemia and the thalassaemias, is discussed by Valenti (this volume; see also Lancet 1977). Given the availability of fetal blood, even if heavily contaminated by maternal cells, the laboratory procedures for the detection of the abnormal status of globin chain synthesis, in the diseases under consideration, seem extremely accurate in expert hands (Alter *et al.* 1976a, b; Kan *et al.* 1977). However, from a strictly technical viewpoint, perhaps the main problem is posed by the methods available for fetal blood sampling, and their safety and more general applicability. If fetal blood sampling became an acceptable procedure one could foresee a number of expert applications not possible at present when one can practically only use amniotic fluid and cells for prenatal diagnosis. In the chemical field possibly the most immediate developments would be the detection of coagulation disorders, such as classical haemophilia A, or of Duchenne's muscular dystrophy. In general, biochemical disorders that are detectable after birth by investigation of the blood or its components may not be too difficult to detect by study of the fetal blood. However, it is clear that much laboratory work and knowledge of fetal systems would be required before a practical application of fetal blood sampling for prenatal diagnosis of other chemical conditions than the haemoglobinopathies were feasible.

A most important practical advance in the biochemistry of prenatal diagnosis is going to be the ability to detect prenatally fibrocystic disease of the pancreas, and to detect simply and reliably heterozygotes in the population. This disease has the high frequency of one in 2000–2500 births among Caucasians, though with interesting variation (Di Sant'Agnese and Davis 1976), and a thirty or forty times lower frequency in Africans and Orientals, a situation similar to that of Tay-Sachs' disease among the Ashkanazim compared with the Sephardim Jews and with the Gentiles.

Various tests have been proposed for carrier detection and indeed for early disease detection (Ward 1976). Especially interesting in the context of prenatal diagnosis is the work of Danes and Bearn on metachromasia of the fibroblasts in culture, a method that, however, has been too temperamental generally, and thus clearly unsuited to prenatal diagnosis. Hösli and his collaborators (1976) have recently claimed that an ultramicro-method can be used to detect, in cystic fibrosis fibroblasts, a high increment of alkaline phosphatase activity following their *in vitro* exposure to a urinary glycoprotein. The prospects for prenatal diagnosis would be clear, but as yet there has been no confirmation of this work. A method like this could only be used in high-

risk pregnancies and not for population screening. However one may predict that in the not too distant future a reasonably simple screening technique for cystic fibrosis will become available. One of the strategies that could then be used would be the screening of pregnant women and, following this, the stepwise detection of their carrier husbands, the prenatal recognition by amniocentesis of their abnormal fetuses and possibly of their hetorozygous fetuses. Conversely, if a test were developed that because of its nature and complexity could be used only to detect the fetus at high risk, the following might be a guide as to what might happen with a retrospective approach of pregnancy testing following the birth of affected children. Given 1100 pregnancies at risk (the theoretical approximate number of matings between heterozygotes in the population), an average of 275 affected children might be born from first pregnancies. Let us assume that in second pregnancies these mothers would avail themselves of the prenatal test. Let us also assume that the other 825 families, unaware that anything was wrong, would have their second babies, one-quarter of whom would be abnormal. Furthermore let us assume that many of the families, given prenatal diagnosis, might aim at two normal children. So some of the mothers would have to have more than two pregnancies and more than one abortion of abnormal fetuses if they desired to reach their quota of normal children. At some stage the 1100 families, using the prenatal diagnostic test coupled with abortion of the affected fetuses, would have produced fewer abnormal children, and, by way of compensation, more normal children, compared with their reproductive performance in the absence of the prenatal diagnostic test (Jones and Bodmer 1974). Of the normal children, two-thirds would be heterozygous carriers of the cystic fibrosis gene.

This brings me to touch upon the future *genetic* effect of prenatal diagnosis in relation to the conditions for which it is being practised. When dealing with autosomal recessive diseases the effect of aborting the affected can, under certain not too implausible assumptions, be considered dysgenic, because the net result can be an increase of the frequency in the population of the genes that underlie these recessive diseases. An extreme situation makes the position clear. Assume that we are dealing with a recessive condition in which the homozygous mutant is infertile or dies relatively early and does not pass on his mutant alleles. Let us assume that prenatal diagnosis can detect the affected. Thirdly let us stipulate that under these circumstances the parents would want to substitute for each affected child one normal one and would aim at having two such children. As already mentioned, two out of every three of these children would be heterozygous carriers. So, for genes in the homozygotes that, by reason of infertility or death before reproduction, would be lost from circulation, there would be substituted genes in carriers that would be immitted into the population to circulate there. Thus the frequency of the abnormal gene would rise. Fraser (1973) has calculated that over a span of many generations and with the assumptions just made, and others, the frequencies of specific genes would rise by some twenty to fifty per cent if pre-

natal screening and elimination of all homozygotes were practiced, but by only some two to four per cent if selective abortion was retrospective to the birth of affected children (see also Smith 1973). Thus, taking a general view of the situation, the rises would be relatively unimportant, would take generations to reach their new equilibria and, indeed, may even be a good thing in respect to those genes for which there is single locus hybrid vigour or other advantageous effects (Polani 1974, Saugstad 1977). The same type of considerations, given the same assumptions, apply to X-linked disease and to those chromosomal situations in which one of the parents is a translocation carrier. With autosomal dominant conditions and with other chromosome disorders there is no dysgenic effect; abortion of the affected fetuses reduces the frequency of the deleterious dominant genes and of the chromosome errors (Smith 1973).

A few comments are required on the future of treatment of genetic disease. The main justification for selective abortion lies in the absence of effective forms of treatment for many of the genetic conditions under consideration, let alone their cure, but methods of treatment are continuously being developed and improved. Treatment is needed at the very least for the management of children born with a genetic disorder for which prenatal or early pregnancy screening is impossible. Nevertheless, the existence or development of forms of treatment raises many ethical and practical issues to which we shall of necessity have to attend increasingly in the future. For example, there may well be genetic diseases in which treatment is best started *in utero*, and in which even a cure may be achieved by intrauterine intervention, possibly on lines that combine the cellular approach of attempts to treat some of the mucopolysaccharidoses (Dean *et al.* 1976) with the intrauterine transfusion approach used in the management of rhesus disease. In such a situation one can easily visualise the problems that could arise in trying to resolve the conflicting options of intrauterine treatment or pregnancy termination. More practically, once fibrocystic disease can be diagnosed prenatally how much effort is going to be applied to the treatment of those who will be born affected on the assumption, for example, that no reliable test will exist for the population detection of carriers? Thus a case can be made for continued research on treatment and consequently for the need for continued research on treatment and consequently for the need for continued financial support for this work. One of the dangers of measuring things too closely in terms of cost-effectiveness is that research and applied work on treatment could be affected. There is a very real risk that this work will stop, and the risk is magnified by the fact that further research on treatment, especially of those diseases that are already prenatally recognisable, will look not only cost-ineffective but may also appear an uninteresting problem, wasteful and purposeless from the purely scientific viewpoint.

Another area that might suffer from the advances in prenatal diagnosis and screening is research on true or primary prevention. It is essential that research on true prevention of birth defects, both clinical and experimental,

be continued. This is clearly the case at the very least for those multifactorial conditions with variable prevalence in different populations, and within a population, where prevention would, *a priori*, seem feasible.

I have taken a rather near-sighted view of the problems underlying prenatal diagnosis and related topics. By implication I have accepted that induced termination of pregnancy of serious, incurable, untreatable genetic disease is here to stay, at least for some time. There does not seem to be an immediate complete alternative to this. There are, of course, hopes that the procedures will be made more effective and, especially, hopes that the necessary action may, in the future, be taken earlier. Nevertheless, it is not impossible that advances will be forthcoming so that one might operate fetal selection *really* early, preferably at the preconceptional stage. As long as one does not accept other reproductive options in certain situations, for example artificial insemination (Richardson 1975), one can see more problems than possible solutions to an approach to prevention by preconceptional methods. However, to take an example, it can be suggested—as indeed it has (Fraser 1973)—that given the ability to separate the X-bearing from the Y-bearing spermatozoa, the use of only X sperm from the husband might circumvent the conception of an affected male when the mother is a carrier of a serious detrimental X-linked recessive gene. Indeed, the efficiency of the method whereby X-bearing sperm might be concentrated would not have to be necessarily very high for the procedure to be useful. All that an imperfect method would require is monitoring of the special pregnancy by amniocentesis.

I said at the very beginning that when considering the future of prenatal diagnosis and genetic screening in addition to technical and scientific issues there were important socio-ethical issues to be considered, both present and future. I will attend briefly to this second dimension of prenatal diagnosis, counselling and especially screening for genetic and related diseases. I have left the topic last not because I believe that it is unimportant, nor because it is easy to deal with, but for the very opposite reasons. Many difficult and troubling problems in genetic screening seem to rest with the socio-ethical issues that they raise. Without wishing to minimise the scientific and technical difficulties or the organisational ones, or the financial problems, options and dilemmas, it is in the socio-ethical field that many serious challenges exist. It seems desirable that the practice of genetic screening—and what I am saying for this activity applies to an extent to prenatal diagnosis of high risk pregnancies as part of genetic counselling—should satisfy a number of practical criteria. Many of these are obvious and, taken together, they add up to the many proposals that have been made from time to time. These are discussed in detail in a monograph published by the Committee for the Study of Inborn Errors of Metabolism of the National Research Council, U.S.A. (1975) and are briefly summarised below. In considering how the recommendations of the Committee would apply to genetic screening in Great Britain, there are many differences due to different attitudes, mores, customs and legal system. I should also stress that the criteria as a whole are addressed to genetic screen-

ing in general and not specifically to prenatal diagnosis. Allowance must be made for these differences. So, for example, not all points made by the Committee would necessarily be relevant if screening for high risk of neural tube defect were to be considered in Great Britain. Nevertheless, within these limitations, the criteria set out by the working party would undoubtedly repay careful study and consideration, side by side with the criteria suggested, for example, by Cochrane and Holland (1971) and by Whitby (1974), as providing sound bases for the adoption of a screening programme. The aims of genetic screening should be precisely spelled out, and the public, and the medical profession, should accept to a large extent both the objectives and the procedures.

Clearly the feasibility of screening should be carefully studied, a pilot study should be done and should show that the tests to be used in screening have the required qualities of being safe, simple, acceptable, precise, sensitive, specific and not disproportionally costly. Obviously laboratory facilities would have to be available and their quality must be of the required standard, proved to be so by a quality control study. There should exist resources and provisions for the necessary counselling, incident upon screening; the methods of communicating information to all concerned should be worked out; and provisions should be made for the essential follow-up, and for monitoring. Attention should be directed particularly at attracting to the screening procedure all sections of the community at risk. It has been said that those who set out to screen are actually advertising. Obviously to advertise a ware without being able to supply the product is foolish. To advertise screening without the required back-up is unethical.

The U.S.A. Committee feels that very special attention must be given to the particularly sensitive areas of privacy, risk of stigmatisation, and to confidentiality. This sensitive step in particular requires public representation and participation in developing the screening programme, though how to achieve this is, in itself, a major problem. The question of consent to take part in a screening programme is a complex and difficult one. The U.S.A. Committee considers it in relation to a screening programme—for example like the one concerned with Tay-Sachs disease—and sees the need for informed consent to screening. In the context of public involvement, it is considered important that attention should be paid to the very real difficulties that the public in general finds in understanding the genetic and other biological principles, which relate both to the disorders for which screening is contemplated and to the screening procedures themselves. The Committee felt that it is only through understanding and discussion that informed participation in, and consent to, screening, and to the screening procedures, has a meaning.

There are many legal implications of some of the points just raised and they are bound to differ from country to country. The most important is that, in general, taking part in a screening programme should not be made compulsory by law. Another requirement should be mentioned: it is that the aims of screening, vis-à-vis actual or potential treatment for the conditions that are

being screened, must be clearly understood (Lappé, Gustarfson and Robbin 1972). As well as a host of practical problems, genetic screening in a wide sense and prenatal diagnosis clearly have profound social implications—which have been touched upon in this volume—and raise fundamental ethical issues, now and in the future. While limitations of competence and space do not allow me to enter into a discussion of the latter, moral issues, I would echo Firth's (1952) views of the essential prerequisites of ideal observers, oriented to absolute ethical decision making: knowledge, perceptivity, impartiality, dispassion, and consistency.

In conclusion, it is perhaps appropriate to look once more at the technological and scientific position. When we consider the impact of scientific thought and technology on society (Ciba Foundation Symposium 1972) the tendency generally, when things are not especially acceptable or palatable, is to represent the scientist as the perpetrator and to cast society in a passive role. It is however notable that other views of the relationship have been put forward from many directions, and bear scrutiny. To speak for these views I will quote from Lord Brain. In *Science and Man*, Lord Brain starts with the obvious premise that a scientist is a member of society, raised within society, educated by society for his work, paid by it, for it. There follows the argument that often it is society that fails to foresee the consequences, and thus that it is society that is 'responsible for what it does, or fails to do with the scientist's discoveries' (Brain 1966). Nevertheless the scientist *has* very special responsibilities, not least the responsibility to 'try to lead scientific advances by positive action' (Florey 1964).

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Fetal Blood Sampling in Early Pregnancy

Inheritable haemoglobinopathies, in particular alpha-thalassaemia, beta-thalassaemia and sickle cell disease, are the most important genetic disorders in the world. The heterozygous conditions, present in millions of people, can be recognised accurately in the laboratory. By prenatal diagnosis the couples at risk could be effectively counselled, so as to prevent the birth of their affected children.

Although they are disorders of the adult haemoglobin, it has been demonstrated that at five weeks' gestational age the human fetus is already capable of producing adult haemoglobin (Walker and Turnbull 1955, Kazazian and Woodhead 1974). The expression of the sickle cell and thalassaemia genes has been detected during the first trimester (Kan *et al.* 1972, Kazazian *et al.* 1972). Techniques aimed at obtaining fetal blood *in utero* have been developed, and the samples successfully analysed for the *in utero* diagnosis of these conditions. Failures to obtain fetal blood, obstetrical complications and diagnostic errors published so far indicate that a considerable amount of improvement and simplification of the methods adopted is necessary before a sizeable preventive programme could be entertained.

The difficulties seem to be concentrated on the obstetrical aspects of the problem related to sickle cell anaemia and beta-thalassaemia. For the prenatal diagnosis of alpha-thalassaemia the fibroblasts cultured from the amniotic fluid have been successfully used, with the technique of DNA-DNA hybridisation, so that this condition escapes the difficulties of fetal blood sampling (Kan, Golbus and Dozy 1976).

Techniques of Fetal Blood Sampling

Fetal blood *in utero* has been sampled during the second trimester by two different approaches: (a) placental venepuncture under direct vision, using an endoscope called an endoamnioscope or fetoscope (the latter term may be confusing, since it is commonly, although erroneously, used in the U.S.A. to indi-

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This paper was received too late for inclusion in the section entitled 'The Diagnosis'.

cate an instrument to listen to the fetal heart!); (b) placental aspiration, with which fetal blood or a mixture of feto-maternal blood may be obtained.

Fetoscopy

The endoscopic instruments used vary in diameter from 10 mm (Westin 1954), to 7 mm (Valenti 1972), to 2.2 mm (Scrimgeour 1973), to 2 mm (Kaback *et al.* 1973). The largest instruments have been introduced through the cervical canal (Westin 1957) or transabdominally, through a small laparotomy incision under general or regional anaesthesia (Scrimgeour 1973; Valenti 1972, 1973, 1974). Westin was able to photograph fetal limbs between the 16th and the 20th week. All his patients underwent termination of pregnancy subsequently.

The cervical route should not be followed, because the chance of rupturing the membranes, with consequent interruption of pregnancy, is very high. The author (Valenti 1972, 1973, 1974, 1976) has experimented with a modified paediatric cystoscope, with an oval 18 French sheath section, provided with microlens fibre optic telescope (American Cystoscope Makers Inc., New York). Skin biopsy forceps and a flexible 27-gauge needle could be housed within the same cannula.

Some sixteen patients undergoing abortive hysterotomy between 14 and 20 weeks of pregnancy, in accordance with the New York State law, underwent fetoscopy at the time of operation, under regional or general anaesthesia. The placenta was carefully localised by ultrasonography prior to surgery. When the placenta was anterior, the posterior placenta-free wall of the uterus was exposed through a longitudinal abdominal incision. When the placenta was posterior a small transverse suprapubic incision through the abdominal wall and the peritoneum was sufficient. Clear visualisation of the fetus was obtained in twelve out of sixteen cases, although not all the parts of the fetus could be visualised. Still photographs were taken and a 16 mm colour motion picture made in five cases. During fetoscopy fetal limbs and their abrupt motions can be seen, as well as the introduction of a fine needle into a small vessel on the fetal aspect of the placenta. Its withdrawal was not followed by any bleeding in these cases. In fact there is a very rapid formation of an intravascular clot, leaving the vessel intact.

Blood samples from fetal vessels on the placenta were obtained in six instances, without complications. The intra-amniotic pressure, higher than the intracapillary pressure, may explain in part the lack of bleeding after the venepuncture. Fetal lymphocytes were set up in culture and provided numerous suitable metaphasic plates for karyotyping within 72 hours. Also, the blood samples proved suitable for biochemical analysis: hexosaminidase was easily and accurately measured (Valenti 1976).

Fetal endoscopy, with a fairly large instrument such as the one described, involves a laparotomy and significant trauma to the uterine wall, which may jeopardise the continuation of pregnancy should the method be applied to a high genetic risk pregnancy. Smaller instruments, which can be introduced through an intact abdominal wall, are available. A modified

version of the needlescope (Valenti 1974, 1976) has an oval cannula measuring 2.3 mm in its maximum diameter and allows the introduction of a flexible 27 gauge needle. Originally developed for the visualisation of joints, the needlescope uses, for the transmission of light, ion-exchange-treated glass rods of special composition, called 'selfoc', which also function as lenses, with a glass fibre light guide. The endoscope itself has a diameter of 1.7 mm and a view angle of 55°.

The instrument has been used by the author for fetal visualisation and blood sampling in nine patients, all of whom were to undergo abortive hysterotomy between 16 and 22 weeks' gestation. All of these patients had a posterior placenta, localised by ultrasonography. In five of the nine cases, visualisation of the fetus was achieved. In the remaining four patients no visualisation was possible because some bleeding in the amniotic sac was enough to render the medium completely opaque. The limiting factor was, in every case, the smallness of the visual field, which did not allow for orientation and recognition of fetal anatomy. Blood sampling under vision from the puncture of fetal vessels on the inner aspect of the placenta, posteriorly located, was successful in three of five cases where it was attempted. Between 0.03 and 0.5 ml of blood was obtained. Twice it was a mixture of maternal and fetal blood, because the needle had penetrated through the small vessel into the intervillous space underneath. The specimens were still suitable for the recognition of the haemoglobin chains by the haemoglobin synthesis technique.

Scrimgeour (1973, 1976) has successfully applied a 2.2 mm fibre optic telescope with an angle of 110° to thirty patients already scheduled for termination of pregnancy by hysterotomy. Six more patients at genetic risk were also studied. All had had two children affected by malformations of the central nervous system. In one case the fetus could not be visualised. The pregnancies that were allowed to continue did so without untoward effects from the fetoscopic manipulations.

Thirteen fetoscopies were carried out by Laurence *et al.* (1974) between the 14th and the 18th week of pregnancy: twelve were immediately followed by hysterotomy or hysterectomy. In one case, at risk for anomalies of the limbs, the fetus was found to be normal, the pregnancy proceeded without complications and a normal fetus was born at term. The instrument used was a 9 mm diameter end-viewing laparoscope, introduced through a laparotomy incision.

Aladjem (1976) also has experimented with a fetoscope (3.5 mm in diameter) and with a needlescope (2.2 mm in diameter) in fourteen patients about to undergo abortive hysterotomy. No fetal blood was obtained, as this was not the object of the experiment.

Hobbins and Mahoney (1976) experimented with fetal blood sampling under direct vision in 58 patients, between the 15th and the 22nd weeks of gestation, about to have therapeutic abortion. They used a cannula measuring 2.2×2.5 mm housing the fetoscope. The intrauterine contents were visualised in 52 patients. The authors attempted to draw fetal blood seventeen times and

succeeded in fourteen. It is remarkable that in four cases the placenta was located on the anterior uterine wall, and twice a fetal blood sample was obtained!

Fetoscopy has also been experimented with by Levine and co-workers (1976) for the purpose of obtaining fetal blood in eight patients: they were successful in three. All patients were due for an abdominal interruption of pregnancy. Wheeless (1976) performed fetoscopy on eight patients about to be aborted, but did not attempt to obtain fetal blood. Alter *et al.* (1976a) have recently published a series of fifteen pregnancies at risk for hereditary haemoglobinopathies, where adequate fetal blood for prenatal diagnosis of the conditions was obtained twelve times: fetoscopy was used in seven cases, all with posterior placentas, and in all of them adequate samples of blood were obtained.

Altogether it seems that fetal blood sampling under direct vision is still at an early stage of experimentation, with a very small number of pregnancies at risk actually studied. For the moment fetoscopy can and should be used when the placenta is located on the posterior uterine wall. When the placenta is anterior the method of placental needle aspiration is, at this time, the method of choice, until and unless flexible small fetoscopes are developed, which can be manœuvred and directed from the outside.

Placental Aspiration

The detailed localisation of the placenta, and of the point of attachment of the umbilical cord to it, is of paramount importance. The extension and thickness of the placenta and the distance of the fetal aspect of it from the mother's abdominal wall are analysed very carefully and the echograms recorded on Polaroid prints. The exact point of entry for the needle is carefully chosen. Under aseptic conditions and with local anaesthesia a 20 gauge, 3.5 in needle is introduced transabdominally into the placenta. Volumes of 0.1 to 0.2 ml of blood are withdrawn into heparinised syringes. The presence of fetal blood is rapidly determined by the analysis of the red blood cell volume with a model ZB1 Coulter Counter with a 'Channelyzer' (Coulter Electronics). Since the mean corpuscular volume of fetal red cells is larger than that of adult cells, the presence of five per cent or more fetal cells in a mixture can be quickly detected. The Coulter counter is placed in the immediate vicinity of the patient, so that the information becomes known to the operator, and the needle is withdrawn without further traumatisation to the patient. An intravenous solution of isoxsuprine was given throughout the procedure to all the patients studied in Cagliari, Italy. Smears from the same samples were stained with the Kleihauer-Betke method (1957) and the percentage of fetal cells determined. The amniotic fluid withdrawn is examined for fetal blood. The haematocrit is determined and the total volume of fetal blood in the amniotic fluid estimated. The amniotic fluid fibroblastic cells are set up in culture and later subjected to chromosome analysis. Alpha-fetoprotein level in the amniotic fluid is also determined.

This method, described by Kan *et al.* (1974), has been applied to a

number of pregnancies at risk for hereditary haemoglobinopathies by different groups of researchers. Kan *et al.* (1975) diagnosed homozygous beta-thalassaemia in two pregnancies, which were then interrupted on that basis. The prenatal diagnosis was confirmed on the abortuses. Subsequently sickle cell anaemia was also diagnosed *in utero* during the second trimester, by the same group (Kan, Golbus and Trecartin 1976) and by Alter *et al.* (1976b). Recently two series of cases have been published: one with fifteen cases, eight of which were subjected to the technique of placental aspiration and seven to fetoscopy (Alter *et al.* 1976b) and the other with 24 cases, all studied by the method of placental aspiration (Kan *et al.* 1977).

The results detailed here have been obtained in a total of 59 cases, including: (a) 24 cases published already by our group (Kan *et al.* 1977); (b) 21 cases studied by the same group in San Francisco since that publication; and (c) 14 cases studied entirely at the Paediatric Department of the University of Cagliari, Italy, under the direction of Professor A. Cao and with the cooperation of Dr M. Furbetta, in charge of the Prenatal Diagnostic Center, and his co-workers, Drs A. Angius, R. Fais and A. Ximenes. The cases are detailed separately: (a) 45 counselled in San Francisco, Cagliari and Rome and actually subjected to the prenatal diagnostic technique in San Francisco; (b) 14 cases studied entirely at the University of Cagliari.

The San Francisco Series

Forty-one pregnancies were monitored for beta-thalassaemia and four for sickle cell anaemia. Thirty-four couples requested the prenatal diagnosis because of one or more children homozygous for beta-thalassaemia; eleven couples, including two at risk for sickle cell anaemia, were in their first pregnancy. The couple signed a consent form, approved by the 'Committee on research of the University of California in San Francisco', after all the details of the method, its related risks and uncertainties had been carefully explained at great length. Genetic counselling was given to some in Rome, to others in Cagliari or San Francisco. The sampling and analysis of fetal blood were done in San Francisco. The results were obtained within 48 hours of the sampling.

Placental aspiration was applied to all of these cases, using a 20 gauge, 3.5 in needle, between the 18th and the 23rd week of pregnancy, according to a technique previously described (Kan *et al.* 1974; Golbus, Kan and Naglich-Craig 1976). Placental localisation was obtained by echography, at first using a Picker model EV9, subsequently a Picker model L80 'grey scale', and recently a real-time scanner (Advanced Diagnostic Research).

Amniotic fluid was always sampled for chromosome analysis and also for the determination of the level of alpha-fetoprotein. Prior to placental aspiration 3 ml of maternal blood were withdrawn and reticulocyte count, blood group and Rh, and the count of erythrocytes containing fetal haemoglobin, done. The presence of fetal blood in the mixture obtained by placental aspiration was determined immediately by using a 'Coulter particle size

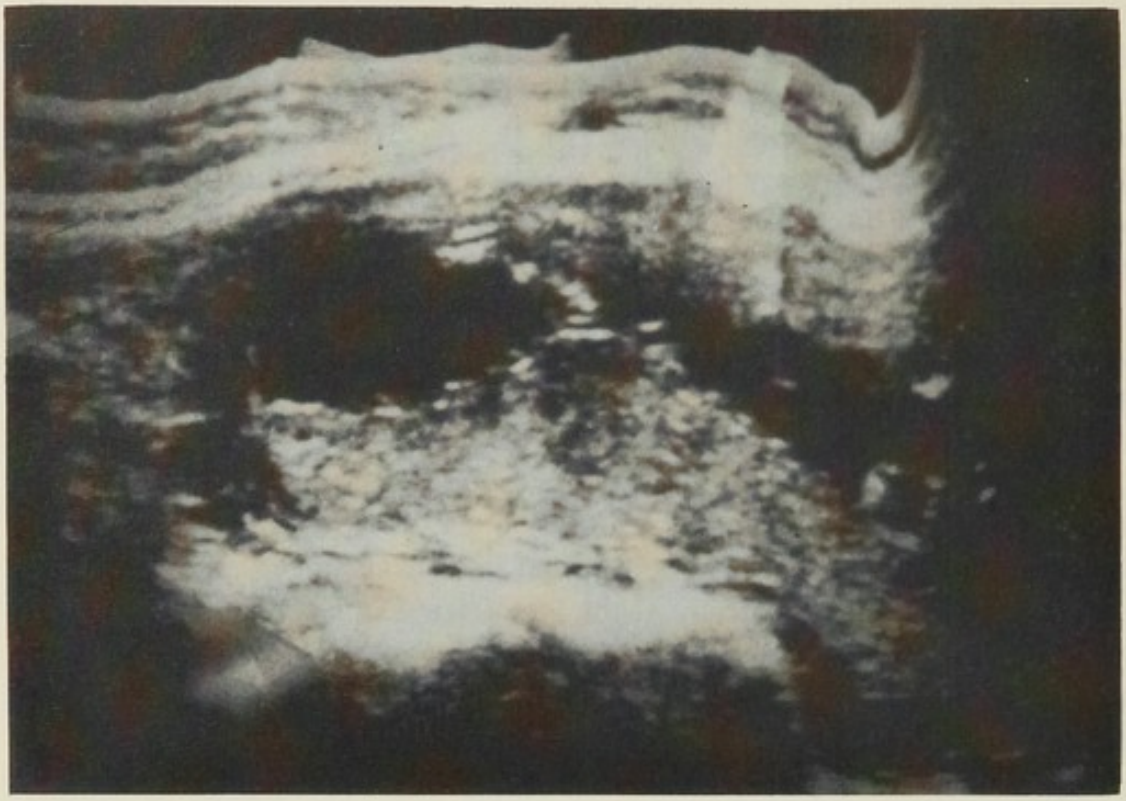

Figure 1. Grey-scale echogram, longitudinal scan, at 20 weeks' gestation. The placenta appears to be located on the posterior uterine wall. The umbilical cord is visible in the centre, in its point of placental attachment. The area of attachment has often a 'conical' shape.

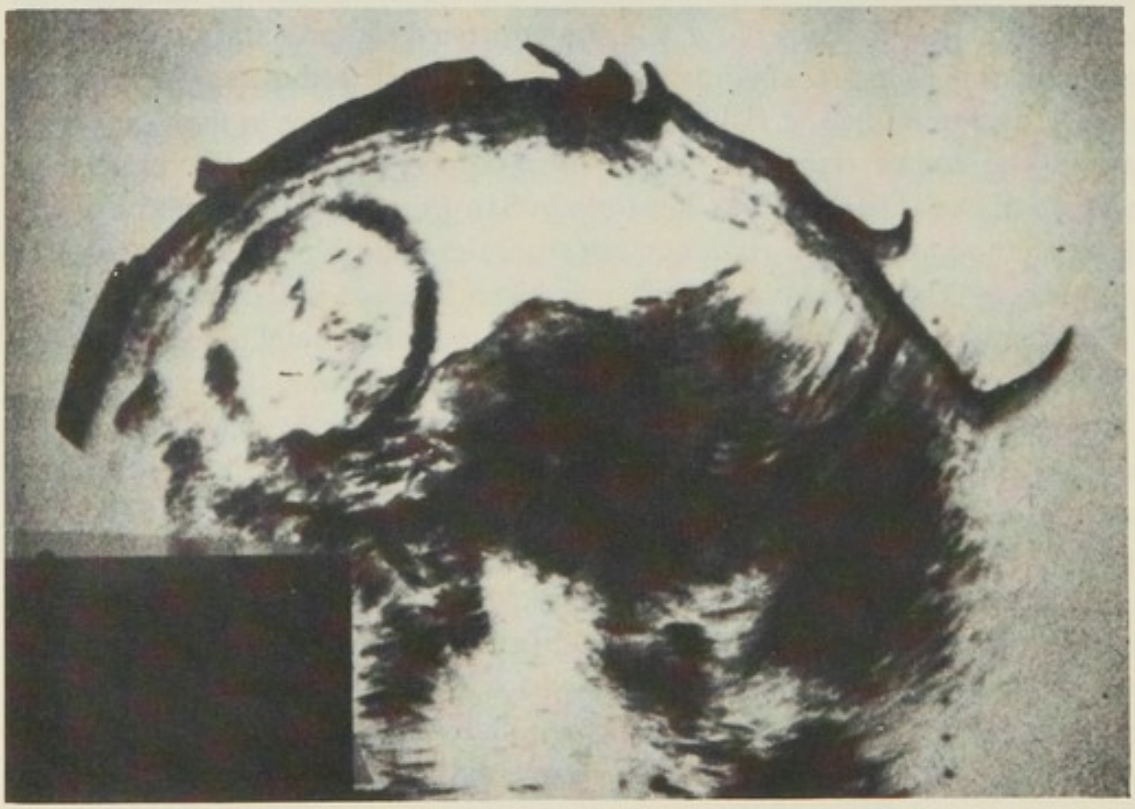

Figure 2. Grey-scale echogram, transverse scan at 21 weeks' gestation. The placenta is located on the posterior uterine wall. To the right of the fetal head a placental 'conical' area is seen, probable point of attachment of the cord.

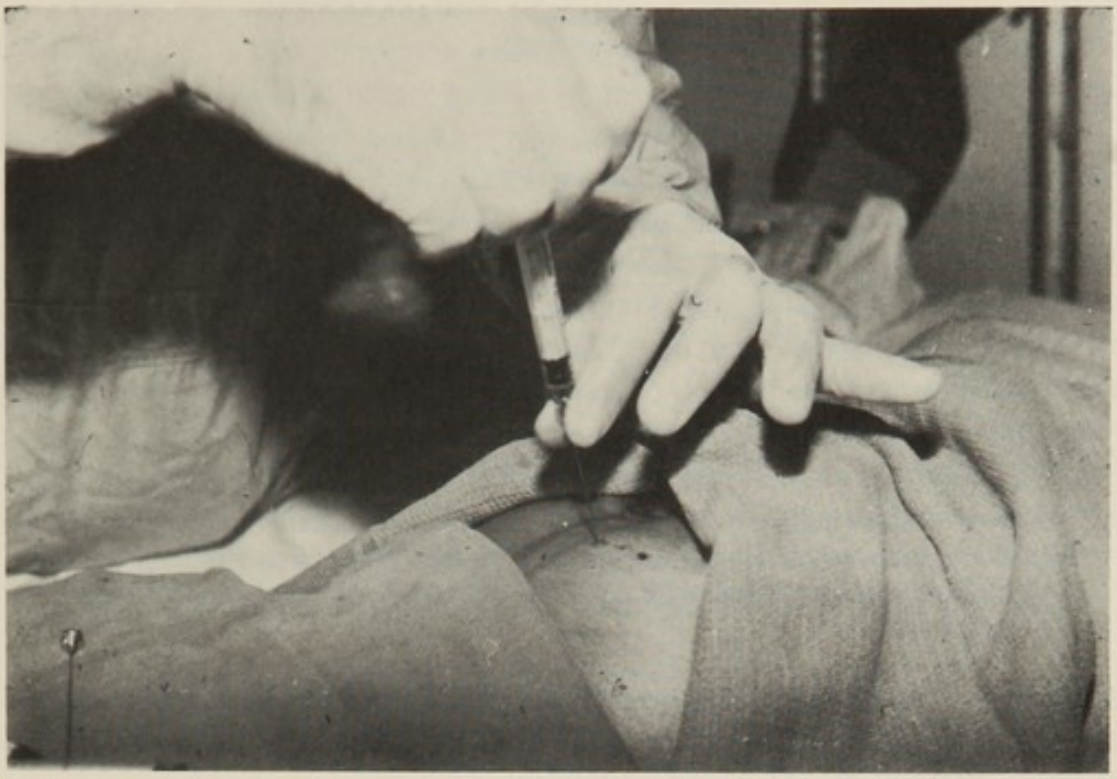

Figure 3. Placental aspiration under strictly aseptic conditions. The placenta is located on the anterior uterine wall.

analyzer' and subsequently checked on smears stained with the Kleihauer-Betke technique (Kleihauer *et al.* 1975).

Within 24 hours of the aspiration, fetal haemoglobin containing erythrocytes were searched for on a sample of maternal blood. Samples containing fetal blood were incubated with ^{3}H-Leucine. When the percentage of fetal blood was less then 50%, fetal erythrocytes were concentrated with anti-i serum (Kan *et al.* 1974). Globin chains were separated on carboxy-methyl-cellulose columns, and the β/γ ratio calculated. The same procedure was applied to blood obtained from the cord of aborted fetuses or newborns, to confirm the prenatal diagnosis (Kan and Nathan 1968, Kan *et al.* 1972). Furthermore, the presence or absence of haemoglobin A and S was checked by electrophoresis on agar at pH 6.2 (Schneider *et al.* 1974).

Results

A suitable sample of fetal blood was obtained from 41 out of 45 cases (93%). The percentage of fetal blood varied from 7 to 99.9% and its volume from 2 to 200 μl. The location of the placenta, anterior in 27 and posterior in 18 cases, did not influence the success in obtaining fetal blood. In four cases not enough fetal blood was obtained: two were at risk for beta-thalassaemia and the other two for sickle cell anaemia. In three instances the patients refused the repetition of the placental tap, and the fourth case was a twin pregnancy, and no attempt was made at sampling fetal blood.

Complications. There were five complications: (a) severe amnionitis. which lead to interruption of pregnancy; (b) fetal haemorrhage and fetal

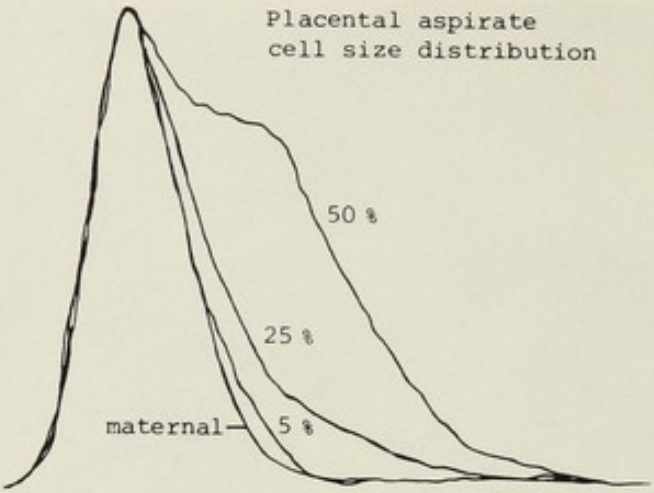

Figure 4. Drawing from a multiple count with a Coulter Counter Channelyzer (particle size analyser) showing, to the right, the curves related to fetal erythrocytes from three aspirates, with 5%, 25% and 50% fetal blood concentration respectively.

death in two cases, due to injury to the umbilical cord by the needle; (c) fetal death due to the puncture of the fetal heart by the needle; (d) spontaneous abortion 20 days after placental aspiration.

In eight cases an increase of fetal cells in the maternal circulation was observed, from 1 to 2%, indicative of fetal haemorrhage less than 10 ml. In two cases an excessive amount of fetal blood was found in the amniotic fluid, which indicated a feto-maternal haemorrhage of about 25 ml. All women were Rh positive, except one, in whom the fetal cells were found to be Rh negative, so that no anti-D antiserum had to be given to the mother.

Haematologic studies. When the percentage of fetal cells in the samples aspirated from the placental site varied from 7 to 50%, differential agglutination with anti-i antiserum made it possible to obtain a concentration up to 54–90%. In these samples the contribution of maternal radioactivity was not significant.

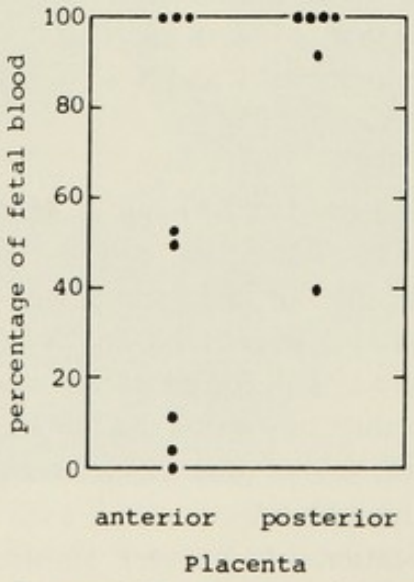

Figure 5. The San Francisco series: results of placental aspiration (45 cases).

Sickle cell anaemia. In both cases the analysis of fetal blood showed synthesis of γ, α and β^S chains, and no synthesis of β^A. The diagnosis of the disease was made and, upon request of the parents, both pregnancies were terminated. The prenatal diagnosis was confirmed on both abortuses.

Beta-thalassaemia. The results in the 39 cases from whom suitable volumes of fetal blood were obtained are reported in figure 6. Twelve pregnancies, where a β/γ ratio varied from 0.066 to 0.128, have reached term and the analysis of cord blood has shown normal ratios. Seven fetuses, with a ratio between 0.037 and 0.094 prenatally, were found to be carriers of the trait after birth, at term. In six, with a β/γ ratio equal to 0, the diagnosis of homozygous beta-thalassaemia was made and, upon request by the parents, pregnancies were interrupted. Blood studies on the abortuses always confirmed the prenatal findings. In those pregnancies which were accidentally interrupted the β/γ ratio varied from 0.033 to 0.106. These results, confirmed on the abortuses, indicate that the fetuses were not homozygotic for beta-thalassaemia.

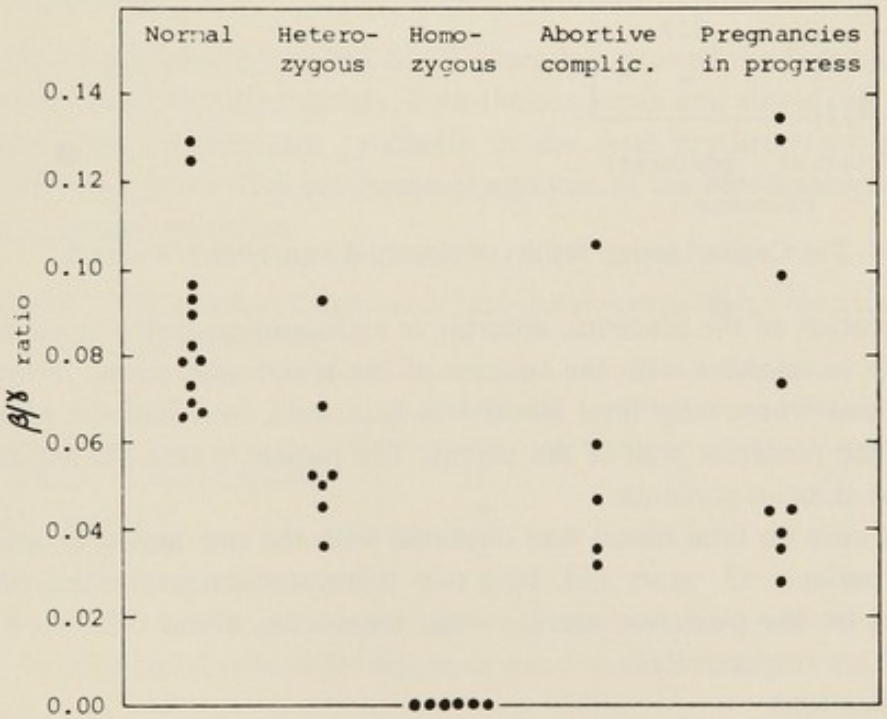

Figure 6. The San Francisco series: prenatal diagnosis of beta-thalassaemia. In 39 of 41 pregnancies at risk, fetal blood was aspirated from the placenta.

Nine pregnancies are still in progress at the time of writing. Based on previous experience it can be reasonably expected that these fetuses are not homozygous for beta-thalassaemia. Those with a β/γ ratio higher than 0.06 will probably be normal; and those with a ratio lower than 0.06 will be carriers of the trait.

The Cagliari Series

Fourteen pregnancies at risk for beta-thalassaemia were studied in Cagliari, Italy. The procedure was exactly the same as the one applied in San Francisco.

The echographic localisation of the placenta was obtained with a Picker model L80. No real-time scanning was used.

Thirteen couples had already had one or more children homozygous for the condition. One had had no children. Suitable samples of fetal blood were obtained from thirteen cases (92.8%). The percentage of fetal blood in the mixture aspirated from the placenta was 99.9% in seven cases, and varied from 6 to 70% in six cases.

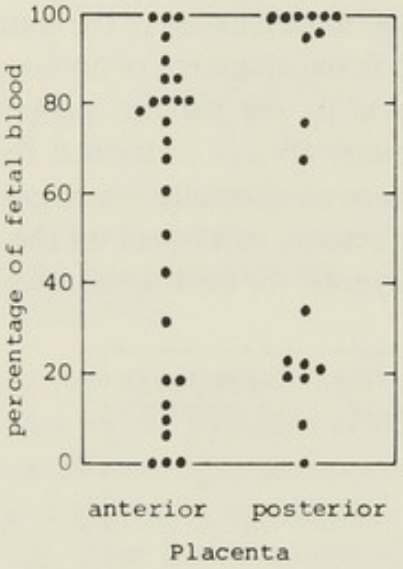

Figure 7. The Cagliari series: results of placental aspiration (14 cases).

The location of the placenta, anterior in eight and posterior in six cases, did not seem to interfere with the success of the blood aspiration: in fact, of the seven cases where only fetal blood was aspirated, four had the placenta situated on the posterior wall of the uterus. The patient where the aspiration failed had an anterior placenta.

In one case no fetal blood was obtained with the one and only attempt made: the patient, 43 years old, had two leiomyomata implanted on the anterior and on the posterior uterine wall, measuring about $10 \times 8 \times 6$ and $14 \times 10 \times 12$ cm respectively.

Complications

The patient with uterine myomata ruptured the membranes some thirty days after the placental aspiration and aborted a 500 g fetus, who died shortly thereafter. The β/γ ratio was 0.040. No sign of damage by the needle to the fetus could be ascertained. It is conceivable that the pregnancy would not have progressed to term even without our attempt at fetal blood sampling.

One patient had a stillbirth some forty days after the placental tap—the β/γ ratio was 0.057 at the time of the tap. On delivery, the fetus was macerated and no haemoglobin synthesis could be elicited in the erythrocytes. This patient had already had a stillbirth at six months' gestation some years previously.

Haematologic Studies

In seven cases fetal blood cells in the samples were 99.9%, and in three

cases over 50%; no differential agglutination was necessary. In three cases the percentage of fetal blood in the mixtures was less than 50%, and by agglutination with anti-i serum it was increased to more than 60%.

Results

In three cases the β/γ ratio was 0. All of the three couples requested the interruption of pregnancy and the studies conducted on the abortuses confirmed their homozygosity for beta-thalassaemia. One pregnancy has come to term, and the globin synthesis on the newborn cord blood confirmed the prenatal diagnosis of heterozygosity for the disease. No fetal injury by the needle could be demonstrated.

For the remaining eight cases the course of pregnancy is uneventful so far. In two cases the β/γ ratio was 0.11 and 0.10 respectively: these fetuses were considered normal. In six pregnancies the fetuses were diagnosed as heterozygous: the β/γ ratios were 0.038, 0.040, 0.045, 0.050, 0.057, and 0.059.

Discussion

Our experience of a total of 59 cases has proved the feasibility of diagnosing *in utero*, in mid-trimester, beta-thalassaemia and sickle cell anaemia by the study of haemoglobin synthesis in the fetal erythrocytes obtained by placental aspiration. The biochemical analysis of the blood samples aspirated has proved very accurate.

Table 1. The prenatal diagnosis of beta-thalassaemia (San Francisco + Cagliari series = 55 pregnancies).

	S. Francisco	Cagliari	Totals
Normal or heterozygous	33	10	43
Homozygous	6	3	9
Failures[1]	2	1	3
Abortive complications	5	2	7

[1] No fetal blood suitable for diagnosis was obtained.

Suitable fetal blood samples were obtained in 54 out of 59 cases (91%). If one excludes the four cases who refused the repetition of the placental tap, and the twin pregnancy, fetal blood in sufficient amounts for the diagnosis was obtained in all of the 54 cases.

The use of the Coulter particle size analyser has proved of paramount importance to identify rapidly the presence and percentage of fetal erythrocytes in the blood mixture aspirated, so that the number of placental punctures could be limited to a minimum.

The quality of the echograms and consequent precision of placental localisation also proved essential. By the identification of the point of placental insertion of the umbilical cord with new ultrasonographic techniques (such as 'grey scale' and real-time scanning) it is to be expected that fetal death from

puncture of the cord will be avoided in the future. Also fetoscopy might be helpful in reducing the risk of direct fetal injuries. This method will be limited by the high frequency of anterior placentas (over 50%), when the fetoscope cannot and should not be used.

The risk of complications with the method of placental aspiration is still high (11.0% in our series), but experience is rather limited, and it is certainly comparable to the risk of mid-trimester amniocentesis in its early stage of experimentation.

Feto-maternal transfusion has been observed, but always at a low level, except for two instances when the cord was punctured.

In our series the biochemical analysis of fetal blood has given excellent results. It has been possible to distinguish the hetero- from the homo-zygous beta-thalassaemia condition.

Altogether, should further experience prove that fetal risk can be lessened by improvements in the obstetrical aspects of the technique, it can be said that placental aspiration is an acceptable method to diagnose *in utero* hereditary haemoglobinopathies. If such a desirable improvement cannot be attained other methods for fetal blood sampling will have to be developed, such as, for example, the stimulation *in vitro* of fibroblasts to the globin synthesis (Harrison 1976).

Summary

Fetal blood in mid-trimester has been obtained by venepuncture under direct vision and by transabdominal placental needle aspiration by several authors. Antenatal diagnosis of sickle cell anaemia and beta-thalassaemia has been achieved in a number of cases.

A series of 59 cases studied in San Francisco, U.S.A., and in Cagliari, Italy, has been illustrated in detail. Placental aspiration has been successful in obtaining fetal blood samples suitable for diagnosis in 54 cases (91.5%). The incidence of serious fetal complications has been high (11%). It is hoped that, with an improvement of the obstetrical technique, such risk can be lowered considerably in the near future.

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List of Participants

ADAM, Dr Robert
Taunton & Somerset Hospital, Taunton, Somerset

AITKEN, Mrs Elizabeth
Department of Obstetrics, Western General Hospital, Edinburgh

AKUSE, Dr James
Maternity Unit, Southampton General Hospital, Southampton

ALBERMAN, Dr Eva
3 Millfield Place, London N6

ARNOLD, Dr & Mrs David
25 Mountain Road, Caerphilly, Glamorgan

ARTHUR, Miss Lesley
68 Marchmont Crescent, Edinburgh

ATHANASSIADES, Dr Nicholas
Royal Hospital for Sick Children, Department of Medical Genetics, Glasgow

ATKINS, Mr John
Middlesbrough Maternity Hospital, Middlesbrough, Cleveland

BAIRD, Sir Dugald
21 Russell Place, Edinburgh

BAIRD, Dr Joyce
Department of Endocrine & Metabolic Diseases,
Western General Hospital, Edinburgh

BARTSCH, Dr Fritz
Obstetric Department, East Hospital, 5–41685 Gothenberg, Sweden

BASSETT, Dr William
Craigshill Health Centre, Livingston, West Lothian

BAULD, Mrs Rhona
Pathology Department, Royal Hospital for Sick Children, Edinburgh

BELL, Dr Jeanne
M.R.C. Clinical & Population Cytogenetics Unit,
Western General Hospital, Edinburgh

BENNETT, Dr Michael
Institute of Obstetrics & Gynaecology,
Queen Charlotte's Maternity Hospital, London

BENZIE, Mr Ronald
2917 Bloor Street West, Toronto, Canada

BERREY, Dr Peter
21 Comely Bank Avenue, Edinburgh

BLAIR, Dr Robert
'Glengarden', 4 Chapland Road, Lanark

BOUÉ, Prof. André & Mrs Joelle
Groupe de Recherches de Biologie Prénatale,
Château de Longchamp, 75016 Paris

BOWSER-RILEY, Mrs Susan
1 Freelands Cottages, Ratho, Midlothian

BRASH, Mr & Mrs John
Department of Obstetrics, St James's Hospital, Leeds

BROCK, Dr David
Department of Human Genetics, Western General Hospital, Edinburgh

BROTHERSTON, Prof. Sir John
26 Mortonhall Road, Edinburgh

BROUGH, Mr Frank
Meadow House, Foxhouses Road, Whitehaven, Cumbria

BROWN, Dr David
Department of Obstetrics and Gynaecology,
Western General Hospital, Edinburgh

BROWN, Dr George
9 Transy Grove, Dunfermline

BUSFIELD, Mr H. Malcolm
133 Manor Road, Littleover, Derby

BUTLER, Mr Leslie
Queen Elizabeth Hospital, London

BUTTERWORTH, Miss Mildred
Maternity Unit, Staincliffe General Hospital, Dewsbury, West Yorkshire

CAMERON, Dr Alastair
Medical Residency, Falkirk Royal Infirmary, Falkirk

CAMERON, Dr Euan
Regional Hormone Laboratory, 12 Bristo Place, Edinburgh

CASE, Miss Barbara
Shaw House, Shaw Lane, Ebchester, Consett, Co. Durham

CASKA, Dr Peter
'The Lodge', Heatherwood Hospital, Ascot

CHAWNER, Mr John
St David's Hospital, Bangor, Gwynedd, North Wales

CHNG, Dr Pang
Aberdeen Maternity Hospital, Aberdeen

CLARK, Dr F. Ronald
Obstetric Unit, Western General Hospital, Edinburgh

CLARKE, Mrs Petronella
Department of Obstetrics, St Bartholomew's Hospital, London

CLIVE, Dr Eric
Department of Scots Law, University of Edinburgh, Edinburgh

COXON, Mr Alan
11 Kenton Road, Gosforth, Newcastle-upon-Tyne

CROMPTON, Dr Gareth
Gwynedd Area Health Authority, Coed Mawr, Bangor

CROWDER, Dr Mary
165 Hamilton Street, Broughty Ferry, Dundee

DASMAHAPATRA, Mr Tapasranjan
Flat 2, Hull Royal Infirmary (Sutton), Salthouse Road, Hull

DAVIDSON, Dr Frank
Scottish Home & Health Department, St Andrew's House, Edinburgh

DAVIDSON, Mr George
4 Penlee Gardens, Devonport, Plymouth, Devon

DAWSON, Mr & Mrs Alexander M.
Muirneag, Rumsam, Barnstaple, North Devon

DENNISS, Mr & Dr Richard
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DILLON, Dr Stanley
Gorton Lodge, Longdon, Rugeley, Staffs

DIXON, Mr Reginald
146 Blackcarr Road, Baguley, Manchester

DONALD, Prof. Ian
9 Hamilton Drive, Glasgow

DOWIE, Mr Robert
36 Mountbarrow Road, Ulverston, Cumbria

DUBUISSON, Dr Jean-Bernard
12 Bd. Desgranges, 92330 Sceaux, France

DUKE, Mr Anthony
Riverdale, Colwich, Stafford, Staffs

DUMEZ, Dr Yves
159 Rue de Charonne, 75011 Paris

DUNCAN, Prof. Archibald
1 Walker Street, Edinburgh

DUNLOP, Prof. Sir Derrick
28 Saxe-Coburg Place, Edinburgh

DURHAM, Mr & Mrs Peter
Mandeville, Westfield Lane, Etchinghill, Folkestone

DUTHIE, Dr & Mrs Ian
21 West Albert Road, Kirkcaldy

EDMOND, Dr Elizabeth
Regional Virus Laboratory, City Hospital, Edinburgh

ELSTEIN, Mr & Mrs Max
Department of Human Reproduction,
South Block, General Hospital, Southampton

EMERY, Prof. Alan
Department of Human Genetics, Western General Hospital, Edinburgh

ESKANDER, Dr
Mayday Hospital, Thornton Heath, Surrey

FAIRWEATHER, Prof. Denys
University College Hospital Medical School, Huntley Street, London

FARR, Dr Valerie
6 Polmuir Road, Aberdeen

FAWDRY, Dr Rupert
Department of Obstetrics and Gynaecology,
Western General Hospital, Edinburgh

FERGUSON-SMITH, Prof. Malcolm & Mrs Marie
Department of Medical Genetics, Royal Hospital for Sick Children, Glasgow

FORD, Dr Gillian
Room 1013, Hannibal House, Department of Health and Social Security,
Elephant & Castle, London

FORFAR, Prof. John
Department of Child Life and Health, 17 Hatton Place, Edinburgh

FRASER, Dr Ian
15 Spoutwells Avenue, Scone, Perth

GALJAARD, Prof. & Mrs Hans
Department of Cell Biology and Genetics, Erasmus University, Rotterdam

GILLESPIE, Mr Alan
Frimley Park Hospital, Frimley, Camberley, Surrey

GLASS, Dr Norman
D.H.S.S., 151 Great Titchfield Street, London

GOMEZ, Dr Gaspar
1 Maryfield Terrace, Dumfries

GOSDEN, Dr C. M.
M.R.C. Clinical & Population Cytogenetics Unit,
Western General Hospital, Edinburgh

GRANT, Dr John
Scottish Home & Health Department, St Andrew's House, Edinburgh

GRANT, Dr Kenneth
36 Dunbar Road, Haddington

GRANT, Mr Michael
6 Montfort Place, Newcastle, Staffs

GORDON, Dr George
Cresswell Maternity Hospital, Dumfries

HALLOWAY, Dr Christopher
Obstetric Department, St Mary's Hospital, Portsmouth

HANNAN, Dr Moina
General Hospital, Scunthorpe, South Humberside

HASSARD, Mr & Mrs Jason
Westfield Lodge, Oakley, Bedford

HENRION, Prof. Roger & Dr Edith
16 Rue Chardon-Lagache, 75016 Paris

HIBBARD, Prof. Bryan
Department of Obstetrics,
Welsh National School of Medicine, Heath Park, Cardiff

HILL, Dr Lyndon M.
1 Jasmin Avenue, Chapel Park, Newcastle

HKAU, Dr Richard
Department of Obstetrics and Gynaecology,
Bangour General Hospital, Broxburn

HURLEY, Prof. R.
Queen Charlotte's Hospital, London

HUTCHEON, Miss Mary
10 Duchy Road, Harrogate, North Yorkshire

HUTCHON, Dr David
72 Craiglockhart Loan, Edinburgh

HWANG, Dr Dong Hoon
Department of Obstetrics, Ninewells Hospital, Dundee

IMRIE, Mr Alastair
12 The Triangle, North Ferriby, North Humberside

JEFFERSON, Dr Wendy
'Nordanay', St Johns Road, Hazlemere, High Wycombe, Bucks

JELEN, Dr Ivan & Mrs P.
Department of Obstetrics and Gynaecology,
Western General Hospital, Edinburgh

JOHNSTONE, Mr Douglas
142 Westley Road, Bury St Edmunds, Suffolk

KENNEDY, Dr David
Fife Area Laboratory, Mayfield Road, Kirkcaldy

KENNEDY, Dr Walter
University Dept. of Obstetrics & Gynaecology, Edinburgh

KHAN, Mr Abdus
Hillingdon Hospital, Uxbridge

KHAN, Dr Mehmood
P.O. Box 407, Durban 4000, South Africa
KING, Mr & Mrs John
11 Kings Avenue, Morpeth, Northumberland

LAURENCE, Prof. K.M.
Child Health Laboratories, The Welsh National School of Medicine, Cardiff
LEVVY, George
Department of Obstetrics & Gynaecology,
Western General Hospital, Edinburgh
LORIMER, Dr J. Derek
62 Holmfield Road, Stoneygate, Leicester
LOUDON, Dr John D.O.
Department of Obstetrics & Gynaecology,
Eastern General Hospital, Edinburgh
LOUDON, Dr Nancy
94 Inverleith Place, Edinburgh
LOW, Dr Robert
391 Albert Drive, Glasgow
LOWLES, Dr Ian
15 Redman Close, R.A.F. Ely, Cambridgeshire

MACAFEE, Dr C.A. Jeremy
Leicester Royal Infirmary, Maternity Hospital, Leicester
MACDONALD, Dr H. & Mrs. R
St James's University Hospital, Leeds
MCGILLIVRAY, Prof. I.
Department of Obstetrics, Foresterhill, Aberdeen
MCINTOSH, Mr A.S.
Newcastle General Hospital, Newcastle-upon-Tyne
MCKENZIE, Dr John
Department of Developmental Biology,
Marischal College, University of Aberdeen
MCLEAN, Mr Graeme
Royal Gwent Hospital, Newport
MCLEOD, Dr
Lothian Health Board, Edinburgh
MCNAUGHTON, Prof. & Mrs Malcolm
Royal Maternity Hospital, Rottenrow, Glasgow
MANAGHAN-ANSLOW, Miss Francesca
Department of Obstetrics, Middlesex Hospital, London
MANN, Dr Christine
Hawthornden House, Lasswade, Midlothian
MARTIN, Dr Margaret
Paisley Maternity Hospital, Paisley

MIKKELSEN, Dr Margareta
John F. Kennedy Institute, DK 2600 Glostrup/Copenhagen, Denmark

MORE, Dr Marion
4 Saxe-Coburg Street, Edinburgh

MORRIS, Miss Susan
25 Jordan Lane, Edinburgh

MORRIS, Dr
Lothian Health Board, Edinburgh

MULDOON, Mr Michael
26 Abbey Park Road, Grimsby, North Humberside

MUNRO, Dr H. D. Ross
Craigshill Health Centre, Livingston, West Lothian

MURRAY, Dr Anne
5 Nelson Street, Edinburgh

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Brocades (G.B.) Ltd., Brocades House, West Byfleet, Weybridge, Surrey

NIXON, Miss Jennifer
8 Cryon View, Gloweth, Truro, Cornwall

PARRY, Dr Elizabeth
12 Henderland Road, Edinburgh

PASSARGE, Prof. E. & Mrs E.
Institut fur Humangenetik, Universitätsklinikum, Hufelandstrasse 55,
D-4300 Essen 1, West Germany

PATEL, Dr Naren
Ninewells Hospital, Dundee

PATHAK, Mr & Mrs Upendra
220 Eccles Old Road, Salford

PATRICK, Dr Desmond
Institute of Child Health, 30 Guilford Street, London

POLANI, Prof. P. E.
Prince Philip Research Laboratories,
Paediatric Research Unit, Guy's Hospital, London

PORTEOUS, Mr Colin
'Charlton', Quarry Drive, Aughton, Ormskirk, Lancs

RAEBURN, Dr John
Department of Human Genetics, Western General Hospital, Edinburgh

RAHMANIE, Dr Nooria
Risedale Maternity Hospital, Barrow-in-Furness, Cumbria

RANDLE, Mr Guy
'Brimley Close', Molescroft, Beverley, Yorks

RAOUF, Dr Monir
King's College Hospital, Denmark Hill, London

RAWLINGS, Dr Ian
31 High Street, Coldstream, Berwickshire

REDPATH, Dr William
88 Thirlestane Road, Edinburgh

REISS, Mr Herbert
20 Harley House, Marylebone Road, London

RICHARDSON, Mr John
2 Prospect Close, Easter Compton, Bristol

ROBERTS, Dr David
Community Medicine, Gwynedd Health Authority, Coed Mawr, Bangor

ROBERTS-THOMSON, Mr Kenneth
8 Larkspur Terrace, Newcastle-upon-Tyne

ROBINSON, Miss Daphne
Department of Obstetrics, Western General Hospital, Edinburgh

ROBINSON, Dr Hugh
Queen Mother's Hospital, Glasgow

ROCKER, Mr Israel
2 Stow Park Circle, Newport, Gwent

ROTHMAN, Dr Doreen
Room 1019 D.H.S.S., Hannibal House, Elephant & Castle, London

RUSSELL, Dr Alan
Department of Obstetrics, Wythenshawe Hospital,
Floats Road, Wythenshawe, Manchester

SALAMA, Dr Fakhry
33 Summerdale, Shotley Bridge, Consett, Co. Durham

SCARISBRICK, Miss Jacqueline
Regional Hormone Laboratory, 12 Bristo Place, Edinburgh

SCHUTZEL, Dr Helga
Schering AG, 1000 Berlin 65, Mueller Str. 170/178.

SCOTT, Miss Elizabeth
6 House O'Hill Road, Edinburgh

SCRIMGEOUR, Dr John
Maternity Unit, Western General Hospital, Edinburgh

SHEPHERD, Mr Richard
St Mary's Hospital, Milton Road, Portsmouth

SHIELDS, Mr Michael
10 Melrose Place, Watford, Herts

SHORT, Prof. Roger
M.R.C. Unit of Reproductive Biology, 2 Forrest Road, Edinburgh

SIGGERS, Dr David
Child Health Unit, Southampton General Hospital, Southampton

SINGER, Dr Jack
Kings College Hospital, Denmark Hill, London

SKINNER, Dr Rosalind
Department of Human Genetics, Western General Hospital, Edinburgh

SMITH, Dr Armand
Lothian Health Board, Edinburgh

SPALDING, Dr Elizabeth
Redhill General Hospital, Redhill, Surrey

SPIERS, Miss Norma
Department of Obstetrics, Western General Hospital, Edinburgh

SRINIVASAPPA, Dr Namagohdlu
Upton Hospital, Slough, Berkshire

STARRITT, Mr Alexander
Court Vean, Werfa Road, Abernant, Aberdare, Glamorgan

STEELE, Mr Stuart
23 Lynwood Road, Ealing, London

STEVEN, Dr J. Douglas
20 Spottiswoode Road, Edinburgh

STRONG, Prof. John
Department of Medicine, Western General Hospital, Edinburgh

STUART, Miss J. Edna
Department of Obstetrics and Gynaecology,
Western General Hospital, Edinburgh

SWAPP, Dr Garden
Aberdeen Maternity Hospital, Aberdeen

TAYLOR, Mr David
Duchy House Nursing Home, Queens Road, Harrogate, North Yorkshire

THOMPSON, Mr Ian
Edinburgh Medical Group Research Project, 24 Buccleuch Place, Edinburgh

TOOP, Dr Kenneth
1 Binghill Crescent, Milltimber, Aberdeen

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22 Stocks Lane, Chester

TURNBULL, Prof. Alexander
John Radcliffe Hospital, Headington, Oxford

VALENTI, Prof. C.
Medical Diagnostic Centre, Rome

WALKER, Prof. James
Department of Obstetrics and Gynaecology, University of Dundee, Dundee

WATSON, Miss Janet
Pathology Department, Royal Hospital for Sick Children, Edinburgh

WATT, Miss Muriel
27 Well Court, Edinburgh

WEIR, Dr Alistair
Royal Infirmary, Stirling

WHITFIELD, Prof. & Mrs Charles
Queen Mother's Hospital, Glasgow

WHITTLE, Dr Martin
Department of Obstetrics, U.H.S.M., Withington, Manchester

WILLIAMS, Dr Anthony
Jersey Maternity Hospital, St Saviours Road, Jersey

WILLIAMSON, Dr Elspeth
Department of Child Health, Southampton General Hospital, Southampton

ZUPANIC, Dr Matko
Liverpool Maternity Hospital, Oxford Street, Liverpool

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