

## Prenatal diagnosis of genetic disorders

M. F. NIERMEIJER, E. S. SACHS, M. JAHODOVA, C. TICHELAAR-KLEPPER,  
W. J. KLEIJER, AND H. GALJAARD

*From the Department of Cell Biology and Genetics, and Department of Obstetrics and Gynecology, Medical Faculty, Erasmus University, Rotterdam, The Netherlands*

**Summary.** Three hundred and fifty pregnancies were monitored by trans-abdominal amniocentesis in the fourteenth to sixteenth week of gestation followed by karyotyping or biochemical assays of cultured amniotic fluid cells and analysis of alpha-fetoprotein in the amniotic fluid supernatant. The pregnancy was interrupted in 36 cases (10%) either because of a fetal abnormality or the presence of a male fetus in pregnancies at risk for an X-linked disease.

Four chromosomal aberrations were found in 87 pregnancies tested because of advanced maternal age. In 101 pregnancies with a recurrence risk of Down's syndrome, 2 fetuses with an abnormal karyotype were detected. In 11 cases, in which 1 parent was a carrier of a balanced translocation, 2 unbalanced fetal karyotypes were found. Fetal chromosome studies in 43 pregnancies at risk for an X-linked disease indicated the presence of a male fetus in 21 cases.

Prenatal diagnosis of 11 different metabolic diseases was performed in a total of 34 cases. Microchemical techniques were used to allow completion of the diagnosis of seven different enzyme deficiencies within 9 to 22 days after amniocentesis. Alpha-fetoprotein assay in the amniotic fluid supernatant of 47 pregnancies at risk for an open neural tube defect resulted in the detection of 3 anencephalic fetuses during the second half of pregnancy.

The safety and reliability of amniocentesis and the possible effects on the outcome of pregnancy are evaluated. Prenatal diagnosis offers a promising alternative for parents who are at risk of having a child with a genetic disease which can be detected in amniotic fluid or in cultured amniotic fluid cells.

Since the first reports of fetal karyotyping (Steele and Breg, 1966; Jacobson and Barter, 1967) and biochemical assays (Nadler, 1968, 1969) after amniocentesis in early pregnancy, increasing numbers of centres have gained experience on prenatal diagnosis of genetic diseases. Recent reviews (Milunsky, 1973; Hsu and Hirschhorn, 1974) show how the application of prenatal diagnosis in genetic counselling has already enabled many parents at risk for genetic disease to have non-affected children. Prenatal chromosome analysis has become routine and the number of inborn errors of metabolism which can be detected in cultured amniotic fluid

cells has steadily increased (Milunsky and Littlefield, 1972; Burton, Gerbie, and Nadler, 1974). Though theoretically over 40 metabolic disorders are now detectable in cultured amniotic fluid cells, only limited experience with each of these diseases has been gained. A few small series have been accumulated: for GM<sub>2</sub> gangliosidosis type 1 (Tay-Sachs' disease) (O'Brien *et al*, 1970; Navon and Padeh, 1971; Ellis *et al*, 1973), glycogenosis type II (Pompe's disease), and the mucopolysaccharidoses (Milunsky, 1973; Niermeijer *et al*, 1974). Conventional biochemical analysis requires relatively large cell numbers, which sometimes resulted in long waiting periods for the parents

(4 to 8 weeks). The development of micro-chemical techniques enabled enzyme assays on small numbers of cultured amniotic fluid cells and a reduction in the time required for parental diagnosis (Galjaard *et al*, 1973; 1974c; 1974d; Wendel *et al*, 1973).

A recent and important contribution to prenatal diagnosis was the finding by Brock and Sutcliffe (1972), that the alpha-fetoprotein level in amniotic fluid is increased when the fetus has an open defect of the neural tube. When relatively new techniques like those of prenatal diagnosis are used full assessment of the scope and risks of the various procedures is possible only when sufficiently large series of diagnoses for different groups of genetic diseases can be analysed, including follow-up studies on children after amniocentesis. The present report describes 350 prenatal diagnoses performed in pregnancies at risk for a chromosomal aberration, X-linked disease, neural tube defect, or 1 of 11 different metabolic diseases.

### Methods and materials

**Clinical methods.** The actual genetic risk was estimated in each woman referred for prenatal diagnosis

by taking the family history. Additional diagnostic tests on previous affected children, parents, or relatives (karyotyping, enzymatic studies, etc.) were performed when necessary in order to make sure that amniocentesis was justified. A risk higher than 1% for a disease amenable to prenatal diagnosis was taken as an indication for amniocentesis (Table I). The indication for advanced maternal age was accordingly limited to women 38 years and older.

The procedure of amniocentesis and its related risks were explained to the patients.

Transabdominal amniocentesis (Queenan, 1970) was performed under local anaesthesia in the fourteenth to sixteenth week after the last menstrual period. Ultrasound examination was carried out to localize the placenta, measure the fetal biparietal diameter, estimate the volume of amniotic fluid, and exclude the presence of twins. Amniotic fluid, 10 to 15 ml, was aspirated into a siliconized glass syringe.

Selective abortion was performed when indicated by intra-amniotic injection of hypertonic saline or by intra- or extraovular infusion of prostaglandin F<sub>2</sub>-alpha. Follow-up studies on children born after amniocentesis were performed using a schedule modified from the British Medical Research Council 'Working Party on Amniocentesis'.

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TABLE I  
PREGNANCIES MONITORED BY AMNIOTIC FLUID (CELL-) ANALYSIS

Indication	No. Tested	No. Abnormal Fetuses	Type of Abnormality
<i>Chromosomal aberrations</i>			
Recurring risk Down's syndrome	101	2	1 trisomy 21; 1 7/21 translocation
Advanced maternal age	87	4	3 trisomy 21; 1 47,XXY
Down's syndrome in relatives	8	—	
Inherited translocations	11	2	1 13/22 unbalanced; 1 G/G unbalanced
Miscellaneous	19	2	1 trisomy 18; 1 47,XXY
	226	10	
<i>X-linked diseases</i>			
Duchenne's muscular dystrophy	18	11	11 ♂ fetuses (1,XO/XY mosaic)
Haemophilia	16	7	7 ♂ fetuses
Others	9	3	3 ♂ fetuses
	43	21	
<i>Metabolic diseases</i>			
Glycogenesis type II	13	3	2 affected; 1 misdiagnosed
GM <sub>2</sub> gangliosidosis (Tay-Sachs)	3	—	
GM <sub>2</sub> gangliosidosis (Sandhoff)	3	—	
GM <sub>1</sub> gangliosidosis	2	—	
Fabry's disease	1	—	
Metachromatic leucodystrophy	3	—	
Krabbe's disease*	1	—	
Hurler's disease	3	—	
Hunter's disease	3	—	
Gaucher's disease	1	—	
Maple syrup urine disease	1	—	
	34	3	
<i>Neural tube defects</i>			
Recurrence risk neural tube defects	28	—	
Hydramnios after 20 weeks	19	3	3 anencephalics
	47	3	
Totals	350	37	

\* Assay performed by Dr A. D. Patrick, London.

analyses were performed by our own group. Samples referred by other centres were transported in siliconized glass flasks. Amniotic fluid samples from normal pregnancies were kindly provided by Dr K. L. Garver, Magee Women's Hospital, Pittsburgh (USA) and stored until use as controls in biochemical studies, according to a procedure described earlier (Niermeijer *et al.*, 1973).

#### Cell culture methods for amniotic fluid cells.

After counting the cells with a haemocytometer the amniotic fluid was centrifuged for 5 minutes at  $80 \times g$  and the supernatant was centrifuged for another 10 minutes at  $1500 \times g$  and stored at  $-70^\circ\text{C}$  until needed. The collected cells were cultured according to different techniques, especially during the initial phase of this study. The procedure used in the last 300 cases is described here.

The cell pellets were resuspended in Ham's F10 medium supplemented with 25% v/v of fetal calf serum (Flow Company), penicillin (50 U/ml), and streptomycin (50  $\mu\text{g/ml}$ ). For chromosome analysis cells were seeded on  $22 \times 22$  mm coverslips in different 35 mm Falcon plastic Petri dishes. Aliquots of 0.75 ml, containing 50 000–100 000 cells, were gently pipetted on the top of the coverslips and left overnight in a fully humidified incubator with 5%  $\text{CO}_2$  in air as the gaseous phase before additional medium was added up to 2 ml. The medium was changed after 5 days and subsequently every second day until sufficient mitotic cells for harvesting were present. Some bloody samples of amniotic fluid were processed according to Lee, Gregson, and Walker (1970); as this did not give significant improvement in cell growth, the method was omitted.

Culture methods for biochemical analysis were similar. Cells cultured in the Petri dishes were used to provide cell homogenates. In addition cells were seeded in Mylar dishes with a thin plastic bottom to enable microchemical studies on groups of 100 to 300 dissected cells (Galjaard *et al.*, 1973; 1974c). For each prenatal diagnosis amniotic fluid cell samples from a comparable gestational period cultivated under similar conditions (medium, pH, cell number seeded, period of cell cultivation after subcultures) were used as controls.

Amniotic fluid cell cultures received from outside centres were grown in Ham's F10 medium for at least 24 hours before harvesting.

#### Chromosome analysis of cultured amniotic fluid cells.

When sufficient mitotic cells were observed in a culture, colchicine (0.02  $\mu\text{g/ml}$ ) was added to the medium for 3.5 to 4 hours. A procedure adapted from P. Hösl (1970, personal communication) was used for *in situ* spreading and gradual fixation to avoid loss of mitotic cells. Q banding was performed with a 0.5% solution of atebriin (Caspersson, Lomakka, and Zech, 1971). Metaphases were studied with a Leitz fluorescence microscope equipped with a vertical illuminator and filter combinations as described by Van der Ploeg and Ploem (1973).

A minimum of 16 metaphases was analysed from at

least two different cultures and a minimum of 3 metaphases was karyotyped (Paris Conference, 1971). Polyploid metaphases were not included. A complete fetal chromosome analysis was used for prenatal sex determination in pregnancies at risk for an X-linked disease.

#### Microchemical analysis of cultured amniotic fluid cells.

Cell homogenates or dissected groups of 100 to 300 freeze-dried cultured cells were prepared as described (Galjaard *et al.*, 1973; 1974c). The procedures for the microchemical assay of  $\alpha$ -galactosidase (Galjaard *et al.*, 1974d), acid  $\alpha$ -1,4-glucosidase (Galjaard *et al.*, 1973), acid  $\beta$ -galactosidase (Galjaard *et al.*, 1974a),  $\beta$ -D-N-acetylglucosaminidase (Galjaard *et al.*, 1974b) and arylsulphatase A (Niermeijer *et al.*, 1974) have been described.  $\alpha$ -L-iduronidase was measured according to Hall and Neufeld (1973) using a final volume of 30  $\mu\text{l}$ .  $\beta$ -glucosidase was assayed using a procedure modified from Beutler *et al.* (1971).

Electrophoresis of  $\beta$ -D-N-acetylglucosaminidase was performed as described (Galjaard *et al.*, 1974b) and heat inactivation studies of  $\beta$ -D-N-acetylglucosaminidase isoenzymes in amniotic fluid supernatant were performed according to O'Brien *et al.* (1970).

The results of assays on cell homogenates were expressed per mg protein as determined according to Lowry *et al.* (1951) using a final volume of 60  $\mu\text{l}$ . When possible the activity of a second, non-related enzyme localized in the same subcellular compartment was measured as a test for the preservation of enzymatic activity during preparation of the sample.

The enzymatic activities were expressed per cell in microchemical assays on dissected groups of freeze-dried cells.

Cultured cells from control amniotic fluids and fibroblasts from a previous affected child and heterozygous carriers were analysed at the same time as the cells for diagnosis, whenever these specific fibroblast strains were available. Studies on the uptake and release of  $^{35}\text{SO}_4$ -sulphate in cultured amniotic fluid cells from pregnancies at risk for mucopolysaccharidoses type I and II were performed according to Fratantoni *et al.* (1969) as modified by E. F. Neufeld (1972, personal communication).

Branched chain ketoacid decarboxylase was assayed in cultured amniotic fluid cells using a microassay described by Wendel *et al.* (1973).

Cultured cells from a pregnancy at risk for Krabbe's disease were assayed for  $\beta$ -galactocerebrosidase activity using the natural substrate by Dr A. D. Patrick (Institute of Child Health, London).

#### Determination of $\alpha$ -fetoprotein (AFP) in amniotic fluid.

The single radial immunodiffusion technique using Partigen plates (Behringwerke) was used and duplicate samples from pregnancies with a recurrence risk for a neural tube defect were analysed by Dr D. J. H. Brock, Department of Human Genetics, Edinburgh, with rocket immunoelectrophoresis. This test has only been used since January, 1974, and in addition to pregnancies at risk for a neural tube defect all

other amniotic fluids were routinely tested. In pregnancies at risk for a neural tube defect a chromosome analysis was performed as well to exclude the presence of a fetus with a chromosome aberration.

**Confirming studies after selective abortion.** Fetal necropsies were performed by Dr J. L. J. Gaillard, Department of Pathology. Cell cultures were initiated from fetal skin. Fetal organs or fetal cells were used for biochemical assays by Dr J. F. Koster (Department of Biochemistry) in the case of metabolic diseases.

## Results

**Prenatal chromosome studies.** The results of 350 pregnancies monitored for a variety of genetic risks are listed in Table I. The period between amniocentesis and diagnosis in 269 prenatal chromosome analyses was 6 to 14 days in 47%, 15 to 21 days in 40%, and 22 to 28 days in 13% of the cases. Most cultures had colonies with varying morphology (epithelioid, fibroblast-like, and intermediate). No 46XX/46XY mosaicism was observed, which might indicate admixture with maternal cells, nor was a male child diagnosed as female.

In 101 pregnancies with a recurrence risk of Down's syndrome 60 mothers had a previous child with cytogenetic evidence of trisomy 21, and 2 had a child with a sporadic translocation. The diagnosis of Down's syndrome in the other children had been made because of the clinical features. In one case of fetal trisomy 21 the mother had had a previous child with the same aberration. Lymphocyte and fibroblast studies in the parents showed no evidence for mosaicism. A second abnormal karyotype found in the group with recurrence risk of Down's syndrome was a sporadic translocation 46,XY,t(7q-,21q+) (Fig. 1). The previous child had had Down's syndrome caused by a 46,XY/47,XY+21 mosaicism; the parents' karyotypes were normal (Dr W. L. Gouw, Dept. Anthropogenetics, Groningen). The parents were informed about

the possible consequences of this type of chromosomal aberration, and they asked for interruption of the pregnancy. Chromosomes studies on fetal cord blood gave similar results to the prenatal karyotype; however the fetus showed no macroscopical abnormalities.

In 87 cases tested because of advanced maternal age, 4 non-disjunctions were detected (Table I). These 4 cases occurred in 68 mothers of 40 years and older. The chromosome abnormalities were confirmed after elective abortion.

Eight patients were referred because one of the sibs of the parents had Down's syndrome. No abnormalities were detected.

In 11 cases one of the parents carried a balanced chromosomal translocation. The type of translocation and the result of prenatal testing are listed in Table II. Two unbalanced karyotypes were observed. After interruption of the pregnancy the prenatal diagnosis was confirmed in Case 4. Case 2 was referred in the twenty-third week of pregnancy

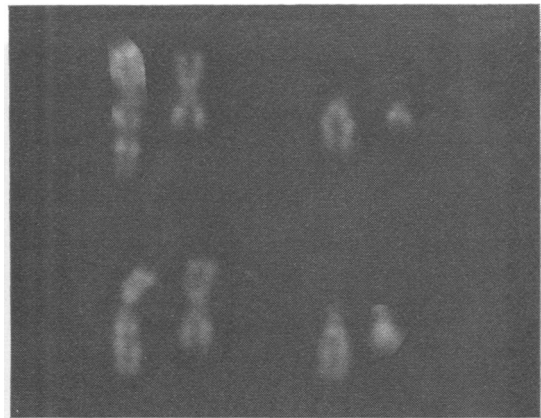


Fig. 1. Prenatal karyotype (Q-banding) 46,XY,t(7q-,21q+) from a pregnancy with a recurrence risk of trisomy 21.

TABLE II  
PREGNANCIES AT RISK FOR AN INHERITED TRANSLOCATION

Case No.	Type of Translocation	Carrier Parent*	Outcome of Pregnancy
1	D-G	M	46,XY
2	13-22	M	46,XY,D-,t(22-13)+
3	14-21	M	Spontaneous abortion 4 weeks after amniocentesis
4	21-22	M	46,XY,t(21-22) selective abortion
5	D-G	M	45,XY,t(D-G)
6	D-G	M	45,XY,t(D-G)
7	D-G	F	45,XY,14-,21-,t(14q;21q)+
8	6-13	M	46,XX
9	t(4;10)(q21;q11)	M	t(4;10)(q21;q11)
10	14-21	F	45,XX,14-,21-,t(14q;21q)+

\* M = mother, F = father.

and the mother was found to be a 22/13 translocation carrier. A 46,XY,-13,+der(22)t(13;22)(q12;q23) karyotype was found in the fetus (Fig. 2) in the twenty-seventh week of pregnancy. A male child weighing 1950 g was born in the thirty-seventh week and died on the second day of life. The following malformations were observed: cleft palate, antimongoloid eye slant, bilateral iris colobomata, broad nasal bridge, dextrocardia, ventricular septal defect, transposition of the aorta, anal atresia with rectourethral fistula, hypospadias, and undescended testes, and a small cystic left kidney. This pattern of malformations resembled the cases of trisomy 22 as described by Punnett *et al* (1973) and Hirschhorn, Lucas, and Wallace (1973).

In Case 3 (Table II) spontaneous abortion occurred 4 weeks after amniocentesis; there had been previous signs of imminent abortion. The amniotic fluid cells failed to grow. Unfortunately, the abortion material was not available for chromosome analysis. Miscellaneous indications for cytogenetic studies in 19 cases included the recurrence risk of the 5p- and 18q- syndromes, maternal mosaicism,

multiple abortions, and severe intrauterine growth retardation in the second half of pregnancy. A trisomy 18 was found in the thirty-fifth week of gestation in the last case. The child was delivered stillborn. A 47,XYY fetus was detected in a pregnancy from a mother who had two previous spontaneous abortions; a number of her relatives were mentally retarded. The parents requested interruption of the pregnancy.

In 34 of the 43 cases in the group at risk for X-linked recessive diseases the mother was a proven or possible carrier of either Duchenne's muscular dystrophy or haemophilia. Other indications for prenatal sex determination were risks for X-linked mental retardation, oculocerebrorenal (Lowe's) syndrome and X-linked agammaglobulinaemia. These parents wanted to limit their offspring to non-affected females, knowing that a male would have a 50% chance of being affected. One couple at risk for Duchenne's muscular dystrophy chose to continue their pregnancy after the prenatal test showed that a male child would be born. In one culture obtained from the fifteenth week of

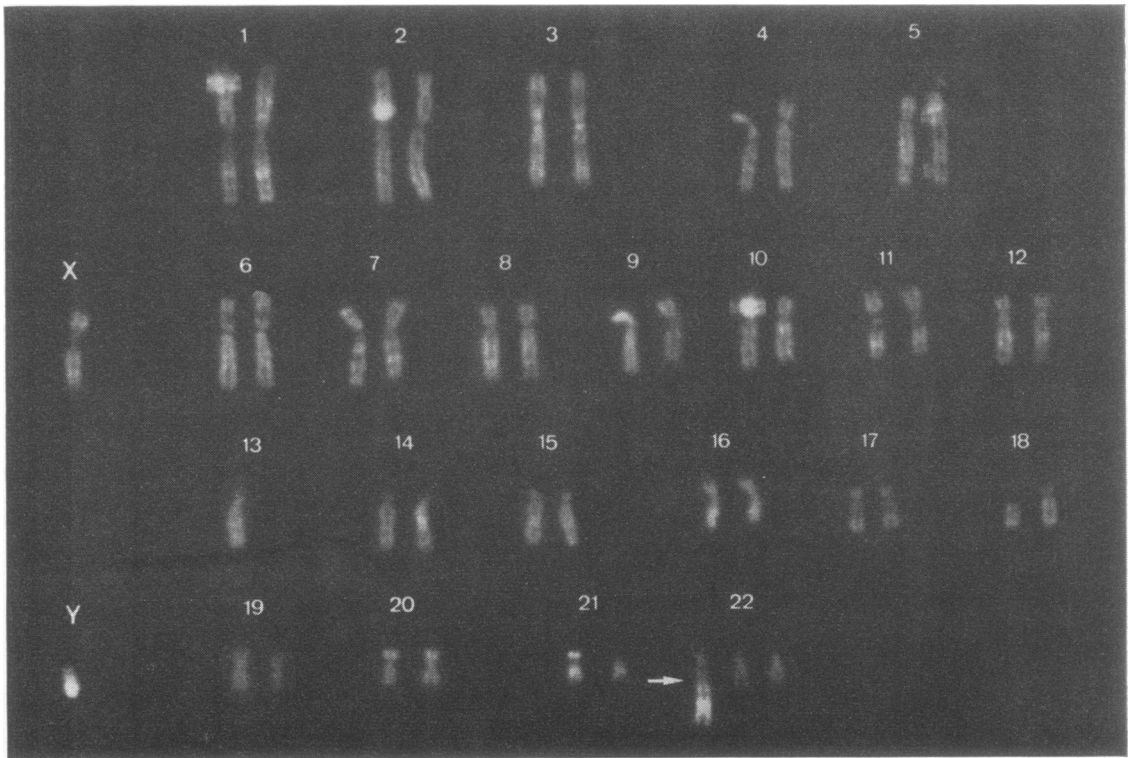


FIG. 2. Prenatal karyotype (Q-banding) 46,XY,D-,t(13;22)(912; 912)+ showing partial trisomy 22.

pregnancy at risk for Duchenne's muscular dystrophy a 45,XO/46,XY mosaicism was found. Cultures initiated from amniotic fluid obtained at termination of the pregnancy all showed 46,XY metaphases. The fetus had a male phenotype and male internal genitals; cells from fetal tissues could not be grown.

#### Prenatal diagnosis of metabolic diseases.

Thirty-four pregnancies were monitored for 11 different metabolic diseases (Table I). Some of these results have been presented in detail elsewhere (Niermeijer *et al*, 1974, 1975; Galjaard *et al*, 1974d).

The diagnosis in a previously affected child was made according to the methods listed in Table III and it is apparent that the enzyme defect had been identified in the majority of cases by studies on leucocytes and fibroblasts. When only necropsy studies were available, the parents were informed that prenatal diagnosis in their cases had certain restrictions.

In the first two pregnancies tested for Pompe's disease insufficient cell growth was obtained and the activity of acid  $\alpha$ -1,4-glucosidase in the amniotic fluid supernatant was normal. At that time (1969) such a test was still considered as reliable (Nadler and Messina, 1969). Unfortunately, one of these children proved to be affected with Pompe's disease. In all 11 subsequent cases prenatal diagnosis was based on assays on cultured cells. In two cases very low activities of acid  $\alpha$ -1,4-glucosidase were observed; after interruption of these pregnancies in the eighteenth week enzyme assays on fetal tissues (liver, muscle, heart, brain, and kidney) confirmed the diagnosis. In one instance a low activity of acid  $\alpha$ -1,4-glucosidase in an early primary amniotic fluid cell culture was erroneously interpreted as

compatible with Pompe's disease in the fetus, and the pregnancy was terminated. Later it was seen that the enzymatic activities in later subcultures of control amniotic fluid cells might be considerably higher than those in early primary cultures. Since then we have used control cells which have been cultured under similar conditions and for the same period as those from the pregnancy at risk (Niermeijer *et al*, 1975). In 8 cases the activities of acid  $\alpha$ -1,4-glucosidase were found to be in the range of controls or heterozygotes and all children born were found to be normal. These prenatal diagnoses were completed in 14 to 22 days after amniocentesis.

Three pregnancies were tested for Tay-Sachs' disease. In one case a normal hexosaminidase A activity was detected on electrophoresis of cultured amniotic fluid cells. A normal electrophoretic pattern in amniotic fluid supernatant was found in the other two cases; this result was confirmed by analysis of cultured cells in other laboratories.

Three amniotic fluid cell cultures were monitored for Sandhoff's disease and all showed the presence of both hexosaminidase A and B by electrophoresis and a normal total hexosaminidase activity. One of these children has been born and has serum hexosaminidase activity in the normal range.

Microchemical assays of  $\beta$ -galactosidase were performed on dissected groups of 10 to 20 freeze-dried cultured cells from two pregnancies at risk for GM<sub>1</sub>-gangliosidosis. The diagnoses were completed 9 and 15 days after amniocentesis and showed normal values (Table IV).  $\beta$ -galactosidase activities obtained from freeze-dried cells showed a good correspondance with values obtained in analyses on cell homogenates prepared from replicate cultures. Activities are expressed per mg protein in cell homogenates but can be calculated per cell by using

TABLE III  
METHODS OF DIAGNOSIS IN PREVIOUS AFFECTED CHILDREN IN PREGNANCIES AT RISK FOR METABOLIC DISEASES

	No. Pregnancies Studied	Diagnosis of Index Patient by Studies on*					
		Leucocytes	Fibroblasts†	Urine	Necropsy	Clinical Features	Pedigree
Glycogenesis type II	13	11	11		1		
GM <sub>2</sub> gangliosidosis (Tay-Sachs)	3	2	1				
GM <sub>2</sub> gangliosidosis (Sandhoff)	3	3					
GM <sub>1</sub> gangliosidosis	2		1		1		
Fabry's disease	1						
Metachromatic leucodystrophy	3	1	1		1		
Krabbe's disease	1						
Hurler's disease	3		2	1			
Hunter's disease	3					3+	3
Gaucher's disease	1	1					
Maple syrup urine disease	1			1			
	34	19	16	2	4	3+	3

\* Data as obtained from centres referring amniotic fluid or amniotic fluid cell cultures for prenatal diagnosis.

† Fibroblasts from index case studied simultaneously with amniotic fluid cells.

TABLE IV  
 PRENATAL DIAGNOSIS FOR GM<sub>1</sub> GANGLIOSIDOSIS USING DIFFERENT MICROTÉCHNIQUES

A. Studies on groups of 10 to 20 freeze-dried cells*		$\beta$ -Galactosidase Activity ( $\times 10^{-13}$ mol/h per cell)
Amniotic fluid cells		
Pregnancy at risk (Mrs E)		2.5
Control		3.6
Fibroblasts		
GM <sub>1</sub> -Gangliosidosis		0.08
B. Studies on cell homogenates†		$\beta$ -D-N-Acetylglucosaminidase (nmol/h per mg P)
Amniotic fluid cells		
Pregnancy at risk (Mrs E)		5 400
Control 1		5 100
Control 2		4 100
Control 3		11 200
Fibroblasts		
Control		780
GM <sub>1</sub> patient E		63

\* Performed 9 days after amniocentesis.

† Performed 12 days after amniocentesis.

the factor 1 mg protein =  $3.10^6$  cells (Galjaard *et al*, 1974c).

Microchemical analyses were also done on freeze-dried cells cultured from a pregnancy at risk for Fabry's disease. The result was available 11 days after amniocentesis. Cell homogenates from replicate cultures were also analysed, both in our laboratory and in the Institute of Medical Genetics, Copenhagen. Normal  $\alpha$ -galactosidase activities and a normal male karyotype were found. A normal boy was born (Galjaard *et al*, 1974d).

In three cases at risk for Hurler's disease  $^{35}\text{SO}_4$ -incorporation and chase studies suggested normal mucopolysaccharide metabolism in the fetus. In one case, however,  $\alpha$ -L-iduronidase activity fell in the same range found in fibroblasts from Hurler patients. According to the suggestion by Hall and Neufeld (1973), who observed a similar phenomenon, we relied upon the results of  $^{35}\text{SO}_4$ -studies and the pregnancy was continued.

In three pregnancies at risk for Hunter's syndrome chromosome analyses were performed along with  $^{35}\text{SO}_4$  incorporation studies. Three female fetuses were detected and all showed normal mucopolysaccharide degradation, as expected. Microchemical assays of arylsulphatase A activity in three pregnancies at risk for metachromatic leucodystrophy were performed 16 and 18 days after amniocentesis and indicated the presence of unaffected fetuses.

In a pregnancy at risk for Krabbe's disease the assay of  $\beta$ -galactocerebrosidase (performed by Dr A. D. Patrick, London) on a homogenate of cultured cells showed a normal activity.

A  $\beta$ -glucosidase activity half of control amniotic

fluid sample but clearly higher than that found in a previous pregnancy was found in cultured amniotic fluid cells from a pregnancy at risk for Gaucher's disease.

The assay of branched-chain ketoacid decarboxylase activity was completed 11 days after amniocentesis in a pregnancy at risk for maple syrup urine disease. Normal activity was found.

In children born after prenatal testing for metabolic defects, biochemical assays on leucocytes or fibroblasts were performed whenever possible and in 15 cases confirmation of the prenatal diagnosis was obtained. The other pregnancies were not completed when this report was written.

**Prenatal diagnosis of neural tube defects.**  $\alpha$ -fetoprotein levels (AFP) were raised (90  $\mu\text{g}/\text{ml}$  in week 27; 13 in week 29 and 90 in week 22, respectively) in 3 of 19 cases where hydramnios developed in the second half of pregnancy. Ultrasound examination showed anencephalic fetuses in these three cases. No abnormal raised levels were found in 28 pregnancies tested for the recurrence risk of a neural tube defect.

In a pregnancy tested for the recurrence risk of trisomy 21 during the sixteenth week an AFP level of 70  $\mu\text{g}/\text{ml}$  was found; this sample, however, showed admixture with fetal red blood cells and a second sample obtained at the nineteenth week of the pregnancy showed a normal AFP level.

**Complications of amniocentesis.** No serious maternal complications were observed. Two patients experienced a transient vaginal leakage of amniotic fluid for a few days after amniocentesis.

Spontaneous abortions within one month after amniocentesis were observed in 3 pregnancies.

*Case 1.* Spontaneous abortion within two weeks after transvaginal amniocentesis, which was performed because the placenta was localized over the complete anterior uterine wall. Fetal tissues showed signs of amnionitis, and since this episode the transvaginal route had never been repeated.

*Case 2.* Pregnancy at risk for Pompe's disease; spontaneous abortion 3 weeks after amniocentesis. Analysis of fetal tissues showed a deficiency of acid  $\alpha$ -1,4-glucosidase activity.

*Case 3.* Balanced carrier of a 14/21 translocation. Spontaneous abortion occurred 4 weeks after amniocentesis. Amniotic fluid cells failed to grow. Fetal tissues were not available for study.

**Failures of amniocentesis and amniotic fluid cell cultivation.** In 14 of 350 cases (4%) attempts to obtain amniotic fluid failed at the first puncture. In every case a repeat puncture was successfully carried out 10 to 14 days later.

In the whole series of 350 pregnancies monitored 'no result' (no prenatal diagnosis established) was obtained in 9 cases (2.5%); in 18 cases (5%) a repeat amniocentesis was indicated because of failure in cell cultivation.

However, in the 198 consecutive cases studied in 1974 the figure for 'no result' was 2/198 or 1%. In both failures (cases at risk for Down's syndrome) either the repeat amniocentesis failed to obtain sufficient amniotic fluid or cell growth was insufficient for karyotyping. The figure for failure in cell cultivation in 1974 dropped to 2.5%. The reasons for these failures were: transport failures (2 cases), insufficient amniotic fluid obtained at the first puncture (1) and insufficient growth in the first sample (2). In one of these latter cases multiple chromosomal breaks were observed in the first sample obtained; in the repeat culture a normal karyotype was found.

At present the failure rate, the result of insufficient growth of amniotic fluid cells, may be estimated as 2/198 or 1%.

**Follow-up of children born after prenatal diagnosis.** The outcome of pregnancy is known in  $\pm 300$  cases. No increase in complications of pregnancy and delivery was found. Children were seen for follow-up studies at different ages by one of the authors (M.J.). No serious damage or malformations were seen which could be related to amniocentesis. Detailed results on the follow-up studies will be presented elsewhere.

## Discussion

**Evaluation of amniocentesis and amniotic fluid cell culture.** Prenatal diagnosis may be evaluated according to several different criteria. The risk of amniocentesis to the mother may be estimated as negligible; the risk to the fetus as measured by the rate of spontaneous abortion after amniocentesis differs from one study to the other: 1:155 (Nadler and Gerbie, 1970), 4:73 (Doran *et al*, 1974), 3:100 (Golbus *et al*, 1974), 1:50 (Prescott *et al*, 1973), 10:128 (Robinson *et al*, 1973), and 7:477 (Milunsky and Atkins, 1974). These variations may be caused by differences in techniques and by different criteria during the follow-up period. For an abortion to be caused by amniocentesis, a time relation between these two events should exist (Wahlström, Bartsch, and Lundberg, 1974). For the period of one month after amniocentesis 3 spontaneous abortions occurred in our series of 350 pregnancies (about 1%); in one of these amniocentesis was clearly the cause of the fetal loss. The average risk of about 1% is not significantly different from the risk of spontaneous abortion in the second trimester, which was estimated in the U.S.A. as 1.2% (Javert, 1957; Shapiro, Jones, and Densen, 1962) and as 1 to 3% in Sweden (Pettersson, 1968). Of the total fetal loss, 32% occurred between 12 and 19 weeks of gestation, and 12% at 20 weeks or more in a study on spontaneous abortion (Shapiro *et al*, 1962).

Follow-up studies on the children born after prenatal diagnosis failed to detect either an increase in congenital malformations or a specific malformation pattern associated with the puncture. Hsu and Hirschhorn (1974), Golbus *et al* (1974), and Allen *et al* (1974) observed similar results.

We failed to obtain amniotic fluid at the first amniocentesis in 4% of the cases in the present series; failure rates of 1% (Nadler and Gerbie, 1970), 6% (Golbus *et al*, 1974; Prescott *et al*, 1973; Robinson *et al*, 1973; Wahlström, Bartsch, and Lundberg, 1974), 8.5% (Doran *et al*, 1974), and 10% (Milunsky and Atkins, 1974) have been reported. The failure rate of amniotic fluid cell culture in the present study is 1%; others reported either similar data (Nadler and Gerbie, 1970) or higher figures: 10% (Milunsky and Atkins, 1974; Allen *et al*, 1974) and 30% (Robinson *et al*, 1973). The length of the waiting period between amniocentesis (in the fourteenth to sixteenth week) and the eventual diagnosis is another criterion for evaluating prenatal diagnosis. This period should not exceed 2 to 3 weeks so that if necessary abortion can be performed before the mother experiences fetal



movements (Ferguson-Smith *et al*, 1971; Golbus *et al*, 1974). In the present study the result of prenatal chromosome analysis was available within 3 weeks in 87% of the cases (47% of the parents knew the result within 2 weeks). These data are similar to those from Nadler and Gerbie (1970), Therkelsen *et al* (1972) and Milunsky and Atkins (1974); Prescott *et al* (1973) and Golbus *et al* (1974) used longer periods.

**Indications and problems in prenatal chromosome analysis.** Prenatal monitoring has become a routine for pregnancies at advanced maternal age and for cases with a recurrence risk of trisomy 21 (Down's syndrome). The recurrence risk of trisomy 21 was found to be 1% in a series of prenatal diagnoses collected by Milunsky (1973) and a similar risk was observed in the present series. In the group of pregnancies at advanced maternal age the risk for chromosomal aberrations (determined from several series of prenatal diagnosis) is 1.5% in mothers between 35 and 39 years and about 3% in the group of 40 years and older (Hsu and Hirschhorn, 1974). In the present series 4 non-disjunctions were found in 68 pregnancies in mothers 40 years and older. These risk figures are higher than those generally given in genetic counselling to older mothers. The risk for a child with a chromosomal aberration is much higher when one of the parents is a carrier of a balanced translocation (Table II). After the detection of the familial nature of a translocation in a family with one child affected with Down's syndrome (Table II, Case 1), several relatives were karyotyped. In this way another high-risk pregnancy was identified in time to give the parents the chance of requesting prenatal diagnosis (Table II, Case 5). Timely identification of translocation carriers is one of the important tasks of a genetic counselling service.

A number of the technical problems in chromosome analysis on cultured amniotic fluid cells has been discussed recently, including chromosomal mosaicism (Kardon *et al*, 1972; Hsu *et al*, 1973; Cox *et al*, 1974) and structural aberrations arising *in vitro* by contamination with mycoplasma (Schneider *et al*, 1974). One case of a 45,XO/46,XY mosaicism in the present study is comparable with the observation of Kardon *et al* (1972) and this mosaicism probably arose *in vitro*; however, cytogenetic studies on the fetus were not possible. In one case inconsistent structural aberrations were detected; metaphases from a repeat culture (obtained after a second amniocentesis) were normal. The role of mycoplasma in this series is difficult to

evaluate. Though all tests were negative, mycoplasma testing was performed only periodically.

Nadler (1972) estimated that maternal cells would be growing in 0.5% of amniotic fluid cell cultures and this would accordingly lead to a number of false diagnoses. Philip *et al* (1974) reported 2 false sex predictions in 93 cases and they advocate the comparative study of Q-banded maternal and fetal karyotypes, since in most cases differences in fluorescent markers might enable a distinction to be made between mother and fetus. In the present series no sex chromosome mosaicism was observed and comparative studies of maternal and fetal cells were not done. Hsu and Hirschhorn (1974) discuss the problem of counselling parents when an apparent balanced reciprocal translocation is found. This rearrangement may be an aneusomy by recombination, though true cases should be detectable by banding techniques (Boué and Boué, 1973). In sporadic balanced reciprocal translocations, which occur in about 1 of every 3000 newborns (Jacobs *et al*, 1974), small deletions, not discernible with present techniques, or a position effect may be present (Skovby and Niebuhr, 1974). In addition, *de novo* balanced structural autosomal rearrangements might be associated or the cause of severe mental retardation (Jacobs, 1974). Our finding of an apparently balanced *de novo* 7-21 translocation is an example of this problem, when the prenatal karyotype leaves doubts about the eventual phenotype. Another counselling problem arises when there is an odd number of sex chromosomes. An XYY karyotype may be expected in 0.09% of newborns, an XXY in 0.1% (Jacobs *et al*, 1974). Even after extensive studies on the clinical aspects of the XYY genotype (see for reviews: Hook, 1973; Borgaonkar and Shah, 1974) the number of unanswered questions warrants counselling of the parents to inform them that the definite risk is not known. The final decision rests with the parents.

#### **Prenatal diagnosis of metabolic disease.**

The prenatal diagnosis of metabolic defects formerly required a period of 4 to 8 weeks after amniocentesis and parents had to be warned about waiting periods of up to 6 weeks, when prenatal diagnosis was discussed (Milunsky, Atkins, and Littlefield, 1972). Microtechniques facilitated the enzymatic analysis of fewer numbers of cells, either small groups of freeze-dried cultured amniotic fluid cells or homogenates of a few thousand cells. Prenatal analysis for Pompe's disease was completed in 11 to 22 days (Niermeijer *et al*, 1975), for Fabry's disease in 11 days (Galjaard *et al*, 1974d) and for GM<sub>1</sub>-gangliosidosis in 9 and 12 days. In previous reports on

prenatal diagnosis of GM<sub>1</sub>-gangliosidosis the result became available after 18 days (Lowden *et al*, 1973) and 4 weeks (Kaback *et al*, 1973; Booth, Gerbie, and Nadler, 1973). The prenatal monitoring for meta-chromatic leucodystrophy has required 3 to 4 weeks (Leroy *et al*, 1973; Van der Hagen *et al*, 1973); in the present study diagnosis was performed in 16 and 18 days in two pregnancies studied. A further reduction in this waiting period may be achieved by decreasing the final volume of the enzymatic reaction to 1 to 5  $\mu$ l and measuring the extinction in a microspectrophotometer (Galjaard *et al*, 1974c). Testing for maple syrup urine disease using a radioactive substrate was completed in 11 days in this study; others have used periods of up to 8 weeks (Elsas *et al*, 1974; Hoo, Latta, and Schaumlöffel, 1974). Using the methods described, the interval between amniocentesis and diagnosis for a number of metabolic diseases is comparable to the period required for prenatal chromosome analysis.

The basic requirements for reliable prenatal diagnosis of metabolic diseases were reviewed by Littlefield (1971), Burton *et al* (1974) and Hsu and Hirschhorn (1974). The present experience indicates that the following points should be considered:

1. The expression of the metabolic defect should be characterized in fibroblasts from a previous affected child or relative from the family under study, and the level of residual enzymatic activity should be studied, since genetic heterogeneity exists within apparently identical metabolic diseases. From Table III one may note, that these data were not available in all cases studied. Many parents will not seek genetic counselling until after the death of an affected child; fibroblast cultures from affected children can be stored in a cell bank to provide control material for a future prenatal diagnosis.

2. A number of variables may influence enzymatic activities in cultured cells and sufficient experience with these factors is essential. The type of tissue culture media (Ryan, Lee, and Nadler, 1972; Butterworth *et al*, 1974), its pH (Eagle, 1973; Lie *et al*, 1973), differences in cell types in amniotic fluid cell cultures (Gerbie *et al*, 1972), and the effect of growth to confluency (Okada *et al*, 1971; Russell, Russell, and Littlefield, 1972; Butterworth *et al*, 1973; Galjaard *et al*, 1974e) may all effect enzymatic activities. The activity of some lysosomal hydrolases may show considerable variations in primary cultures of amniotic fluid cells; in later subcultures the activities increased during prolonged cultivation (Niermeijer *et al*, 1975). Amniotic fluid cell cultures used as controls in prenatal diagnosis should, therefore, be carefully matched for their growth stage and passage. Since fibroblast cultures and amniotic fluid

cultures have important enzymatic differences, fibroblasts should not be used as the sole controls (Kaback, Leonard, and Parmley, 1971).

#### **Prenatal diagnosis of neural tube defects.**

The prenatal diagnosis of open tube defects by raised levels of  $\alpha$ -fetoprotein in the amniotic fluid (Brock and Sutcliffe, 1972) offers new perspectives in genetic counselling. The risk of open neural tube defects will become a common indication for prenatal diagnosis, since neural tube defects and Down's syndrome have a comparable frequency. Closed lesions or smaller open defects possibly remain undetectable by AFP levels, and the parents should be so informed; however, the clinical effects of these lesions may be milder in some cases (Laurence *et al*, 1973; Harris *et al*, 1974). The reliability of the method has been proven in different centres (Allan *et al*, 1973; Milunsky and Alpert, 1974; Brock, Scrimgeour, and Nelson, 1975). Aspecific rises in second trimester amniotic fluids may be caused by admixture with fetal blood, which has a high AFP concentration (Field *et al*, 1974; Ward and Stewart 1974). In the case observed in this study the amniotic fluid AFP level returned to normal levels in 10 days. In our laboratory blood-stained amniotic fluids were tested for fetal Hb in cases with a recurrence risk for neural tube defects.

In our series of 350 pregnancies, selective abortion was performed as a result of the prenatal diagnosis in 36 cases. In 90% of the cases the pregnancies were continued knowing that the risk of a particular genetic disease was eliminated. Ferguson-Smith (1974) stated: 'unfortunately, many parents at risk do not know that the option of prenatal diagnosis is available to them, and a very few are actively discouraged from seeking it by their medical advisers, who may feel that the risks of foetal abnormality are too small to justify the risk of the procedure.' Results in the present series again show that these latter fears are unjustified. Improvement of communications between genetic counsellors and family doctors might contribute to a better application of present knowledge.

#### **Conclusions**

1. Transabdominal amniocentesis in the fourteenth to sixteenth week is harmless to the mother and carries a risk for abortion which is as yet unknown but may be estimated as  $\pm 1\%$ .

2. There is a 5% chance that a second amniocentesis after 10 to 14 days will be necessary (4% because of a first 'dry tap' and 1% for culture

failure). The chance that no diagnosis will be obtained after a repeat amniocentesis is about 1%.

3. When sufficient experience in cell cultivation and chromosome identification techniques are available, prenatal chromosome analysis may be considered a routine technique. The same applies to AFP analysis for open neural tube defects.

4. Prenatal diagnosis of metabolic disorders should be carried out only in centres that have sufficient experience with biochemical analysis of cultured human cells. Experience with the biochemistry of the metabolic diseases studied is a prerequisite as is the availability of the proper control material in a cell bank. Microchemical techniques have the advantage of reducing the waiting periods for the parents and should be available in the diagnostic centre.

Collaboration between different centres is required because of the relative infrequency of most metabolic diseases and the facilities and know-how required.

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#### Addendum

Since this paper was accepted further pregnancies have been monitored—635 up to December 1975. Thirteen chromosomal non-disjunctions (7%) were found in 190 pregnancies tested because of advanced maternal age ( $\geq 38$  years) (10 trisomies 21, 2 trisomies 18 and 1 47 XXY). This confirms the observation that the risk for a non-disjunction in a pregnancy at this age is higher than previously estimated from retrospective data. In a

survey of experiences with 6000 prenatal diagnoses made by different European centres (Galjaard, 1976, in press) a risk of 4.6% for a fetal chromosomal abnormality was found in mothers of  $\geq 38$  to 40 years.

The recurrence risk of Down's syndrome (other than by inherited translocation) was monitored in 145 pregnancies; 3 fetal chromosome abnormalities as indicated in the paper were observed. Sixty-one pregnancies were monitored for metabolic diseases: 55 for 13 different lysosomal storage diseases, 2 for Maple syrup urine disease, and 4 for the Lesch-Nyhan syndrome.

More than half of these cases were referred by centres in different European countries, some of these as duplicates. Affected fetuses were found in 9 cases (Pompe's disease, Hurler's disease, Maple syrup urine disease, and the Lesch-Nyhan syndrome).

Results of biochemical studies on children born in 1975 after prenatal diagnosis for a metabolic disease confirmed the prenatal prediction.

In 91 pregnancies tested for a recurrences risk of neural tube defects 1 case of fetal meningomyelocele was detected in the 16th week of pregnancy by raised levels of amniotic fluid AFP.

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