PAPERS AND ORIGINALS

Chromosome Analysis before Birth and its Value in Genetic Counselling

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Summary

Chromosome analysis of amniotic cell cultures was achieved in 29 out of 30 consecutive patients who were referred for genetic counselling during pregnancy. Amniocentesis was performed without any apparent untoward maternal or fetal complication. The only pregnancy terminated was that of a carrier of X-linked granulomatous disease, in whom the amniotic cells showed that the fetus was male and also had Down's syndrome (trisomy G). Chromosome analysis in the remaining 28 patients showed normal karyotypes. The interval between amniocentesis and a definitive karyotype varied from 7 to 31 (average 18.4) days.

The reliability of chromosome analysis from amniotic cell culture and of fetal sex determination by means of the sex chromatin and Y-fluorescence techniques was studied further in amniotic fluid from cases of therapeutic abortion and of rhesus incompatibility. The fetal sex was correctly determined in all cases. It is concluded that antenatal diagnosis of genetic disease by amniocentesis now permits a more practical approach to genetic counselling.

Introduction

The Abortion Act 1967 affords mothers with a substantial risk of bearing a seriously handicapped child the choice of having the pregnancy terminated. Many such parents are now referred to genetic clinics for assessment of this risk. Even with a high risk—for example, in autosomal recessive disorders—the decision to terminate will result on average in the sacrifice of

three normal for every affected fetus. In an increasing number of genetic disorders this situation can be avoided by antenatal diagnosis (Jeffcoate *et al.*, 1965; Jacobson and Barter, 1967; Nadler, 1969; Fratantoni *et al.*, 1969) and selective termination only of the pregnancies in which the fetus is affected.

The diagnosis of genetic defects in the fetus is possible by cytological and biochemical analysis of the amniotic fluid obtained by transabdominal amniocentesis. As chromosome aberrations are numerically the most important indication for antenatal diagnosis our efforts have been directed particularly at the chromosome analysis of cultured amniotic fluid cells (Ferguson-Smith and Ferguson-Smith, 1970). This report describes our experience of 30 consecutive patients referred for genetic counselling during pregnancy.

Materials and Methods

Specimens of amniotic fluid from the 30 patients referred for antenatal diagnosis were obtained by transabdominal amniocentesis. In addition, amniotic cell cultures were attempted in 24 pregnancies terminated by hysterotomy and in one specimen of amniotic fluid obtained in late pregnancy because of rhesus incompatibility. In the therapeutic abortions the fluid was aspirated by syringe from the intact sac shortly after hysterotomy. Fifteen of the specimens for antenatal diagnosis were transported to Glasgow by air on the day of amniocentesis (B3, B19, B37, B41, B47, B58, B67, B68, B70, B71, B72, and B73 from Belfast; B25 and B65 from Oxford; and B46 from Manchester). One specimen (B60 from Newcastle) arrived the following day by rail, and one specimen (B75 from Oxford) arrived by post two days later.

In 39 of the 55 specimens 1-2 ml of the fluid was used also for sex chromatin analysis. An additional 30 specimens of amniotic fluid, obtained specifically for the evaluation of the sex chromatin test, have been included in the series. In all cases the sex chromatin status was determined before confirmation by chromosome analysis of cultured amniotic fluid cells, by chromosome analysis of fibroblast cultures from fetal biopsy material, or by subsequent examination of the child at birth.

Our culture technique, based on that of Nadler (1968), has been modified by previous experience (Ferguson-Smith and

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Ferguson-Smith, 1970). After allocating 1-2 ml of amniotic fluid for sex chromatin analysis, 10-15 ml in a sterile polystyrene container is centrifuged at 1,000 r.p.m. for 10 minutes. The supernatant is removed, leaving 1 ml in which to resuspend the cell deposit, of which 3-4 drops are added to each of four 30-mm Petri dishes. The cells are covered with a coverslip and allowed to settle for two minutes before adding 2 ml of culture medium (Ham's F.10, containing penicillin, streptomycin, and nystatin, +30% fetal calf serum) to each dish. The cultures are incubated at 36.5°C in 5% CO₂ and the medium is replaced every three days. At five to seven days the coverslips are inspected for the attachment of epithelioid cells: if absent, the amniocentesis is repeated without further delay; if present, the cultures are continued until an active monolayer is apparent (average 10-15 days). The coverslip is then placed in a fresh Petri dish with 2 ml of fresh medium and inspected to ensure that the cells are attached to the coverslip rather than to the Petri dish. The coverslip is harvested 24 hours later by adding 8 µg of demecolcine to the medium for five to six hours. It is then removed from the Petri dish, immersed gently in 1-12% sodium citrate at 36.5°C for 20 minutes, fixed in fresh 3:1 absolute ethanol-glacial-acetic acid for 15 minutes (after first being held over the fixative for 30-60 seconds), dried in air, stained in 2% aceto-orcein, and mounted on a slide.

The cells which remain attached to the Petri dish after removal of the cover slip are redistributed by trypsinization. The medium is replaced by 0.75 ml of 0.25% trypsin in edetic acid buffer and incubated for five to six minutes. Then trypsinization is arrested by adding 2 ml of fresh medium. A new coverslip is placed in the dish and the medium is changed 24 hours later. After one to two days sufficient cells are usually attached to the coverslip to allow another harvesting.

In 25 of the cases referred for antenatal diagnosis and in 14 of the hysterotomy series enough amniotic fluid was available for sex chromatin analysis. After centrifuging 1-2 ml of amniotic fluid the cell deposit is fixed in 3:1 ethanol-acetic acid, spread on microscope slides, allowed to dry, and then stained in 1% cresyl echt violet for sex chromatin, and/or in 0.5% aqueous quinacrine dihydrochloride for the fluorescent Y body (Pearson *et al.*, 1970). Latterly, improved sex chromatin preparations were obtained by first filtering through an SM Millipore filter, fixing in situ in 95% ethanol, and then staining with 1% cresyl echt violet.

Summaries of the histories of the 30 patients referred for antenatal diagnosis are given in the Appendix.

Results

The results of chromosome analysis of amniotic cell cultures in the 30 referrals are shown in Table I. The chromosome constitution of the fetus was determined in all except one case (97%), though a second specimen of amniotic fluid was required in five patients. The success rate in terms of satisfactory cultures is thus 29 of 35 samples (83%). The interval between amniocentesis and a definitive report on the karyotype (days in culture) varied from 7 to 31 days (average 18.4 days).

In two patients (B19 and B28) the indication for antenatal diagnosis was the high risk of a serious X-linked disease. The aim of cytogenetic examination was to determine the sex of the fetus. In the case of Hunter's syndrome carrier (B19), as the fetus was male the amniotic cell cultures were also examined for cellular metachromasia with toluidine blue (Danes and Bearn, 1966). Metachromasia was not detected in a confluent primary coverslip culture taken at four weeks or in two confluent coverslip preparations following one subculture at five weeks after amniocentesis, while positive and negative control cells subcultured at the same time gave the expected reactions. It was decided that the fetus was an unaffected male and the pregnancy allowed to continue to term. The patient had an unaffected son.

The pregnancy of the carrier of X-linked granulomatous disease (B28) was the only one to be terminated. This decision was taken on sex chromatin analysis of amniotic fluid cells at 16 weeks' gestation which showed a male fetus, which thus had a 50% chance of being affected. The pregnancy was terminated two days after amniocentesis. The fetus was male with a crown-rump length of 12.5 cm and an extra digit on the radial aspect of the right thumb. There were no gross lesions in the thoracic or abdominal viscera or the brain. Microscopical examination showed no abnormality in adrenals, spleen, pancreas, thymus, lung, liver, kidneys, testes, or placenta. Subsequent chromosome analysis of the amniotic cell cultures showed an abnormal karyotype—47,XY,G+. It was concluded that the fetus had Down's syndrome (trisomy G).

Twenty-five of the other pregnancies continued to term with a favourable outcome. In each the phenotype of the fetus

TABLE I—Culture of Amniotic Cells Obtained by Amniocentesis from 30 Patients Referred for Antenatal Diagnosis

Case No.	Age	Indication	Gestation (Weeks)	Volume of Fluid (ml)	Karyotype	No. of Cells Analysed	Days in Culture	Continued or Terminated
A38 ..	32	Previous Down's syndrome	20	30	46,XY	10	30	C
B3 ..	26	D/G translocation	19	20	46,XX	5	11	C
B19 ..	29	X-linked mucopolysaccharidosis	18	20	46,XY	9	14	C
B21 ..	32	Previous Down's syndrome	30	18	46,XX	4	17	C
B22 ..	35	D/D translocation	33	40	46,XY	18	11	C
B25 ..	28	2/D translocation	17	8	46,XX	20	15	C
B28 ..	37	X-linked granulomatous disease	16	20	47,XY,G+	12	31	Terminated
B30 ..	40	Maternal age >40	15	15	46,XY	16	17	C
B33 ..	30	Previous Down's syndrome	20	30	46,XY	5	10	C
B37 ..	41	Previous Down's syndrome	17	7.5	46,XY	22	24	C
B39 ..	32	Previous Down's syndrome	16	10	46,XX	17	19	C
B40 ..	29	Previous Down's syndrome	20	20	46,XY	13	27	C
B41 ..	30	Sib with Down's syndrome	15	16	46,XX	15	31	C
B42 ..	23	Iodine-131 exposure	16	15	46,XY	30	26	C
B45 ..	35	Previous Down's syndrome	16	20	46,XX	15	20	C
B46 ..	36	Maternal trisomy mosaicism	16	10	46,XX	13	17	C
B47 ..	25	Previous Down's syndrome	18	10	46,XY	20	15	C
B58 ..	30	Previous Down's syndrome	17	10	46,XY	5	22	C
B59 ..	38	Sib with Down's syndrome	16	20	46,XX	10	15	C
B60 ..	40	Previous Down's syndrome	18	20	—	—	—	C
B62 ..	35	Previous Down's syndrome	16	15	46,XY	6	28	C
B65 ..	26	Two previous children with trisomic Down's syndrome	18	15	46,XX	18	19	C
B67 ..	26	B/D translocation	24	10	46,XX	12	25	C
B68 ..	30	Previous chromosome aberration	16	10	46,XX	10	10	C
B70 ..	40	Maternal age >40	16	10	46,XY	4	8	C
B71 ..	39	Previous Down's syndrome	16	10	46,XX	5	7	C
B72 ..	30	Previous Down's syndrome	14	10	46,XY	17	20	C
B73 ..	29	Family history of Down's syndrome	13	13	46,XX	10	20	C
B75 ..	41	Maternal age >40	14	6	46,XY	10	12	C
B76 ..	46	Maternal age >40	22	20	46,XY	25	12	C

corresponded to the previously determined karyotype. The mother and fetus showed no apparent ill effects from the amniocentesis. In the three patients who have not yet delivered, the pregnancies are progressing normally.

HYSTEROTOMY SERIES

The results of amniotic cell culture in the 24 hysterotomies and in the case of amniocentesis for rhesus incompatibility are shown in Table II. Satisfactory growth of amniotic cells was obtained in 21 samples (84%) with karyotype analysis in 17 (68%). The problems connected with obtaining amniotic fluid from hysterotomy specimens probably account for the lower rate of successful chromosome analysis. However, unsuccessful samples were from conceptions at 7, 8, 10, 10, 11, 15, and 32 weeks gestation. This confirms our observation in earlier studies (Ferguson-Smith and Ferguson-Smith, 1970) that amniotic specimens obtained before 14 weeks and possibly after 30 weeks are less satisfactory than specimens obtained at 14 to 30 weeks. This is probably related to the low cell count in early pregnancy and the large number of anucleate cells in later pregnancy (Votta *et al.*, 1968; Wachtel *et al.*, 1969).

One culture (B29) is of special interest as all mitoses were tetraploid. The occurrence of high frequencies of tetraploid

TABLE II—Culture of Amniotic Cells Obtained from Hysterotomies

Case No.	Gestation (Weeks)	Growth in Culture	Karyotype	No. of Cells Analysed	Days in Culture
B2	16	+	46,XY	9	15
B4*	32	+	—	—	—
B5	7	—	—	—	—
B6	11	+	—	—	—
B7	8	+	46,XY	9	21
B8	17	+	46,XX	3	18
B10	10	+	—	—	—
B11	15	+	46,XX	5	16
B12	15	+	—	—	—
B13	19	+	46,XY	10	14
B14	10	—	—	—	—
B15	18	+	46,XY	2	14
B16	8	—	—	—	—
B17	15	+	46,XX	2	13
B18	14	+	46,XY	6	28
B20	18	+	46,XY	2	43
B24	12	+	46,XY	6	5
B27	12	+	46,XY	3	11
B29	16	+	92,XXXXYY	10	17
B31	13	+	46,XY	7	19
B32	15	+	46,XY	1	28
B34	17	+	46,XY	5	23
B35	10	—	—	—	—
B36	17	+	46,XX	6	27
B38	22	+	46,XY	5	15

*Amniocentesis from patient with rhesus incompatibility.

TABLE III—Results of Sex Chromatin and Fluorescent Y-Body Analysis

Case No.	Indication	Gestation (Weeks)	Sex Chromatin		Fluorescent-Y % Positive	Confirmation of Sex		
			% Positive	Positive or Negative		Amniotic Cells	Fetal Biopsy	Sex at Birth
A1	Rh disease	40	0	—				M.
A2	Rh disease	40	23	+				F.
A3	Rh disease	40	24	+				F.
A4	Rh disease	40	0	—				M.
A5	Rh disease	40	31	+				F.
A8	Rh disease	41	34	+				F.
A9	Rh disease	41	8	—				M.
A10	Rh disease	40	3	—				M.
A21	Therapeutic abortion	14	28	+		XX	XX	
A27	Therapeutic abortion	10	0	—		XY		
A28	Therapeutic abortion	16	0	—		XY		
A30	Therapeutic abortion	17	28	+			XX	
A31	Therapeutic abortion	15	0	—		XY	XY	
A32	Rh disease	33	0	—				M.
A38	Previous Down's syndrome	20	0	—		XY		M.
A40	Rh disease	26	2	—				M.
A41	Therapeutic abortion	11	0	—				
A43	Therapeutic abortion	16	30	+			XY	
A45	Rh disease	30	0	—				M.
A51	Rh disease	34	0	—				M.
A60	Rh disease	31	0	—				M.
A64	X-linked muscular dystrophy	32	0	—				M.
B1	Therapeutic abortion	16	0	—		XY		
B3	D/G translocation	19	22	+		XX		F.
B6	Therapeutic abortion	11	38	+			XX	
B8	Therapeutic abortion	17	32	+		XX		
B10	Therapeutic abortion	10	0	—			XY	
B11	Therapeutic abortion	15	24	+		XX		
B13	Therapeutic abortion	19	0	—		XY		
B15	Therapeutic abortion	18	0	—		XY		
B17	Therapeutic abortion	15	30	+		XX		
B18	Therapeutic abortion	14	0	—		XY		
B19	X-linked muscular dystrophy	18	0	—		XY		M.
B20	Therapeutic abortion	18	3	—		XY		
B21	Previous Down's syndrome	30	22	+		XX		F.
B22	D/D translocation	33	0	—		XY		M.
B25	2/D translocation	17	27	+		XX		F.
B28	X-linked gran. dis.	16	3	—		XY		
B31	Therapeutic abortion	13	0	—		XY		
B32	Therapeutic abortion	15	0	—		XY		
B33	Previous Down's syndrome	20	0	—		XY		M.
B34	Therapeutic abortion	17	0	—		XY		
B36	Therapeutic abortion	17	34	+		XX		
B37	Previous Down's syndrome	17	0	—		XY		M.
B38	Therapeutic abortion	22			52	XY		
B39	Previous Down's syndrome	16			4	XX		F.
B40	Previous Down's syndrome	15	0	—		XY		M.
B41	Sib with Down's syndrome	17	44	+		XX		F.
B42	Iodine-131 exposure	16			95	XX		F.
B45	Previous Down's syndrome	16	44	+		XX		F.
B46	Maternal trisomy mosaicism	16	39	+		XX		F.
B52	Homocystinuria	16	0	—		XY		M.
B54	Therapeutic abortion	17	36	+		XX		
B55	Galactosaemia	21	20	+		XX		F.
B57	X-linked muscular dystrophy	15	35	+		XY		F.
B58	Previous Down's syndrome	17	0	—		XY		M.
B59	Sib with Down's syndrome	16	37	+		XX		F.
B61	X-linked agammaglobulinaemia	26	27	+		XY		F.
B62	Previous Down's syndrome	16	0	—	6	XY		M.
B65	2 previous children with Down's syndrome	18	28	+	80	XX		F.
B67	B/D translocation	24	24	+		XX		F.
B68	Previous chromosome aberration	16	25	+		XX		F.
B69	Spontaneous abortion	14	20	+		XX	XX	
B70	Maternal age >40	16	0	—	75	XY		M.
B71	Previous Down's syndrome	16	32	+	5	XX		F.
B72	Previous Down's syndrome	14	3	—	60	XY		
B73	Family history of Down's syndrome	13	24	+	0	XX		
B75	Maternal age >40	14	0	—	45	XY		M.
B76	Maternal age >40	22	0	—	58	XY		

cells in amniotic cell cultures from normal embryos is well recognized (Walker *et al.*, 1970), though the absence of any diploid cells after only 17 days in culture in our case might suggest that the embryo had been tetraploid from conception. We were unable to obtain further evidence on this point as tissue taken from the apparently normal fetus failed to grow in culture. The frequency of completely tetraploid embryos in early pregnancy must be low, judging from chromosome studies in spontaneous abortion (Carr, 1965), which suggest a rate of about 1.5 per 1,000.

With this one exception, the cultures in the hysterotomy series had normal karyotypes.

ANALYSIS OF SEX CHROMATIN AND FLUORESCENT Y BODIES

The fetal sex was correctly diagnosed by sex chromatin analysis in the 66 cases examined (Table III). In the female fetus 20 to 44% of the amniotic cells were chromatin-positive, while in the male fetus they were almost consistently chromatin-negative. In six male conceptions a low count (2 to 8%) of chromatin-positive nuclei were found, suggesting contamination of the amniotic fluid with maternal cells; the alternative possibility of sex chromosome mosaicism in the fetus is considered unlikely.

The fetal sex was also accurately predicted in the 13 cases examined by the quinacrine fluorescence methods for the fluorescent Y body (Y chromosome) in interphase nuclei. The low count of fluorescent bodies in B39, B61, and B71 is within the normal range (about 0-10%) of female somatic cell nuclei observed by ourselves (unpublished results) and others (Polani and Mutton, 1971). In our experience with male somatic cell nuclei the normal range of fluorescent Y bodies is 30 to 80%.

Discussion

This study confirms that antenatal diagnosis has an important role in genetic counselling. Each of the patients was extremely anxious about the outcome of her pregnancy, but in every case this was alleviated by antenatal diagnosis. As several patients had initially requested termination, the overall effect of amniocentesis has been to save pregnancies. The only pregnancy terminated was that of a carrier of fatal X-linked granulomatous disease where the fetus was found to be male with a 50% risk of being affected. Subsequently the fetus was found also to have Down's syndrome (trisomy G). The risk to a woman aged 37 years having an infant with Down's syndrome is about 1 in 262 (Carter and Evans, 1961).

Few studies of genetic counselling based on antenatal diagnosis have been reported (Jacobson and Barter, 1967; Valenti and Kehaty, 1969; Fratantoni *et al.*, 1969; Butler and Reiss, 1970; Gertner *et al.*, 1970; Nadler and Gerbie, 1970; Gerbie

et al., 1971). These studies are shown in Table IV and compared with the present series. Interestingly, about 79% of the total reported cases are from Chicago (Nadler and Gerbie, 1970; Gerbie *et al.*, 1971). In about half of their patients the indication for antenatal diagnosis was the risk of chromosome abnormalities with advancing maternal age—that is, mothers over 40 years. In all series a total of 115 mothers over 40 years was studied; five fetuses in this group had a chromosome abnormality (four had Down's syndrome and one Klinefelter's syndrome).

When either parent is a known translocation carrier or has chromosomal mosaicism with a high risk of having a chromosomally abnormal fetus, amniocentesis is mandatory. Though for D/G and G/G (21/22) translocations the risk for both male and female heterozygotes is much less than the theoretically expected 33% recurrence risk (Hamerton, 1970), in practice (Table IV) one-third of such conceptions had an unbalanced chromosome constitution.

In families who already have a child with Down's syndrome (trisomy G) there is an increased risk for recurrence of Down's syndrome (Carter and Evans, 1961), but the finding of three affected fetuses in 69 mothers who had a previous trisomy G infant (Table IV) is much higher than expected. Antenatal diagnosis in patients over 40 years or with a previous child with Down's syndrome thus seems justified provided the risk to the mother and fetus is small (see below).

Two further indications for antenatal diagnosis are X-linked and autosomal recessive diseases. The management of mothers who are carriers of X-linked disorders depends on the sex of the fetus. The purpose of antenatal diagnosis is to distinguish the male fetus with a 50% risk of being affected from the unaffected female fetus. As only two X-linked disorders—the Hunter and the Lesch-Nyhan syndromes—at present can be specifically diagnosed in utero, it is expected that termination in this group will be high. This is confirmed in Table IV; of 30 pregnancies to carrier mothers, 11 were terminated. Similarly, in autosomal recessive disorders in which the in utero diagnosis is possible by assay of the enzyme activity in cells cultured from amniotic fluid termination of 25% affected homozygous fetuses is to be expected. Of 35 pregnancies studied nine were terminated because the fetus was affected (Table IV).

Though amniocentesis caused little discomfort in our patients it is potentially dangerous and should be used only when there is a substantial risk of severe fetal abnormality. Among possible fetal complications are deaths from abruptio placentae, infection, fetal haemorrhage, and puncture of the fetus. Maternal complications may include maternal haemorrhage, peritonitis, rhesus isoimmunization, and premature labour. So far none of these complications have been observed in cases for genetic counselling. Gerbie *et al.* (1971) had no fetal or maternal complications attributable to the amniocentesis in their series of 238 pregnancies. They also have information

TABLE IV—Cases in which Successful Antenatal Diagnosis by Amniocentesis has been used in Genetic Counselling

Indication	Jacobson and Barter (1967)	Valenti and Kehaty (1969)	Fratantoni <i>et al.</i> (1968)	Butler and Reiss (1970)	Gertner <i>et al.</i> (1970)	Gerbie <i>et al.</i> (1971)	Others*	Present Series	Total Pregnancies Examined	Total Pregnancies Affected	Total Pregnancies Terminated
Previous trisomic Down's syndrome	1	—	—	—	5 (1)	50† (1)	—	13	69	3	2
Other previous chromosome abnormality	—	—	—	—	—	—	—	1	1	0	0
Chromosomal translocation	2	1 (1)	—	2 (1)	—	28 (9)	1 (1)	4	38	12	12
Maternal mosaicism	—	—	—	—	—	—	—	1	1	0	0
Advanced maternal age	—	1	—	—	6 (1)†	104 (4)	—	4	115	5	5
Family history of relatives with Down's syndrome	—	—	—	—	1	—	—	3	4	0	0
Exposure to mutagens	—	2	—	—	6	—	—	1	9	0	0
X-linked disease	—	—	1 (1)	—	2 (1)	24* (8)	1	2 (1)	30	12	11
Autosomal disease	—	—	—	—	2	32 (8)	1 (1)	—	35	9	9
Total	3	4 (1)	1 (1)	2 (1)	22 (3)	238 (30)	3 (2)	29 (1)	302	41	39

Figures in parentheses indicate the number of pregnancies terminated.

*Fujiimoto *et al.* (1968); Schneck *et al.* (1970); Kersey *et al.* (1971).

†Including one fetus affected but pregnancy not terminated.

‡Fetus had Klinefelter's syndrome (47,XXY).

that a further 170 patients have had amniocentesis for antenatal diagnosis in four other centres, all without fetal or maternal complications. Experience with amniocentesis in the third trimester shows that the risk of fetal and maternal complications is greater when the placenta is located on the anterior wall of the uterus. This was a cause of failure to obtain amniotic fluid in one of our cases (A38). We have therefore used ultrasonography whenever possible to locate the placental site and to exclude multiple pregnancy before attempting amniocentesis. It is important that amniocentesis should not be undertaken in a twin pregnancy or for trivial purposes such as choosing the sex of offspring.

EARLY DIAGNOSIS

In agreement with other studies (Nadler, 1968; Valenti and Kehaty, 1969; Abbo and Zellweger, 1970; Ferguson-Smith and Ferguson-Smith, 1970), successful chromosome analysis was achieved from primary culture of amniotic cells grown on coverslips. Of the various modifications suggested to give the shortest delay from amniocentesis to chromosome analysis, those in which the cells are fixed and stained on the coverslip tend to be quicker than air-dried preparations made from cell suspensions after subculture. In the series of patients referred for antenatal diagnosis, the interval between amniocentesis and the cytogenetic diagnosis was, on average, 18.4 days (Table I). The quickest result was obtained seven days after amniocentesis.

The aim is to have a definite antenatal diagnosis as early as possible in the pregnancy to permit selective abortion before fetal movements are pronounced, and ideally before 20 weeks' gestation. This was achieved in 20 patients in the present series in whom amniocentesis was performed before 19 weeks. In three patients the result was not available until 21 weeks, in another three not until 24 weeks, and in one it was not known until 27 weeks. In the remaining two cases amniocentesis was delayed to 30 and 33 weeks by which time termination could not be contemplated (amniocentesis was performed because of maternal anxiety). As trans-abdominal amniocentesis is usually not practical before the 14th week of pregnancy, the optimal time for amniocentesis for antenatal diagnosis seems to be between 14 and 17 weeks' gestation.

Though chromosome preparations made direct from the primary growth of amniotic cells on coverslips gave the quickest result, the number of mitoses in the initial analysis was in eight cases fewer than 10. Nevertheless, the quality of the mitoses was uniformly high, and a firm decision on the karyotype was possible on as few as four cells (B21). The probability of detecting chromosomal mosaicism is therefore low, but this eventuality can never be excluded even with the larger number of cells obtained later by subculture.

DETERMINATION OF SEX

The sex of the fetus was determined accurately by sex chromatin analysis in each of the 66 cases studied. Nelson and Emery (1970) found that accurate sex prediction was possible in only 91% of their cases. The technique is simple and reliable, though the examination of air-dried preparations is tedious as many cells have small pyknotic nuclei and are thus unsuitable for analysis. The introduction of the Millipore filtration technique has resulted in improved fixation and an increased yield of cells, enabling a smaller sample of fluid to be used. The filtration modification is also much less time-consuming.

In 13 cases, in which air-dried amniotic cells were used, the sex of the fetus was determined correctly by the presence or absence of Y bodies in interphase nuclei stained by the quinacrine fluorescence method. Similar observations have

been noted in the three cases reported by Polani and Mutton (1971). The technique is useful for confirming fetal sex. However, it is probably less reliable than the sex chromatin method because female nuclei commonly have a small proportion of fluorescent bodies indistinguishable from the Y body and because the Y body may be extremely variable in size (Borgaonkar and Hollander, 1971).

Antenatal detection of genetic diseases by amniocentesis now permits a more practical approach to genetic counselling. The parents of a child with a genetic disease which has a high risk of recurrence in future offspring are often faced with the difficult decision of whether or not to risk another pregnancy. Antenatal diagnosis now offers such parents a real hope. Should the test show an abnormal fetus, the medical geneticist can present the parents with the available alternatives. If the genetic disease is amenable to treatment, as in the case of congenital adrenal hyperplasia and galactosaemia, the in utero diagnosis will allow immediate treatment at birth. If no treatment is available, as with Down's syndrome and Duchenne muscular dystrophy, the parents can be offered a termination of pregnancy. Thus antenatal diagnosis now affords such parents the chance of having healthy children.

Undoubtedly, the number of genetic diseases detectable in utero will increase, particularly in the group of inborn errors of metabolism. However, these disorders are too rare to permit routine screening, but in the chromosome disorders and X-linked diseases (which are numerically more important) screening of particular at-risk groups may be possible in the future. The antenatal diagnosis of genetic disease not only provides a valuable aid to genetic counselling but also affords the opportunity to reduce genetic disease in the community.

Appendix. Case Histories

A38, 32 years old para 1+0, had a son with Down's syndrome (trisomy G) and was anxious about the risk of recurrence. Amniocentesis was difficult owing to an anterior placenta, and the first specimen taken at 16 weeks' gestation was heavily blood-stained and failed to grow. At 20 weeks' gestation, after ultrasonography, a second specimen was obtained which provided successful amniotic cell cultures.

B3, aged 26 and para 2+0, was found to be heterozygous for a balanced D/G translocation during her third pregnancy. She had a normal 5-year-old boy and a 3-year-old girl with D/G translocation Down's syndrome. No other relatives were affected.

B19 aged 29 and para 4+0, had two sons with X-linked Hunter's syndrome (mucopolysaccharidosis II) and two normal daughters. Review of four generations of the family failed to detect any other affected relative.

B21, aged 32 and para 1+1, had a son with Down's syndrome (trisomy G) who died aged 6 weeks after an operation for intestinal obstruction. Her first pregnancy miscarried at 11 weeks. Her own karyotype was normal.

B22, aged 35 and para 2+1, was found to be the carrier of a 13/14 translocation in an extensive family ascertained through the birth of a nephew with the trisomy D syndrome due to a 13/14 translocation. The patient's two sons, three of her six sibs, and seven of her 10 nephews and nieces were also translocation heterozygotes. In view of a previous miscarriage amniocentesis was performed.

B25, aged 28 and para 3+0, had a grossly malformed child with clinical features resembling the trisomy D syndrome. Chromosome analysis of the child and his father showed 46,XY,2q+pat and 46,XY,t(2q+Dq-) karyotypes respectively; the patient's chromosomes were normal but her two older daughters were translocation heterozygotes similar to their father (Bobrow, 1971). Five of the father's seven sibs, the paternal grandmother, and three of the paternal grandfather's seven sibs had normal karyotypes.

B28, aged 37 and para 3+0, had two normal daughters and had recently lost a son with X-linked granulomatous disease. The abnormal phagocytic function in this boy and his mother has been reported by Macfarlane *et al.* (1967). The present pregnancy was unplanned and termination was requested because the mother was a known carrier and there was a 50% risk to male offspring.

B30, aged 40 and para 2+1, had two normal children followed by a miscarriage. Antenatal diagnosis was requested because of her acute anxiety concerning the risk of chromosome aberrations with advancing maternal age.

B33, aged 30 and para 1+0, had a son with Down's syndrome (trisomy G) and was concerned about the risk of recurrence. She and her husband had normal karyotypes.

B37, aged 41 and para 4+1, had a healthy daughter aged 17 years and two normal sons aged 9 and 6 years. A 19-month-old son with Down's syndrome (trisomy G) had died a year previously. The parents had normal karyotypes.

B39, aged 32 and para 1+0, had a 5-year-old daughter with Down's syndrome (trisomy G) and spina bifida. Both husband and wife had normal karyotypes.

B40, aged 29 and para 1+0, had a 3-year-old son with Down's syndrome (trisomy G). Both husband and wife had normal karyotypes. As the amniotic fluid obtained at 15 weeks was blood-stained and all four cultures failed, amniocentesis was repeated at 20 weeks' gestation.

B41, aged 30 and para 2+0, had a brother and a sister-in-law both with Down's syndrome (trisomy G). Her two children were normal.

B42, aged 23 and para 2+0, received a test dose of five microcuries of iodine-131 at seven weeks' gestation. The ^{131}I uptake was 34% of the dose. A request was made to examine amniotic cells to determine evidence of radiation damage to the fetus. (Chromosome breakage was not observed.)

B45, aged 35 and para 3+1, had a 2-year-old son with Down's syndrome (trisomy G) and congenital heart disease. The patient and her husband had normal karyotypes.

B46, aged 36 and para 1+0, had one 10-year-old son with Down's syndrome (trisomy G). Early in her present pregnancy lymphocyte cultures showed clear evidence of normal/trisomy G mosaicism. This is one of the rare examples of Down's syndrome due to maternal mosaicism and is reported in more detail by Timson *et al.* (1971).

B47, aged 25 and para 1+0, had a son with Down's syndrome (trisomy G) who died during her present pregnancy. Amniocentesis was performed at 16 weeks' gestation because of the extreme anxiety about the risk of recurrence. Unfortunately no growth was obtained and amniocentesis was repeated at 18 weeks' gestation.

B58, aged 30 and para 1+0, had a daughter who died one week after birth with Down's syndrome, cor triloculare, and duodenal atresia. Both parents had normal karyotypes.

B59, aged 38 and para 1+0, had a normal daughter, now 10 years old, by a previous marriage. She was initially referred for possible termination of pregnancy in view of her age and because her brother had Down's syndrome.

B60, aged 40 and para 5+1, had four normal children and one miscarriage followed by a child with Down's syndrome. The amniotic cultures were lost by contamination with infected tissue culture medium and amniocentesis could not be repeated.

B62, aged 35 and para 1+0, had a daughter who died shortly after birth with Down's syndrome and congenital heart disease. For nine years she had avoided pregnancy because of the fear that another child might be similarly affected.

B65, aged 26 and para 3+0, had one stillbirth, the details of which were unknown, followed by two children with Down's syndrome (trisomy G). The patient and her husband had normal karyotypes without evidence of mosaicism in lymphocyte cultures (Bobrow, 1971).

B67, aged 26 and para 2+0, was heterozygous for a reciprocal translocation between the long arm of a group D chromosome and the short arm of a group B chromosome. Her father, her first child, and one of three sibs carried the same translocation. Her second child died seven months after birth with partial D trisomy due to the presence of the smaller of the two translocation chromosomes in addition to an otherwise normal female karyotype.

B68, aged 30 and para 3+0, had one stillbirth, a normal daughter, and a son who died two weeks after birth with multiple malformations including tracheo-oesophageal fistula and congenital heart disease. The parents both had normal karyotypes but the defective child was found to have an extra small acrocentric chromosome thought to represent a deleted D or E chromosome.

B70, aged 40 and para 2+0, had a normal daughter and a son who had severe mental retardation of unknown cause; his karyo-

type was normal. Amniocentesis was performed at 14 and again at 16 weeks as the first cultures failed.

B71, aged 39 and para 1+0. Her only child, who had Down's syndrome (trisomy G), died during the present pregnancy. A maternal half-cousin also had Down's syndrome. Amniocentesis was performed at 14 weeks' and at 16 weeks' gestation as the first cultures failed.

B72, aged 30 and para 2+0, had one son with mosaic Down's syndrome and another with pyloric stenosis. Both parents had normal karyotypes.

B73, aged 29 and para 1+0, had one normal daughter and a maternal first cousin once removed who had Down's syndrome (trisomy G and D/D translocation). Her husband had a maternal first cousin and a maternal first cousin once removed who had Down's syndrome.

B75, aged 41 and para 0, was referred for termination in early pregnancy because of acute anxiety about the risk of Down's syndrome with increasing maternal age. There was no family history of Down's syndrome.

B76, aged 46 and para 2+0, had children now aged 23 and 18 years. Her pregnancy was unplanned and she requested termination in view of her age.

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