Sixteen years' experience of counselling, diagnosis, and prenatal detection in one Genetic Centre: progress, results, and problems

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SUMMARY The work of one Genetic Centre over 16 years, covering about 14 000 kinships, is described. The numbers registered in a year increased from an average of 477 in the early 1960s to 1612 in 1976/1977. The increase is largely, but not entirely, attributable to the advent of prenatal diagnosis, and an account is given of our experience with this. In 1916 patients who had a successful amniocentesis, results indicative of fetal abnormality were found in 4.3% and a balanced translocation was found in an additional 0.9%. Results indicative of fetal abnormality were found in 3.5% of mothers referred because of a maternal age of 40 or more, 3.9% referred because of a high risk of neural tube defect, and 19.3% referred because of a high risk of an inborn error of metabolism. A number of cases with difficult diagnostic problems are described.

The Paediatric Research Unit was set up with the aim of preventing congenital disorders, primarily by pursuing research into causes and by the subsequent application of the research findings through counselling and diagnostic services.

Since the Unit's inception in 1960 about 14 000 kinships have been registered with it, either because one or more members had been referred for diagnosis or advice, or following the discovery of abnormalities in the course of specific population surveys. The latter group was always offered, and often accepted, genetic counselling. From 1971 the chromosome diagnostic work was partly supported by the Department of Health and Social Security on a service basis, and from 1973 the support was extended to amniotic cytology. The South-East Thames Regional Genetics Centre was established in the Unit in 1976 and the Supraregional Laboratory for Tissue Enzymes in 1973.

The following is an account of the work of this Unit, and is offered in the hope that the experiences described may be helpful to others who are planning Genetic Centres.

The major causes for referral by kinship

Table 1 gives a broad classification of the causes for referral between 1960 and 1977, and shows how the number of families registered per year has increased since the inception of the Unit. These data are related to workload, but are not identical to it, since many members of one kinship may be seen, several visits paid by one family. In either case, the families are counted only once, so that the workload is substantially larger than the figures given. On the other hand, not all of those registered have had counselling appointments, since requests for chromosome analysis may be confined to the analysis of a blood sample sent by post. From 1975 to 1977 approximately 50% of those registered were seen in the Unit. The proportion was greater in earlier years.

Before the introduction of prenatal diagnosis or of carrier detection, genetic advice was limited to probability counselling; that is, explaining to families at risk the chances of producing further affected children. The major change which has occurred has been the advent of prenatal diagnosis, but the background work, other than during pregnancy, of cytogenetic diagnosis, biochemistry, and genetic counselling of families has also continued

Table 1 New kinship registrations by 5-year periods by reason for genetic counselling or diagnosis

Referral period	All	Diagnosis or counselling without prenatal diagnosis							Prenatal diagnosis	Population surveys (chromosomes only)	Average registrations per year (excluding population surveys in brackets)
		Chromosomes		Single genes		Multi-factorial	Malignancy	Miscellaneous			
		Down's	Other	Sex	Autosomes						
1.10.60–30.6.65	2383	469	561	11	115	164	22	396	—	645	477 (348)
1.7.65–30.6.70	3844	559	1025	14	280	339	27	390	4	1206	769 (528)
1.7.70–30.6.75	4438	374	916	47	350	411	105	334	1247	654	888 (757)
1.7.75–30.6.77 (2 y only)	3330	172	607	57	270	233	130	229	1526	106	1665 (1612)

increase. It should be noted, however, that in a substantial proportion of families at risk for genetic disease, probability counselling still remains the only option.

Throughout this time about one quarter of the kinships registered had been derived from research chromosome surveys of selected populations, but this proportion has decreased over the years. The fall in the referrals for the diagnosis of Down's syndrome reflects the expansion of laboratory facilities in other hospitals in the region. In contrast, referrals for conditions caused by single genes have doubled, for advice on multifactorial conditions trebled, and for karyotyping in patients with malignancy, for example, leukaemia, have increased even more.

Several of the techniques required for diagnosis of metabolic disorders in cultured fibroblasts or amniotic fluid cells were developed in the Unit (Fensom *et al.*, 1974, 1976, 1977, 1978; Stirling *et al.*, 1978), or exploited for the first time (Ramsay *et al.*, 1974; F. G. Giannelli, 1976, personal communication), so that referrals for these conditions have also increased.

Prenatal diagnosis

DEVELOPMENT AND POLICIES IN THE UNIT

From the autumn of 1969, the Unit began to offer a prenatal diagnosis service for chromosome disorders and soon followed this with the offer of prenatal diagnosis of inborn errors of metabolism. From 1972 onwards, a diagnostic service for neural tube defects (NTD) was offered, with the measurement of alphafetoprotein (AFP) in amniotic fluid. Some of the findings have already been published as individual case reports including Sellar (1975), Campbell *et al.* (1976), Blunt *et al.* (1977), and 546 amniocentesis patients (and their controls) are included in the Medical Research Council (MRC) Working Party on Amniocentesis Report (1978).

The policy regarding the tests done on a specimen of amniotic fluid changed somewhat over the years. Before 1972 the only 'service' investigations were those done for chromosome analysis. Estimations of AFP levels in all fluids received were carried out immediately after the first published indication of its importance for the diagnosis of NTD (Brock and Sutcliffe, 1972). Since AFP studies were being done in the Unit (Adinolfi *et al.*, 1975) at that time, this application was a natural extension of our work. For a few months all fluid taken primarily for this test also had a chromosome analysis performed on it. This soon overstretched the cytogenetic resources of the Unit because of the concurrent increase in demand for karyotyping alone, and from early 1973 amniotic fluid cells from pregnancies at risk for NTD were only cultured where the mother's age, or history, put her at risk of a fetal chromosomal anomaly. As AFP estimations on amniotic fluid are much simpler and cheaper, these have always been done routinely on every amniotic fluid received, regardless of the indication for the test. The amniotic fluid samples of all fetuses investigated for biochemical errors were studied chromosomally. The Fig. gives some indication of the increase in the numbers of amniotic fluids examined.

From the summer of 1977, the Unit has been able to carry out prenatal diagnosis for all 40 metabolic diseases which can be detected reliably in the United Kingdom. Occasionally, samples are divided between ourselves and another laboratory, particularly when techniques are, or were, still experimental, for example, for specific biochemical tests. For simplicity all split samples have been included in the data presented.

MOTHERS REFERRED FOR AMNIOCENTESIS

Referrals for prenatal diagnosis come from obstetricians, family doctors, and paediatricians, both from within and without the Regional Health Authority in which the Unit is situated. By the end of December

Fig. Indications for amniocentesis by year.

1976, 2605 mothers had been referred for this reason, but only 2068 (79.4%) of these later came to amniocentesis. Between 1 January 1977 and the end of 1977 we had a further 1144 referrals in early pregnancy, of which 884 (77.3%) have had amniocentesis. The hospitals where amniocentesis was done varied with time, but for the sample described below 34% were carried out in Guy's. In the remainder, the fluids were sent to the Unit for analysis, usually by rail or car, rarely by post.

The reasons for which amniocentesis was not carried out were investigated in a sub-sample of 1277 referrals between the beginning of July 1975 to the end of December 1976. In 13 of these, it appears that amniocentesis was done elsewhere, but we have no record of the results. Of the remaining 1264, amniocentesis was not attempted in 237 (18.7%), a similar proportion to that in all referrals. The reasons were as follows: in 6.7% there was no clinical indication for amniocentesis; 6.5% refused after counselling, often because they were at relatively low risk of producing an affected child, so that this risk approached that of the amniocentesis itself; 4.8% aborted spontaneously before the amniocentesis was arranged; and in 0.7% the obstetrician advised against the operation.

INDICATIONS FOR AMNIOCENTESIS AND ASSOCIATED PROCEDURES

The Fig. shows how the proportions of amniocenteses carried out for cytology, AFP, and biochemistry other than AFP have changed over the years. The rapid rise in the proportion done for AFP estimations was associated with the dissemination of information on its value in identifying affected pregnancies.

A more detailed breakdown by indication is shown in Table 2 and 3. As can be seen, some women had

multiple indications. Of all indications, 38% were for chromosome analysis because of maternal age, and 35% for AFP because of NTD. Eighty-three (4%) amniocenteses were carried out for inborn errors of metabolism; in 32 of these, cells were both cultured and tested in the Unit (Table 3). Before prenatal diagnosis for metabolic disorders can be undertaken, it is important to confirm the specific enzyme defect in the propositus if possible, or in the carrier parents (or mother in X-linked disorders). This is a considerable proportion of the biochemical workload and has increased considerably each year since 1973 (Table 4). Of the total of 724 enzyme assays performed, 314 were on cultured fibroblasts involving a heavy laboratory workload. Moreover, since it is essential to do control assays, cells from one affected and two normal subjects are cultured and assayed at the same time as the test cells, so that four times as many cell cultures, or about 1250, have actually been assayed biochemically.

SUCCESS RATES OF AMNIOTIC PUNCTURE AND ANALYSIS OF FLUID

The results reported here are those from mothers presenting between 1.9.69 to 31.12.76. Inevitably, we do not yet have complete outcome data on pregnancies presenting in 1977.

Of the 2068 mothers who came to amniocentesis, 1814 had one tap only (Table 5), and the fluid was obtained and successfully analysed in 1694 (93.4%). The remaining 120, in whom no laboratory result was obtained, are described below. Of the 250 gravidae who had two or more taps, fluid was obtained and successfully analysed in 222 (87.4%). Thus, the amniocentesis and laboratory failure rates are nearly double those of the mothers requiring only one tap ($P < 0.01$).

In slightly over half of the 152 (120 one tap, 32 two or more) in whom no result was ever obtained, the failure was the result of a blood-stained fluid or a dry tap. In the remainder, there was a laboratory failure to establish diagnostically satisfactory amniotic cell cultures, both types of failure occurring more frequently in the earlier years.

In the 120 mothers who had only one attempt at diagnosis (Table 5 and 6), no further action was taken in 16 (13%) because fetal death had occurred. There was evidence suggesting that in some of these the fetus had been dead or dying at the time of the first tap. In ten of these, the tap had succeeded but amniotic cell culture failed. However, in two, a brown (but not bloody) supernatant fluid had been observed; in another three, the AFP level had been abnormally high, while in the sixth, the AFP level was so low that fetal death and resorption had been suspected. Of the remaining six mothers without results who had

a fetal loss and no second amniocentesis, four had a dry tap, and in one of these the sonicaid fetal heart monitor did not detect any heart sounds and a

brown vaginal loss was present. The remaining two aborted after a blood-stained tap. The other 104 outcomes are described in Table 6.

Table 2 Individual indications for amniocentesis and information found at amniocentesis from referrals between 1.9.69–31.12.76

<i>Indication for amniocentesis</i>		<i>No of indications*</i>	<i>Results indicative of fetal abnormality</i>		<i>Balanced translocation carriers</i>		<i>Sex at risk but estimated normal</i>	
			<i>No</i>	<i>%</i>	<i>No</i>	<i>%</i>	<i>No</i>	<i>%</i>
<i>For chromosomal investigation</i>								
Maternal age	<35	5	0	0		—		—
	35–39	310	5	1.6		—		—
	40+	507	18	3.5		—		—
Previous conceptus with trisomy or other numerical anomaly		323	3	0.9	1	0.3		—
Kinship trisomy		39	1	2.6		—		—
Previous conceptus with other chromosome anomaly		19	0	0		—		—
Familial translocations	Down's	16	3	18.8	7	43.8		—
	Others	17	0	0	9	52.9		—
<i>For AFP</i>								
Increased risk of neural tube defect		740	28	3.9		—		—
<i>For inborn errors of metabolism</i>								
Sexed for X-linked errors		26	5††	19.2		—	5	19.2
Others tested directly		83	16	19.3		—		—
<i>Miscellaneous</i>								
Includes abnormal at ultrasound and one Fanconi's anaemia		51	4†	7.8		—		—
All indications		2136	83	—	17	—	5	—
All patients tested		2068	—	—	—	—	—	—
All patients with results		1916	83	4.3	17	0.9	5	0.3

*61 cases entered both in chromosomal indication and previous neural tube defect; 7 cases entered twice in 'miscellaneous' because 2 indications; †all high AFP; ††estimated to be abnormal, ie, half of sex at risk.

Table 3 Prenatal diagnosis of metabolic disease

Disease	No of pregnancies Referrals between 1.9.69–31.12.76		Jan–Dec 1977		Total	
	Fluids tested	Fetuses affected	Fluids tested	Fetuses affected	Fluids tested	Fetuses affected
Galactosaemia	4	1	1	0	5	1
Pompe's disease (glycogenosis type 2)	5	1	1	0	6	1
Hurler's disease (MPS 1H)	6	2	—	—	6	2
Hunter's disease (MPS II)	6	1	3	—	9	1
Sanfilippo disease (MPS IIIA)	2	0	—	—	2	0
Tay-Sachs disease	3	0	2	1	5	1
Metachromatic leucodystrophy	1	0	—	—	1	0
Gaucher's disease	1	1	—	—	1	1
Maple syrup urine disease	1	0	1	0	2	0
Xeroderma pigmentosum	3	2	—	—	3	2
Maroteaux Lamy disease (MPS VI)	—	—	1	0	1	0
Sandhoff's disease	—	—	1	0	1	0
Methylmalonic acidemia	—	—	1	0	1	0
Total	32	8*	11	1	43	9

*These are included in Table 2 and 6.

Table 4 Number of assays performed up to 31.12.77

Year ending 31 December	Homozygotes or hemizygotes	Heterozygotes	Prenatal	Post mortem	Total
1973	8	26	3	0	37
1974	57	19	10	4	90
1975	131	21	8	4	164
1976	150	28	11	3	192
1977	183	46	11	1	241
Total	529	140	43	12	724

Table 5 Amniotic punctures and results from referrals between 1.9.69–31.12.76

	No of pregnancies	Results from	No results from
Single tap	1814	1694	120 (6.6%)
Two taps	222	193	29 (13.0%)
Three taps	29	26	3 (10.3%)
Four taps	3	3	—
Total	2068	1916	152 (7.4%)

Table 6 Outcome by result or no result of amniocentesis from referrals between 1.9.69–31.12.76

Outcome	Test result known (%)		Test result not known				All not known (Subtotal) (%)		Total	
			One tap	(%)	2 or more taps (%)					
Terminated for medical indication	82	4.3	1*	0.8	0	0	1	0.7	83	4.0
Terminated on other grounds	6	0.3	2	1.7	0	0	2	1.3	8	0.4
Fetal or neonatal loss	81	4.2	18†	15.0	5	15.6	23	15.1	104	5.0
Survivor	1653	86.3	84	70.0	24	75.0	108	71.1	1761	85.2
Outcome not known	94	4.9	15	12.5	3	9.4	18	11.8	112	5.4
All	1916	100	120	100	32	100	152	100	2068	100

*Split specimen with abnormal result obtained elsewhere;

†16 aborted or diagnosed as IUD before repeat could be organised (see text) and 2 neonatal deaths.

RESULTS BY INDICATION

Table 2 shows the results of the successful amniocenteses according to the indication for the test. Overall, 4.3% of all successful amniocenteses produced evidence of fetal abnormality, and an additional 0.9% detected a fetus with a balanced structural chromosome anomaly.

As expected, the highest yield of cytogenetic anomalies was in those pregnancies with familial translocations, of which balanced carriers were found in nearly half the karyotyped fetuses and unbalanced abnormalities in 9.4%. The yield of chromosomal abnormalities in mothers referred for maternal age was much lower, 2.8% in all mothers aged 35 or more, and 3.5% in those aged 40 or more. A similar proportion of abnormality, 3.9%, was obtained in mothers tested because of a previous infant with NTD. Sixteen (19.3%) of the 83 fetuses tested because of a family history of an inborn error of metabolism were affected.

From the biochemical tests carried out entirely in the Unit before 1977 (Table 3), of 26 pregnancies at high risk of an autosomal recessive disorder the fetus was judged to be heterozygous in 4 (15%), but discrimination between carriers and normal homozygous amniotic cells is unsatisfactory for several of these conditions and is of academic importance only.

ACTION TAKEN AS A CONSEQUENCE OF AMNIOCENTESIS RESULTS AND VALIDATION OF RESULTS

There were 105 mothers in whom the result was either frankly abnormal, or showed a balanced chromosome anomaly, or showed the sex at high risk for a sex-linked abnormality. The position in regard to heterozygotes for recessive inborn errors has already been discussed. Of the pregnancies in which a result was obtained up to the end of 1976, 83 (4.3%) were terminated. These are described in detail below. If patients referred in 1977 are considered, a further 26 were found to have an abnormal result and were terminated, reflecting the increasing rate of referral.

Table 7 Chromosome anomalies found in amniotic cells from referrals 31.12.69–31.12.76

Trisomy or other abnormality	Primary trisomy 21 Other
Balanced translocation	Centric fusion involving no 21 Other
Total	

Chromosome anomalies

Table 7 shows the distribution of these. Of the 36 pregnancies with unbalanced anomalies shown in the Table, 27 were terminated. In six instances the fetus was not available for study or tissue culture failed but in one of these the fetus had features of Down's syndrome. In 19, fetal karyotyping confirmed the amniotic fluid findings. In one case, the amniotic fluid showed mosaicism, but no trisomic cells were found in fetal tissue. This is discussed later. Finally, one of the 27 was terminated after an amniotic fluid diagnosis of trisomy 21, and was unexpectedly found to be twins, one with trisomy 21, the other with a normal karyotype. This happened in 1973 when experience in ultrasound diagnosis was limited.

In the three pregnancies which were not terminated, the amniotic fluid had shown karyotype 47,XXX, 47,XXX, and 46,XX/47,XX+fragment, respectively (see below). In each, the parents elected to continue the pregnancy and the children were of normal appearance and behaviour on follow-up.

Of the 17 pregnancies in which the fetus was shown to carry a balanced translocation, 15 continued to term. The sixteenth aborted spontaneously some time after amniocentesis and the last was terminated at another centre because of maternal anxiety. The amniotic fluid findings were confirmed in the latter.

Raised alpha-fetoprotein

Raised AFP levels were found in amniotic fluids from 42 mothers. Of these, 7 subsequently aborted.

macerated fetus, and in every case the fluid was a dark brown colour (Seller, 1977), suggesting that the high value was accounted for by intrauterine death (3 have been mentioned earlier). In 3, the raised AFP level was associated with severe fresh blood contamination of the fluid (Table 8). However, second samples obtained a week after the first were clear and had a normal AFP value, so the pregnancies were allowed to continue to term and normal infants were delivered. One continued to term because it was a twin pregnancy with one normal and one affected fetus (Campbell *et al.*, 1976). A total of 31 pregnancies were terminated and in 28 the fetus showed an abnormality (27 NTD including 2 with Meckel's syndrome, one other with multiple anomalies including omphalocele). Of the remaining three, one fetus appeared normal but the placenta was grossly abnormal. In the second, the fetus and placenta were normal; two successive amniotic fluid samples had had raised AFP values, but both were severely blood-stained. At that time we did not recognise the significance of fetal blood contamination. The third was a fluid assayed to confirm the findings of a collaborating centre, and though we confirmed their high AFP value they reported finding a normal fetus on termination.

If we count all these cases, including patients of another centre, the unexplained false-positive rate would be 1 in just over 2000 fluids tested for AFP. On the other hand, as far as our own patients are concerned, the two with raised AFP but no fetal abnormality could be accounted for as described above.

Inborn errors and directly detected X-linked conditions

Of 16 affected fetuses detected by direct testing (Table 2), 8 tested in conjunction with other laboratories were terminated, but are not further considered here. Of the remaining 8, 7 pregnancies were terminated and the diagnosis confirmed (Table 3), while the eighth (galactosaemia) proceeded to term and the diagnosis was confirmed by enzyme assay on cord blood. Where direct testing was not possible, all 10 pregnancies found to have a fetus of the high risk sex were terminated.

OUTCOME OF PREGNANCY AFTER AMNIOCENTESIS

An important part of the prenatal diagnosis evaluation is to obtain reports on the outcome of all pregnancies. The outcome of the pregnancies that came to amniocentesis, distinguishing those in whom the result of the amniocentesis was and was not known, is given in Table 6. Information on outcome has been obtained on 95% of all pregnancies receiving amniocentesis up to the end of 1976.

Overall, at least 85.2% of all pregnancies ended with a live baby. In those in whom the test result was known, this figure was 86.3% and in whom outcome was known, 90%. No case is known where a chromosomally abnormal fetus was diagnosed as normal, but in one case AFP was reported as normal in a patient who later aborted a fetus with a skin-covered encephalocele. The Unit does not routinely have follow-up information on these children and cannot therefore report on their current health, but

Table 8 *Problem results: referrals between 1.9.69–31.12.76*

Patient no	Finding		Action	Fetus or infant		
	First sample	Second sample		Macroscopic appearance	Chromosomes	Phenotypic sex
(a) Chromosomes						
7708*	46,XX/47,XX,+G _A	46,XX/47,XX,+?G _A	Terminated	? Abnormal dermatoglyphs	46,XX	F
8649*	46,XX/47,XX,+ frag	No repeat	Continued	Normal	46,XX	F
8380	46,XY/46,XX	No repeat	Premature neonatal death	Abnormal	—	M
10588	46,XY/46,XX	No repeat	Continued	Normal	—	M
11114*	46,XX/47,XX,+9/47,XX,+ ?r	As first	Terminated	Abnormal	46,XX/47,XX,+9	F
11801	92,XXXX	No repeat	Continued	Normal	—	F
11909*	46,XX/47,XX,+ frag	No repeat	Terminated	Normal	46,XX/47,XX,+ r	F
11969	45,X	46,XX	Continued	Normal	—	F
12271*	46,XY/46,X,dic(Y)(q11)/46,X,del(Y)(q11)	As first	Terminated	Epididymal cyst	46,XY/46,X,dic(Y)(q11)/46,X,del(Y)(q11)	M
(b) AFP						
9217*	Fluid bloody, AFP raised	Fluid bloody, AFP raised	Terminated	Normal	—	M
11642		Fluid clear, AFP normal	Continued	Normal	—	F
AV49/MJS			Continued	Normal	—	M
11885			Continued	Normal	—	M

*Entered as abnormal result in Table 2.

many of these are included in the MRC study on outcome of amniocentesis, in which postnatal information was specifically sought.

Some pregnancies with unknown outcome are from mothers resident abroad whom we have been unable to trace, while others resident in this country may still be traced. As one might expect, the highest proportion of unknown pregnancy outcomes (11.8%) were in those pregnancies where no result was obtained on amniocentesis. It has been shown (Table 6) that in these the risk of fetal or neonatal death was highest, over three times the risk in those in whom results were obtained. This group of unknown results also included the only live birth with a chromosomal abnormality, trisomy 18, missed because amniotic cell culture had failed. There were four (normal) cases in which the sex at birth was different from that reported at amniocentesis; twice it was reported as female where a male was delivered, and twice a male where a female was delivered. In three of these, chromosome analysis of the newborn gave a result in accordance with the phenotypic sex, while the fourth (a boy) has not been karyotyped as he was initially rejected. The former two errors could have been the result of maternal cell contamination, the latter must have been a labelling or laboratory error.

All pregnancies tested because of risk of biochemical disorders have been followed up by enzyme assays on the newborn, and all predictions up to the time of writing (September 1978) have proven accurate.

PROBLEMS OF DIAGNOSIS

Certain puzzling situations presented which are reported here in the hope that the experience may be of use to others. The majority are set out in Table 8 and it can be seen that mosaicism is the chief problem. The two fluids containing both normal male and female cells produced male offspring, so maternal contamination is the most likely explanation. Mosaicism with an abnormal cell line was found five times. In the first, at culture the proportion of trisomic cells was small (3 of 200), the pregnancy was terminated, but no trisomic cells were identified in the fetus. However, the dermatoglyphic findings were compatible with mosaicism (Polani and Polani, 1969). In the second pregnancy, 10% amniotic cells contained an extra fragment. The parents elected to continue the pregnancy and a normal girl was born who had a normal peripheral blood chromosome complement; a fibroblast culture was not sought. In two other mosaics (11114 and 11909) and in the mosaic with a Y dicentric, the proportions of abnormal cells were high, the pregnancies were terminated, and the amniotic findings confirmed in

the fetuses, though in the fetal cells of 11909 became clear that the small 'fragment' was a ring chromosome of unidentified origin.

Tetraploidy is a recognised artefact of amniotic fluid culture (Kohn and Robinson, 1970), so this finding did not cause undue anxiety. Of more concern was the 45,X result in a mother aged 40. This is a rare anomaly among survivors, though the commonest among conceptions, and is not associated with advancing maternal age. The cells had been difficult to maintain in culture, so that a cultural artefact was suspected, particularly as 45,X cells are known to have a rapid cell cycle and might have outgrown the original cells from which they had been derived. Amniocentesis was repeated and showed cells with a karyotype of 46,XX. The mother was reassured and a normal girl delivered at term. Not included in Table 8 are four apparently balanced translocations (three reciprocal, one centric fusion 13/14) in the amniotic fluid cells. Of these, 3 were found in fluid taken from mothers who had had a previous Down's syndrome infant. In 3, examination of the parental karyotype showed one or other parent to carry the reciprocal translocation and a good prognosis was given. These have been included under 'familial translocation other than Down's'. One fetus was found to have a reciprocal translocation involving chromosomes 2q and 18p that must have arisen *de novo* assuming paternity was as stated. Though it appeared balanced, it was difficult to be altogether reassuring regarding the prognosis. As an excessive incidence of apparently balanced translocations is found in mentally retarded populations, each *de novo* translocation must be regarded with suspicion as its effect on the phenotype is unknown. The parents elected to continue the pregnancy and a normal boy was born. When seen at age 2½ years he was physically and developmentally normal and his unusual karyotype was confirmed.

In contrast to chromosomal analysis, in which there is agreement on the recognition of most abnormalities, the cut-off points distinguishing normal from abnormal levels of AFP are largely statistical estimates and their significance can be determined by experience only. In the early years there were little data on the normal AFP range, and each laboratory tended to use as the cut-off points its own highest estimation in mothers later delivering a normal baby. Moreover, AFP results may be difficult to interpret, particularly when the fluid is contaminated with fetal blood. A Kleihauer test is now always performed on blood-stained fluids to determine the source of the contaminating blood. Detailed ultrasound scanning (by Professor Stuart Campbell) has provided helpful confirmation of an abnormal result in many cases.

Overall, 15 (0.8%) mothers of the 1916 (Table 2) in whom results were obtained had to be recalled and a possibly ambiguous result discussed with them. This inevitably causes considerable distress. With the use of chromosome banding techniques it is possible that this problem will increase, while greater experience with AFP results in individual laboratories may lead to a diminishing proportion of doubtful findings.

Discussion

Although the major part of the data in this account relates to prenatal diagnosis, we must stress that the results reported here should not be construed to indicate the safety or otherwise of amniocentesis. Information on this can only be derived from *ad hoc* studies with appropriate controls, such as the one by the National Institute of Child Health and Human Development (1976) or that conducted by the MRC (1978).

CHANGES IN REFERRALS OVER THE YEARS

The wide spread of diagnoses for which patients have been referred (Table 1) reflect the technical advances that took place between 1960 and 1976, and their increasing numbers mirror the changes in attitudes of the public and doctors to genetic counselling and its attendant diagnostic procedures. During these 16 years, in keeping with changes in genetic practice, we moved from a chromosome diagnostic and largely risk-giving type of counselling to a situation where direct information could frequently be given on a high risk pregnancy. There has also been a shift towards a more outgoing approach, more effort being directed towards informing the professions and the public of genetic services available by means of circulars, lectures, and the provision of library and information services.

In keeping with national policy these efforts have been concentrated on our own region. It is difficult to ascertain the degree to which we are meeting the needs of the region for genetic services, except in one field, namely prenatal diagnosis on age grounds alone. We know that very few patients are referred elsewhere for this indication (Maryon-Davis, 1978) and we know how many mothers resident in the region we are seeing. We had estimated that in 1971 to 1976 no more than 2% of mothers giving birth in the 35 to 39 age group, and 12% of the older mothers, had been referred for prenatal diagnosis (Polani *et al.*, 1976). We now have accurate data for the whole of 1976 and it has been estimated that these proportions were 3% for the 35 to 39 group and 23% for the 40 and over group; preliminary figures for 1977 show that about 48% of the latter

group had been referred (Alberman *et al.*, 1979). The low figure for the younger age group reflects our policy of not accepting patients aged less than 38 unless there is some indication other than age. There are little similar data from elsewhere, but Milunsky (1975) estimated that in 1974 only 4.1% of pregnant women in Massachusetts over 35 years of age had an amniocentesis done for prenatal diagnosis. Mikkelsen (1976) reported that in 1974 this was true in 14.7% of women having children when they were 40 or more in Denmark, though this proportion had apparently doubled in 1975, and Bartsch (1978) found that, in 1976, 25% of mothers of 35 or more in Göteborg, Sweden, had undergone amniocentesis.

AMNIOCENTESIS UPTAKE

We can use the present data on prenatal diagnosis to make some other points. About 1 in 5 pregnant women seeking advice on prenatal diagnosis did not undergo the test. About a quarter of these had aborted spontaneously before the test was carried out. In the remainder, the test was not carried out mostly because it was not indicated for the risk specified, or because the counselling odds suggested a relatively tolerable risk.

SUCCESS RATE

In 7% of those undergoing amniocentesis for any of the indications, no result was obtained. This figure was lower in those who needed only a single tap. Galjaard (1976) (also in Niermeijer *et al.*, 1976) quotes lower failure rates based on an inquiry covering 46 centres in 8 countries in Western Europe and a much lower proportion of repeat taps (4.3% against our 12%). We too find that dry taps or blood-stained fluid are the commonest causes of failure, followed, in order of importance, by failure to establish satisfactory cell cultures. However, some of these failures were related to a suspected sick or dying fetus. We also suspect that culture failure may not be independent of cellular abnormality and therefore does not occur at random. This clearly is important in dictating the course of action when cultures fail in otherwise satisfactory samples of fluid.

RESULTS

The overall fetal abnormality rate agrees with the proportions detected in the 6000 amniocenteses reviewed by Galjaard (1976), with the annotation in the Lancet (1977), and with the 10 000 amniocenteses reviewed by Golbus (1978).

As already reported a number of times (Ferguson-Smith, 1976; Niermeijer *et al.*, 1976; Polani *et al.*, 1976; Simpson *et al.*, 1976; Sachs *et al.*, 1977; Lancet, 1978; Mikkelsen *et al.*, 1978; Proceedings of III European Prenatal Diagnosis Conference, 1978),

the proportions of trisomic fetuses (including trisomy 21) related to maternal age is higher than that suggested by data on surviving infants, especially with Down's syndrome. This has been variously attributed, to inaccurate data on survivors, selective mortality of affected fetuses during later pregnancy and in the neonatal period, and biased sampling or biased reporting. Clearly, clusters of these factors are likely to operate, possibly in different combinations and proportions in different populations.

We find a similar frequency to that described in published reports of trisomic fetuses in pregnant women who had previously had a trisomic fetus (J. Hamerton, 1976, personal communication; Galjaard, 1976; Golbus, 1978). For pregnancies where a parent was a translocation carrier, our findings are difficult to compare with published data, in which generally centric fusions involving chromosome 21 are not distinguished from interchanges of other types (Lancet, 1977; Proceedings of III European Prenatal Diagnosis Conference, 1978).

The results of AFP studies for high risk of NTD yielded almost 4% abnormal fetuses. This agrees well with the expectation of risk of recurrence of neural tube defects, at least those compatible with fetal survival to the end of the fourth month of pregnancy, in an area of relatively low population prevalence like south-east England. However, in this account we are reporting our total workload and its results. Because of our involvement with amniotic fluid AFP investigation from its inception, we have often received, and include, split samples from collaborating centres developing the technique. This may have resulted in an overrepresentation of cases of raised AFP either because of fetal abnormality or for other reasons.

Nearly 20% of fetuses studied for biochemical abnormalities turned out to be abnormal. This agrees with expectation for the disorders under investigation, which behave as recessives. Golbus (1978), in his review, assembled 565 pregnancies that had been investigated for biochemical errors; 132 (23.4%) of them were shown to be abnormal. Galjaard (1978) has summarised data on amniotic studies for inborn errors in North America and Europe. Of 251 investigations in European laboratories in which the biochemical status of the fetus was precisely determined in respect of the object of the test, 54 (22%) amniotic cell studies turned out to give an abnormal test result.

The importance of validating the results obtained when a pregnancy terminates needs to be stressed again. Apart from one example of missed trisomy 18 because of culture failure, we committed four errors of sexing in almost 2000 pregnancies, fortunately when the problem of sex was not relevant to the pre-

natal diagnosis. J. Hamerton (1976, personal communication) collected information on 3315 pregnancies from 7 studies including the 2 North American ones. Ten errors (3 per 1000) were reported, in 8 instances errors of sex recognition (2.4 per 1000) and in 2 failure to recognise trisomy 21. Two of the sexing errors were, like two of ours, misrecognition of a female fetus, an error which clearly must be attributed to a labelling or similar mistake.

In conclusion we find that the proportion of amniocenteses which gave informative results, the proportion of abnormalities according to the indication for the test, and the outcomes of pregnancy are similar to those described in reports from other centres. Where outcome was known, 90% of the pregnancies screened ended in live born babies, 4% were terminated for fetal abnormality, less than 1% for non-medical reasons, and the remaining 6% ended as a fetal loss or neonatal death.

As can be expected from a subject that is still new and not fully explored, details are changing and emphasis is shifting. Clearly, for example, the findings of the UK Collaborative Study (1977) on serum AFP will alter the approach to the prenatal diagnosis of NTD. Equally, the changing pattern of parental age at reproduction in most Western societies will shift the age criteria for the selection of pregnancies at high risk for chromosome disorders (Philip *et al.*, 1977; Holmes, 1978), while the development of better understanding of some inborn errors and of fetal blood sampling, will enlarge the scope of direct counselling for genetic biochemical anomalies. The major problem in this field that will still remain relates to those abnormalities of structure in which no correlated biochemical or cytological tests exist at present.

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