Karyotypes of Cultured Cells from Foetal Membranes of Normal Newborns

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It has been reported that a substantial percentage (20–30%) of spontaneously aborted human embryos possess deviant karyotypes (Carr, 1965; Schlegel *et al.*, 1966; Polani, 1966). The three most common types of chromosomal abnormalities observed were trisomy, triploidy, and absence of a sex chromosome.

Several investigators have compiled data on the association between abnormal foetal karyotypes and spontaneous abortions, but though these are important there are not sufficient data to allow statistical and epidemiological interpretations to be made. The value of the published series is diminished because all the investigations have been of the *ad hoc* type. In addition, very early abortions (by the sixth week) are as yet inaccessible for chromosome studies; and later abortions have yielded useful material for chromosome analyses in only 50% of cases. This, however, may not always constitute a serious bias (Carr, Bateman, and Murray, 1966).

It is doubtful whether one is justified in basing the diagnosis of the karyotype of the foetus on cultured cells derived from its amnion, chorion, or placenta when, as often happens, the aborted material contains no identifiable foetal parts. Further, few observations have been reported on the karyotypes of these tissues from pregnancies with normal fetuses. Foetal membranes are of particular interest as such material is available in the majority of spontaneous abortions. In fact, some reports (e.g. Klinger and Schwarzscher, 1958) give indirect evidence of a high incidence of tetra- and octoploid cells in human amnion epithelium. Klinger and Schwarzscher (1960) claim that apparently normal amnions contain from 5 to 10% tetraploid cells. Schlegel *et al.* (1966) included a series of 8 normal full-term amnions in their chromosome investigations on spontaneous abortions and examined 13

cultured cells, and all were found to have normal karyotypes.

Normal full-term foetal membranes were used in this work because, if the occurrence of a heterogeneous cell population is a frequent phenomenon in foetal membrane tissue, heterogeneity is likely to increase with gestation time. Apparently important factors are ageing (Jonas and Caunt, 1965) and a relatively low selection pressure in a tissue of moderate differentiation. In consequence, a test of full-time specimens of foetal membranes would be likely to reveal a maximum of karyotype heterogeneity.

Material and Methods

A prospective survey is being carried out in this Institute. All women, registering at the local maternity clinic during the first month of gestation, are followed until their pregnancies terminate in either abortion or childbirth. Chromosome studies are made on wives, husbands, spontaneous abortions, and abnormal children. As a preliminary to this project, examinations have been made on the karyotypes of cultured cells derived from foetal membrane specimens taken at the deliveries of an unselected series of 25 apparently normal children and these results are reported here. The membrane specimens were collected immediately after delivery in tubes containing Parker's Medium 199 and transferred to the laboratory.

After rinsing in Hanks' solution cell cultures were processed according to a method described in a previous report (Böök, Santesson, and Zetterqvist, 1961), with the modification that pronase instead of trypsin was used to loosen the cells from the glass surface. Successful cultures were obtained from 22 different specimens. Suitable monolayers of cells appeared after 6–10 days. Only the primary cultures were used for chromosome analyses. Preparations for microscopical examination of mitotic metaphase chromosomes were made by the air-drying or squash methods and stained in orcein.

Before leaving the hospital all the children were examined by a consultant paediatrician, and were reported

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TABLE
CHROMOSOME ANALYSES OF CELLS OF 22 SETS OF SUCCESSFUL *IN VITRO* CULTURES FROM FOETAL MEMBRANE SPECIMENS FROM NORMAL NEWBORNS OUT OF AN UNSELECTED SERIES OF 25 SUCH CASES *

File No.	Sex of Child	Chromosome Analysis of Cultured Cells						
		Counted			Karyotyped	Estimated Ploidy Level		
		45	46	47		2 X	4 X	% 4 X
05-6-65	M	3	34	—	3	159	1	0.6
05-7-65	F	2	46	1	4	270	5	1.8
05-8-65	M	1	22	—	5	179	6	3.2
05-9-65	F	2	25	3	6	230	4	1.7
05-11-65	M	—	23	—	3	219	6	2.7
05-12-65	F	1	42	1	4	674	48	6.6
05-13-65	M	—	42	1	5	428	8	1.8
05-1-66	M	—	40	—	5	61	0	0
05-2-66	F	—	34	—	6	87	0	0
05-4-66	M	1	28	1	2	100	2	2.0
05-5-66	M	—	29	1	5	82	1	1.2
05-6-66	M	1	33	—	3	52	0	0
05-7-66	F	1	21	—	2	182	9	4.7
05-8-66	M	2	21	1	4	82	2	2.4
05-9-66	M	—	20	—	4	84	0	0
05-10-66	F	—	21	1	4	177	7	3.8
05-13-66	M	2	13	—	2	36	0	0
05-14-66	M	—	22	—	4	148	1	0.7
05-15-66	F	—	2	97	19	177	3	1.7
05-16-66	M	—	22	—	6	253	10	3.8
05-17-66	M	1	18	1	1	113	0	0
05-18-66	M	—	21	—	5	74	0	0
Totals		17	579	108	102	3867	113	2.8

* Inoculated tissue material from 3 different specimens failed to grow *in vitro*.

to have no signs or symptoms of physical malformations or illnesses.

Results

Successful growth *in vitro* of cells from the membrane specimens was obtained in about 80% of the prepared cultures. In our experience this rate is lower than for explants of normal foetal tissue but higher than for those of normal adult skin. In consequence, it seems reasonable to assume that the three instances of no growth were accidental. The results of the chromosome analyses are shown in the Table. For 21 of the 22 specimens the diploid modal chromosome number was 46 and the karyotypes were normal, 46,XX or 46,XY. The only deviation observed was the occurrence of tetraploid cells, the frequency of which varied from 0 to about 6% (mean 2.8%). As a small percentage of tetraploid cells occurs regularly in all kinds of cell cultures derived from human tissues and lymphocytes, this finding should be of no significance. Our results, therefore, confirm that the 21 foetal membrane cultures gave normal karyotypes. The sex chromosomes of these karyotypes corresponded in each case with the sex of the child.

In one case, 05-15-66 (Table), a diploid modal chromosome number of 47 was found. All 47 chromosome cells showed a consistent karyotype of 22 normal chromosome pairs, two X chromosomes, and in addition, a metacentric chromosome smaller

than those of the G group (Fig. A). Examination of a blood culture taken later from this child showed that the growing lymphocytes had exactly the same deviant karyotype with 47 chromosomes (Fig. B).

Comment

The finding of 21 normal and one abnormal karyotype in 22 cell cultures derived from full-term foetal membranes, all of which corresponded with the child's karyotype using blood cultures, justified the use of cultured cells from foetal membranes for the interpretation of the foetal chromosome complement.

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FIG. A. 47 chromosome karyotype of cultured cells derived from the foetal membranes of a full-term apparently normal female newborn child. Note extra small metacentric chromosome.

FIG. B. The identical karyotype from cultured lymphocytes of a blood sample from the same child.

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