Spontaneous Chromosome Breakages Observed in a Phenotypically Normal Woman and Her Two Abortuses[1)]

By

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(With 6 Text-figures and 3 Tables)

While working on chromosomal surveys of induced and spontaneous abortions, the authors had a chance to observe a high incidence of chromosome breakages in two abortuses from one and the same woman, one from a spontaneous abortion and the other from an induced abortion. The former was preliminarily reported in a previous paper (Makino *et al.*, 1967). Further investigation has revealed that a similar chromosome condition has occurred in leucocyte cultures from the same woman, clinically normal. The present paper reports the results of chromosomal studies in the woman and her two abortuses with some remarks on a possible genetic significance of chromosome breakages in relation to abortion.

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Materials and methods

A woman concerned here is 26 years old, and clinically normal and healthy. She had a clinical history of six induced abortions and one spontaneous abortion with threatened termination, without any livebirth. The conceptuses from the last two successive abortions constituted subjects for the present study.

Her sixth pregnancy was resulted in spontaneous abortion with threatened termination at 9 weeks after the last menstrual period. The products of conception (SA-16, corresponding to case 16 in the report of Makino *et al.*, 1967) consisted of only an empty sac, about 2 cm in diameter, and the normal in appearance. Since the chorionic sac was damaged, whether it was an intact empty sac, or not, was uncertain.

Five months following her sixth pregnancy, she became pregnant and received curettage at the 11th week of gestation. The conceptuses (IA-230) involved a ruptured choronic sac and a highly broken embryo, though its legs and arms retained normal morphology without damage.

Clinically, she has received neither known excessive radiation exposure, viral infection nor known cytotoxic agents during or immediately prior to the pesent pregnancies. Further, there were no records for the evidence that the members of her family and her

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Table 1. Chromosome-number distribution and frequencies of chromosome aberrations in leucocyte cultures from a woman studied

Date of sampling	Chrom. no. distr.						No. of cells count.	No. of cells with							No. of cells obs.	No. of abnormal cells obs. (%)
	<45	45	46	47	68	4n		OB	1B′	2B′	1B″	2B″	Fr	D		
6/22/66	4	11*	114	8**	1	3	141	162	37	7	7	1	4	0	223	54 (24.2%)
11/ 9/66	0	1	21	1**	0	0	24	88	8	0	4	0	1	1	101	13 (12.8%)

*: random missing, **: most of them having an acentric fragment,
B′: a chromatid-type breakage, Fr: an acentric fragment,
B″: a chromosome-type breakage, D: a dicentric chromosome.

neighbourhood were infected with epidemic diseases, though measles showed an epidemic record in Hokkaido district, in a period from May to August 1966.

Blood samples were collected twice from this woman, on about a month after the sixth abortion and immediately after the seventh one. In case SA-16, two cell cultures were set up, one from the chorionic membrane involving villi and the other from the amnion. The former grew sufficiently well to permit chromosome analysis. Chromosome slides were prepared on the 3rd day after cultivation. For case IA–230, both skin culture and cultures of ribs with muscle were available for chromosomal studies. Further, two sets of chromosome slides were made on the 7th and 9th day after cultivation in skin cultures. The culture procedures followed the method of Sasaki *et al.* (1966). Chromosome slides were made according to the routine air-drying method.

Results

Table 1 provides the results of chromosomal studies in leucocyte cultures from the woman under study. It was shown that the cell populations showed an unusually high incidence of aberrant chromosomes involving breakages, fragments and a probable dicentric chromosome. In the first sample, 54 cells, or 24.2 per cent, of 223 were found to have abnormal chromosomes (Fig. 4), while the second sample showed 13 metaphases, or 12.8 per cent, of 101 were chromosomally abnormal (Figs. 5 and 6). Karyotype analyses made in 3 excellent metaphases showing no breakage revealed the occurrence of a normal chromosome complement of the female type.

Similar chromosome conditions were found to occur in two conceptuses from the recent successive abortions, cases SA–16 and IA–230. Chromosome-number distribution and frequencies of abnormal chromosomes in these abortuses are given in Tables 2 and 3, respectively.

Case SA–16 showed that 43 metaphases (19.7%) out of 182 cells from the chorion culture contained one or more chromosome breaks, including simple breaks, fragments, and dicentric chromosomes (Fig. 1). Three of 6 cells with 47 chromosomes had an extra chromosome in group D. Four excellent metaphases showing 46 chromosomes had a normal male chromosome complement.

The chromosomes of case IA–230 were examined in three separate cultures

Table 2. Chromosome-number distribution in two abortuses obtained from the same woman concerned in this study

Case no.	Date of sampling	Gestat. age (weeks)	Tissue source	Sex chro-mation	Days in vitro	Chrom. no. distr.					No. of cells obs.	Karyotype
						<45	45	46	47	4n		
SA-16	5/27/66	9	Chorion	0%	3	3	11*	89	6**	3	112	46, XY
IA-230	11/ 9/66	11	Skin	14%	7	1	2	11	0	0	14	46, XX
					9	0	1	10	1	0	12	
			Ribs & muscle	—	7	0	0	14	0	0	14	

*: random missing, **: 3 cells having an acentric fragments and the remaining 3 cells having an extra member in D group chromosomes.

Table 3. Frequencies of chromosome aberrations in two abortuses obtained from the same woman concerned in this study

Case no.	Tissue source	Days in vitro	No. of cells with									No.of cells obs.	No. of abnormal cells obs. (%)
			OB	1B′	2B′	3B′	1B″	2B″	1Fr	2Fr	D		
SA-16	Chorion	3	139	26	8	2	0	0	2	0	2	182	43 (19.7%)
IA-230	Skin	7	57	10	1	0	2	0	3	0	0	73	16 (21.8%)
		9	65	7	0	1	1	0	3	1	0	78	13 (16.7%)
	Ribs & muscle	7	61	9	0	0	2	0	0	0	0	78	12 (16.4%)

derived from two different tissues. In skin cultures, on the 7th and 9th days, 16 (21.8%) of 73 and 13 (16.7%) of 78 cells studied showed one or more chromosome breaks and fragments (Figs. 4 and 5), respectively. Cultured cells from ribs were also remarkable by a high incidence of chromosomal aberrations involving breaks and fragments, 12 (16.4%) metaphases of 78 being abnormal. Karyotype analyses in two good metaphase cells in each tissue disclosed that this embryo had a normal female chromosome complement (46, XX).

Discussion

Current reports indicate that various types of chromosome aberrations involving breakage, fragment, dicentrics and interchange, are induced by environmental factors such as irradiation, certain viruses and chemicals. On the other hand, generally such chromosome abnormalities were known to occur spontaneously in cultured cells from normal human subjects with a very low incidence, about 1 per cent in the Edinburgh Study (Court Brown *et al.*, 1966). And also, a rather high incidence of breaks has been reported to occur frequently in some of normal individuals (Nichols *et al.*, 1966, Lubs and Samuelson,

1967). In our chromosomal surveys in human abortuses, chromosomal aberrations of over 10 per cent frequency are extremely infrequent, except four cases: they are four embryos from induced or spontaneous abortions which showed 21 to 38 per cent of chromosome aberrations (Makino *et al.*, 1962, 1963, 1967; Sasaki *et al.*, 1967).

The fact merits special attention in the present study that a high incidence of chromosome breakages has occurred not only in two repeated leucocyte cultures from a woman, but also in several tissues of her two abortuses. There was no evidence for environmental factors in relation to chromosome breaks in her two pregnancies. Culture conditions such as media, serum, and flasks, may not

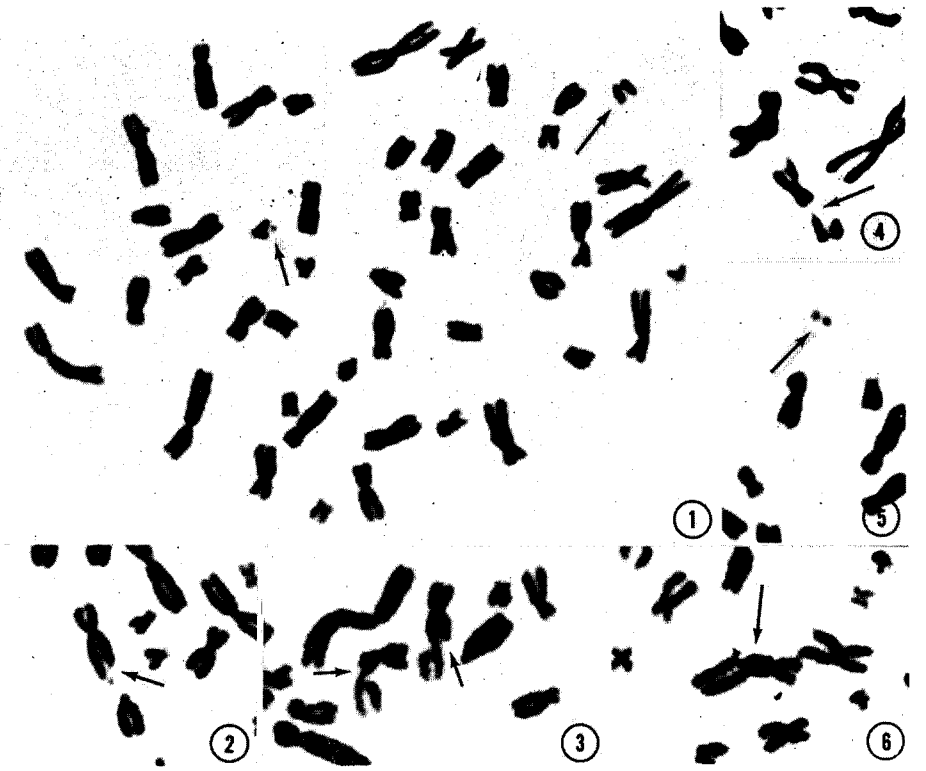

Fig. 1. A metaphase plate from chorion culture of case SA–16, showing a chromatid-type break and an acentric fragment.

Figs. 2 and 3. Partial metaphase plates from case IA-230; 2, from culture of ribs with muscle, showing a chromatid-type break; 3, from skin culture, showing two chromatid-type breaks.

Figs. 4–6. Partial metaphase plates from repeated leucocyte cultures of the woman; 4, from the first sample, showing a chromosome-type break; 5 and 6, from the second sample, showing a minute acentric fragment and a probable dicentric chromosome, respectively.

constitute any cause of an increase of chromosome breaks, because immediately before and after cultivation no case showed an unusually high frequency of breakages. Further, it seems also unlikely that the frequent occurrence of chromosome breaks in the present materials was a chance effect. Here, as a possible interpretation for the present findings, the authors would like to consider a certain intrinsic factor of the woman and her abortuses themselves. Very probably, it would be a genetically determined tendency to produce chromosome breakages, or otherwise a vertical transmission of certain viruses through the placenta.

A similar consideration having attracted the attention of some recent cytogenetists and clinicians has been offered by some workers: in Bloom's syndrome by German *et al.* (1965) and Landau *et al.* (1966), and in Fanconi's anemia by Schroeder *et al.* (1964) and Bloom *et al.* (1966). These two clinical disorders are known to be genetically determined disorders presumably dependent on autosomal genes, and to have in common a high frequency of chromosome breakages and rearrangements and a high prevalence of malignancy, especially of leukemia. German *et al.* (1964) have described that the high incidence of chromosome breakages in Bloom's syndrome seems to be associated with the genetic abnormality and is possibly related to an apparent increase in frequency of malignant neoplasia. Further, Bloom *et al.* (1966) who dealt with Fanconi's anemia suggested that chromosome breaks could be the primary effect of a supposed gene mutation. For final conclusion of the above features, further chromosomal studies are to be required in related subjects with familial surveys.

Many current reports indicate that certain numerical or structural chromosome anomalies are important factors to cause spontaneous abortion (Geneva Conference 1967). On the other hand, Makino *et al.* (1967) reported a case of spontaneous abortions which showed a high frequency of cells with chromatid-type breakages. Aula and Hjelt (1962) found a large dicentric chromosome in 5 of 35 cells derived from a spontaneously aborted fetus. Recently, Sasaki (personal communication) found that some individuals out of couples with repeated spontaneous abortions showed a high frequency of chromsome breaks. It is likely that abortuses from a mother who had certain treatments or viral infection in her early pregnancy may show chromosome breaks at a significantly high incidence, and that in some cases, they may lead to spontaneous abortion or birth defect. At the present status of study in this field, our knowledge is still too poor to discuss a significant role of chromosome breakages in relation to spontaneous abortion.

Summary

Two abortuses from a phenotypically normal woman showed a high incidence of chromosome breakages in cells from several organs. A similar chromosomal feature was observed in two repeated blood cultures from the same woman. She had no clinical history in relation to irradiation, viral infection or chemotherapy during her pregnancy.

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