STABILITY OF ABNORMAL KARYOTYPES IN CELL CULTURE[1]

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INTRODUCTION

FORD (1964) has provided and reviewed evidence for great karyotypic stability in normal somatic tissues. In discussing selection pressure in mammalian cell populations he summarized the available evidence thus "In normal somatic tissues selection can be said to be *conservative*, in irradiated somatic tissues *conservative* and *competitive*, and in neoplastic tissues and cultured cell lines *progressive*". In the same review, FORD underlined the scarcity of information on the nature of selective processes in newly derived cell cultures with a finite lifetime, the "cell strains" of HAYFLICK and MOORHEAD (1964). The work of HAYFLICK and MOORHEAD (*l. c.*) on normal diploid strains and some few data on long term cultures of cells with abnormal karyotypes [*see* TJIO *et al.* (1959) and FRACCARO *et al.* (1960 b) for persistence of the XO karyotype in vitro and FRANCESCHINI *et al.* (1964) for persistence of a deleted chromosome all indicate a high degree of karyotypic stability.

The study of the fate of karyotypic mosaics in vitro has obvious bearing on the problem of selection pressure in cell populations. Few pertinent data found in the literature are summarized in Table 1.

In this paper we report on an investigation of karyotypic variation in long term cultures derived from skin biopsies of two human mosaics, an iso-chromosome for the long arm of X (45, X/46, XXqi) and a mosaic mongol (46, XX/47, XX, G+).

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TABLE 1. *The fate of karyotypic mosaics in long-term cell cultures.*

Type of mosaic	Type of culture	Karyotypes at last determination (days in culture/N° of passages)	Reference
45, X/46, XX	Bone marrow	Persistence of both karyotypes. Decrease of XO cells. (35/5)	FRACCARO *et al.* (1960 a)
46, XY/47, XXY	Skin	Persistence of both karyotypes (62/3)	KLINGER and SCHWARZACHER (1962)
69, XXY/46, XY	Skin	Persistence of both karyotypes Decrease of triploid cells (?/3)	BÖÖK *et al.* (1962)
45, X/46, XXr	Bone marrow	Disappearance of ring (30/1) Decrease of cells with ring (30/2)	FRACCARO and LINDSTEN (1964)
45, X/46, XX/46, XXqi	Skin	Disappearance of isochromosome (360/?)	NUZZO *et al.* (1964)
45 X/46, XXqi	Skin	Persistence of both karyotypes (325/18)	FRACCARO and MANNINI (1966)
46, XY/46, XY, Cr	Skin	Five separate cell lines: disappearance of ring in two, decreased persistence in three lines. (300/70*)	SHAW and KROOTH (1966)

* This indicates cell generations.

Materials and methods

The first mosaic subject was a 15 year-old female with a clinical diagnosis of Turner's syndrome. A blood culture had revealed a mosaic composed of XO cells and of cells with an iso-chromosome for the long arm of X, in the proportions entered in Table 2. The second one was a two year-old female. A blood culture revealed a mosaic of normal and trisomic 21 cells (Table 2). This is case 1 in FRACCARO *et al.* (1967).

Tiny fragments of skin were cultured in a plasma clot and the first subcultures prepared from a cell suspension obtained by trypsinization

TABLE 2. *Chromosome analyses in fibroblast cultures of the isochromosome mosaic (45, X/46, XXqi).*

Days in serial culture	No. of passages	Chromosome number and karyotype						Total
		45, Xqi	45, XO	45, Xqi	46, XXqi	47, XXqi	47	
		(a)	(b)	(c)	(d)	(e)	(f)	
44	2	—	2	—	9	—	—	11
98	5	—	—	—	3	—	1	4
248	12	1	3	1	76	2	4	87
319	17	4	—	2	22	—	2	30
353	20	1	—	2	34	1	—	38
414	25	—	1	—	6	—	1	8
462	28	—	—	—	3	—	—	3
Blood culture		—	19	—	21	—	—	40

Column (a): all were Xqi and (-C, -C, -D); (-E, -G); (-C, -C); (-C-E); (-E, -D, + mar); (-F, -F, + r), respectively.
Column (b): all were (-C)
Column (c): all were Xqi and (-C); (-C); (-D); (-C); (-C), respectively.
Column (d): 248 days — Seven cells had at least one chromatid break
319 days — One cell with breaks
353 days — Four cells with breaks. Two cells were (-B, -D, + mar, + mar) and (-B, -C, + mar, + mar) See Figs 2, 3.
Column (e): (+ r); (+ r); (+ mar), respectively.
Column (f): All had about 92 chromosomes.

of the halos of cell outgrowing from the explants. The cells were cultured in T-flasks with a medium consisting of 25 per cent calf serum in Hank's, supplemented by 0.5 per cent in weight of lactalbumine hydrolysate and 0.04 per cent in weight of yeast extract and with the addition of 200 U/ml of penicillin and 200 μg/ml of streptomycin. The cultures were transferred by trypsinization as soon as the cells had formed a confluent sheet, at a 1 : 2 split ratio initially and at a lower ratio towards the end of the culture period. Chromosome preparations were made either on cells grown on coverslips or from cell suspensions obtained by trypsinization. Sex chromatin was investigated by the method described by FRACCARO and LINDSTEN (1959). Autoradiography was performed on cells exposed continuously for 4 hours to 1 μc/ml of tritiated thymidine.

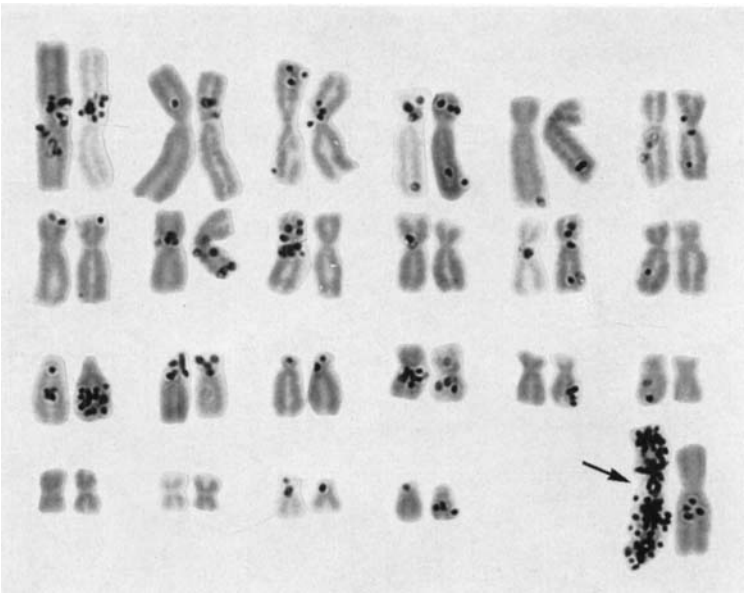

Fig. 1. Iso-chromosome mosaic. Karyotype from an autoradiography of a metaphase with 46 chromosomes. The iso-chromosome X (arrow) is late-replicating.

RESULTS

1. Iso-chromosome mosaic

This strain was kept for 569 days. During the last 100 days, however, the cell population was practically stationary in number and very little or no cell multiplication was taking place. The last chromosome determination was possible at 462 days at the 28th passage. Four more passages were performed but no analysable mitoses were obtained. The results of chromosome analyses are shown in Table 2. The cells with 45 chromosomes were few and those missing a C chromosome could be tentatively interpreted as XO cells (column (b) of Table 2). All the metaphases with 46 chromosomes were analysed and had the iso-chromosome. Autoradiography, performed at 248 days of culture, showed that the iso-chromosome was late replicating at the end of the S period (Fig. 1). Interphase nuclei were stained with Feulgen at the 12th and 19th passages: a high proportion of cells were sex chromatin positive. At the twelwth passage, at 248 days of culture, a proportion of cell had chromatin breaks and two cells had an extra, tiny ring

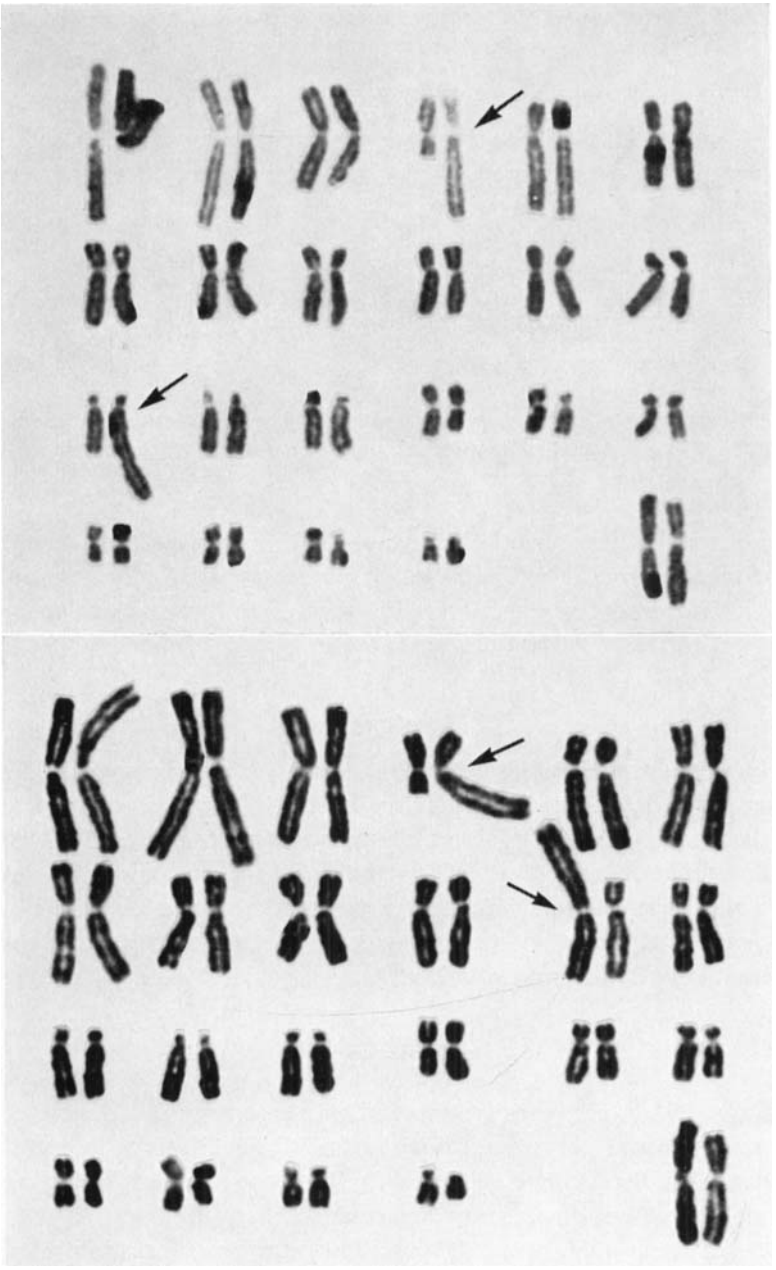

Fig. 2. Iso-chromosome mosaic. Two cells with 46 chromosomes, the iso-chromosome and structural re-arrangements, presumptive translocation. The arrows point to the marker chromosomes.

TABLE 3. *Chromosome analyses in fibroblast cultures of the mongol mosaic (46, XX/47, XX, G+).*

Days in serial culture	No. of passages	Chromosome number				Total
		45	46	47	>47	
23	2	3	10	14	—	27
93	5	3	58	48	3	112
124	8	2	36	32	2	70
148	12	—	—	7	—	7
Blood culture		—	33	32	—	65

Among the cells with 45 chromosomes, six had four and two had five G-chromosomes. The five cells >47 were not analysable with accuracy. All had about 90 chromosomes.

chromosome. At the twentieth passage, 353 days of culture, two of the 46 chromosomes cells with the iso-chromosome, had each two obvious marker chromosomes. These were interpreted as the result of reciprocal translocation, as tentatively represented in Figure 2.

2. Mosaic mongol

This strain was kept for 219 days by 16 serial passages. The last chromosome determination was made at 148 days of culture at the twelfth passage. No analysable metaphases were found in the last four transfers. The proportions of normal and trisomic karyotypes are shown in Table 3. No structural rearrangements were observed in any of the cells, but at the eighth passage, at 124 days of culture, six of the 70 scored cells had chromatid breaks.

DISCUSSION

The results of this investigation indicate a remarkable degree of stability of abnormal karyotypes during their finite life-time in vitro. The persistence of the iso-chromosome for the long arm of X during 460 days of culture confirms and strengthens the similar results obtained by FRACCARO and MANNINI (1966). The number of presumptive XO cells in the fibroblast cultures was so low as to make uncertain their very existence. However, the fact that no XO cells originated *de novo* during the culture indicates that the iso-chromosome had a high degree

of stability, which requires in turn an efficient mechanics of division. It should be noted that in this, as in the previous case of FRACCARO and MANNINI, structural rearrangements appeared during the late phases of culture but none was seen to involve the iso-chromosome. DE LA CHAPELLE *et al.* (1966) and OCKEY *et al.* (1966) reported the existence of two types of iso-chromosomes, a "normal" one and a second one characterized by morphological characteristics that appeared to the authors to indicate a dicentric nature. The criteria by which the second type of iso-chromosome is diagnosed are beyond our capabilities, but one could assume that the present case and the one of FRACCARO and MANNINI are not of this type. In fact, one should expect an iso-chromosome of the type postulated by DE LA CHAPELLE *et al.* (1966) to be unstable *in vitro*. On the other hand, disappearance of the iso-chromosome in long term culture was indeed reported by NUZZO *et al.* (see Table 1). In the cultures from the female mosaic mongol, both karyotypes the normal and the trisomic, persisted in approximately the same proportions. The relative frequencies of the two karyotypes in the first three rows of Table 3 are statistically homogeneous.

FORD (1967, and personal communications) postulated that, in mosaic mongols, one would expect a higher proportion of normal cells in blood and bone-marrows cultures than in fibroblast cultures, due to a relatively high rate of selection in favour of the normal cells in the former, because of their higher mitotic rate. The data so far collected by FORD seems to agree, on average, with this assumption. Our results indicate that, during the limited lifetime of the cultures there was no detectable selective advantage of the normal over the trisomic cells. On the whole, adding the present results to those entered in Table 1 we can conclude that in mosaics *in vitro* there is a remarkable persistence of both numerically and structurally abnormal karyotypes and little or no evidence of selective advantage of one cell type over the other. An exception is the decrease of triploid cells in the mosaic described by BÖÖK *et al.* (1962). Conversely, and not unexpectedly, instability *in vitro* was found in the cases of ring-chromosomes entered in Table 1.

The appearance of chromosomal breakage towards the end of the culture period was observed in both instances. The presence of the occasional cell with new marker chromosomes was observed in the iso-chromosome cultures, both in the present as in the previous case of FRACCARO and MANNINI. The phenomenon could well be the indication of a chromosomal instability typical of ageing cultures that results in

TABLE 4. *The composition of the ring-X mosaic (X/XXr/XXrXr) in a series of blood cultures made at intervals during a 6-years time-lapse.*

B L was made in late 1961 and B 10 in October 1967.

Cultures	Karyotypes			Total
	45, XO	46, XXr,	47, XXrXr	
B 1	22	11	—	33
B 2	78	38	2	118
B 3	21	13	1	35
B 4	37	11	2	50
B 5	58	20	—	78
B 6	31	16	1	48
B 7	88	33	1	122
B 8	31	9	—	40
B 9	17	8	—	25
B 10	16	8	1	25

reproductive impairment and death, *via* the formation of new unfavourable cell genotypes. The chromosomal interchanges represented in Figure 2 could be the representation of such an event. It is generally assumed that the exceptional event of "transformation" and subsequent unlimited proliferation capacity of the cell population (the "cell lines" of HAYFLICK and MOORHEAD, 1964) is due to a similar chain of events, but leading to the exceptional favourable genotype(s).

Does karyotypic stability in culture indicate a similar stability *in vivo*? Information on the stability of mosaic karyotypes *in vivo* is scanty and partly contradictory. FERRIER (1966) observed stability of a 45, X/46, XY mosaic, both karyotypes being present in two examinations performed 5 years apart. REITALU (1968) reported a consistent proportion of the two karyotypes over a six-years period in a 47, XXY/48, XXXq-Y mosaic. We have observed the same proportion of trisomic and normal cells in peripheral blood of a mosaic mongol (LINDSTEN *et al.*, 1962), re-examined after four years (FRACCARO *et al.*, 1967). Similarly, in the case of the ring X-chromosome firstly described by LINDSTEN (LINDSTEN and TILLINGER, 1962; LINDSTEN, 1963) the ring has been present in approximately the same proportions in repeated blood cultures examined during a span of 6 years, as shown in Table 4. Conversely, the case of LA MARCHE *et al.* (1967) indicates the possibility of a disappearing mosaicism possibly due to selective advantage of normal over abnormal cells: only normal cells were found at 10 months

of age in a girl who at birth had 90 per cent of the cells trisomic for chromosome 18. It is obviously impossible to draw firm conclusions from such data. The variables involved are numerous and affected by factors such as age of the individual, problems of sampling, differentials in the natural dynamics of cell populations in different tissue systems.

We can conclude that in newly derived cell cultures with a finite lifetime selection is, in FORD's therminology, mainly conservative also for abnormal karyotypes. This is of practical importance because it indicates that the karyotypes sampled from long term cultures are, in most instances, the true representation of the karyotypic composition of the tissue of origin. Selection seems to be conservative also *in vivo*, at least in a proportion of karyotypic mosaics.

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SUMMARY

Karyotypic variation was investigated in long term fibroblast cultures derived from skin biopsies of two human mosaic, an iso-chromosome for the long arm of X (45, X/46, XXqi) and a mosaic mongol (46, XX/47, XX, G+). Both cell strains showed karyotypic stability. From this and similar observations it was concluded that in newly derived cell cultures with a finite lifetime selection is mainly conservative. Selection seems to be conservative also in vivo, at least in a proportion of karyotypic mosaic.

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Note added in proofs

Since this paper was submitted for publication we had the opportunity to culture a skin biopsy from a patient with a ring chromosome 18 discovered by Dr N. RICCI of the University of Ferrara. The ring chromosome persisted up to 207 days of culture, as shown in the accompanying table.

Days in serial culture	No. of passages	Chromosome nummer					Total
		45r	46r	47r	48r	>48r[1])	
34	primary	—	3	—	—	2	5
64	primary	—	15	—	—	—	15
84	2	—	5	—	—	1	6
96	3	—	4	—	1	—	5
107	4	1	15	—	—	—	16
144	5	2	8	1	1	1	13
171	7	—	6	—	—	2	8
207	11	—	1	—	—	—	1
313	14	no mitoses					

[1] r=presence of ring 18. The cells indicated >48r all had about 90 chromosomes and were not analysable with accuracy.