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CHROMOSOME COUNTS OF ^{90}Sr-INDUCED OSTEOSARCOMAS IN MICE

II. Variation of the chromosome counts of slow and fast growing tumours in hyper- and nonhyperimmunized hosts

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Osteosarcomas induced by ^{90}Sr have been examined for different characteristics previously (NILSSON 1962, 1966, 1969, NILSSON & RÖNNBÄCK 1973). Data indicating that such osteosarcomas possess specific transplantation antigens were presented by NILSSON et coll. (1972). It was then observed that the incidence of progressively growing tumours was significantly greater in whole-body-irradiated (4 Gy; 400 R) than in unirradiated mice. Furthermore, heavy irradiation (150 Gy; 15 000 R) of tumour cells before transplantation decreased the frequency of progressively growing tumours in comparison to treatment with heavily irradiated normal tissues.

Chromosome number variation in serially transplanted ^{90}Sr-induced bone tumours in CBA mice has been reported by BERGMAN & NILSSON (Part I). Previous data have shown that, when relating the outgrowth period of primary tumours to the chromosome pattern (KATO 1968, MARK 1969, MITELMAN 1971), fast growing tumours exhibited a normal chromosome distribution, while tumours with deviating chromosome numbers were slower growing. Therefore, a primary aim of the present investigation was to test whether a connection may exist between the outgrowth period and the variation in chromosome pattern of transplanted ^{90}Sr-induced tumours. For this purpose separate serial transplantation was performed from both a fast and a slow growing tumour of one and the same transfer generation. Furthermore, in order to obtain a prolonged outgrowth period, transplantation was also carried out to recipients with a changed immunologic response, so called hyperimmunized mice. Besides outgrowth period, tumour size and the presence of metacentric chromosomes were recorded. Histologic characterization of the primary tumour and transplanted tumours was also performed.

Material and Methods

Transplantation procedures. Transplantation was always performed to the same sex of the highly inbred strain of CBA mice in order to minimize factors that may favour changes. Therefore, only 60±5 days old male mice were used. From a mouse which had received an injection of 14.8 kBq (0.4 μCi) ^{90}Sr/g body weight, a radiation induced osteosarcoma of predominantly fibroblastic type appeared after 403 days. By serial transplantation in vivo from the primary tumour (NILSSON & ULLBERG 1962), the numerical chromosome progression of 34 succeeding transfer generations was analysed as B-series, which had been established previously (Part I). Thus, from the second transfer generation (B2), separate serial transplantation (Fig. 1), was performed from a tumour with an outgrowth period of 21 days (transfer series B) and from a slower growing tumour with an outgrowth period of 44 days (transfer series b). Transplantation was per-

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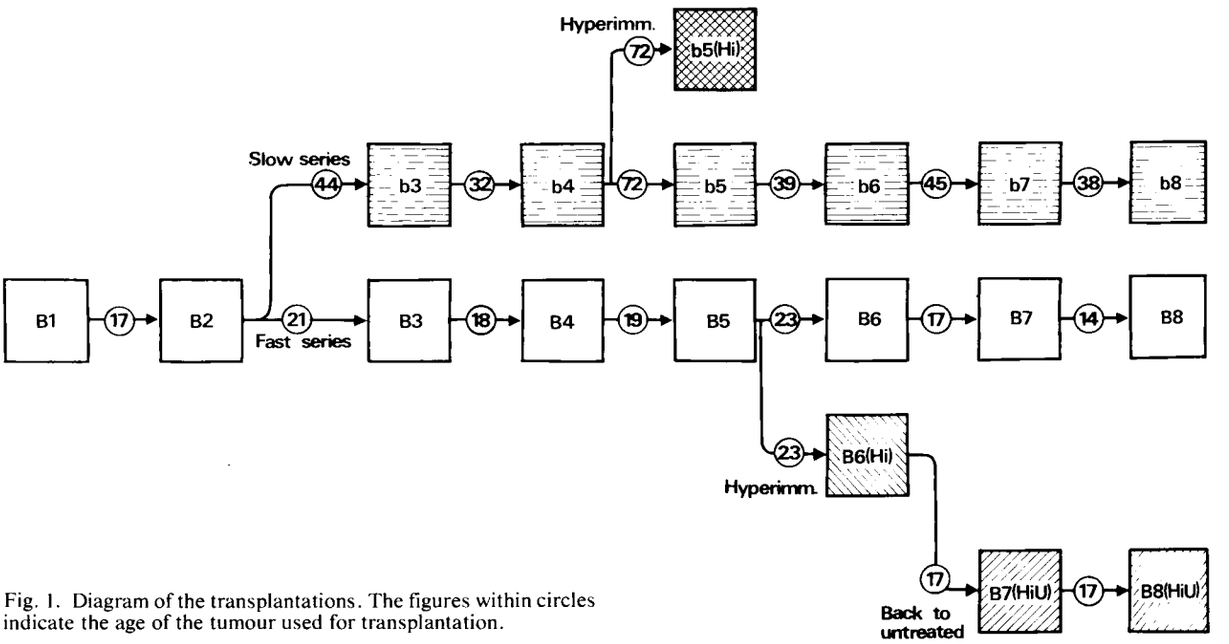

Fig. 1. Diagram of the transplantations. The figures within circles indicate the age of the tumour used for transplantation.

Table 1

The percentile chromosome distribution. B = Fast series. b = Slob series. b5 (Hi) and B6 (Hi) = Transplantation performed to hyperimmunized recipients. B7 (HiU) = Transplantation performed from hyperimmunized to untreated recipients. B8 (HiU) = Continued transplantation to untreated recipients

Transfer generation	No. of tumours examined	No. of cells counted	Chromosome number															
			39	40	41	43	47	48	49	50	51	52	53	54	55	56	57	58
B1	5	125		65.6	1.6												1.6	1.6
B2	5	125		25.6						0.8						2.4	7.2	10.4
B3	5	125	2.4	22.4									0.8				3.2	6.4
B4	5	125		51.2									0.8	1.6	4.0	5.6	4.8	2.4
B5	5	125		33.6										0.8	0.8	4.8	0.8	
B6	5	125		73.6														
B7	5	125		39.2														0.8
B8	5	125		55.2													0.8	
b3	2	48		33.3											2.1	4.2	22.9	4.2
b4	4	100		59.0										2.0	9.0	5.0	20.0	3.0
b5	5	125		24.0							0.8		1.6	1.6	18.4	44.0	8.8	
b6	5	125		23.2								0.8		4.8	43.2	25.6	0.8	0.8
b7	5	125	1.6	36.0		0.8				0.8		5.6	7.2	36.0	4.0	5.6		
b8	5	125		16.0	0.8	2.4	0.8	1.6	2.4	4.0	3.2	6.4	13.6	30.4	14.4	1.6	2.4	
b5 (Hi)	5	125		16.0										0.8	4.0	24.8	41.6	9.6
B6 (Hi)	5	125	0.8	36.8												0.8	2.4	0.8
B7 (HiU)	5	125	0.8	56.0								0.8						
B8 (HiU)	5	125		66.4												0.8	0.8	

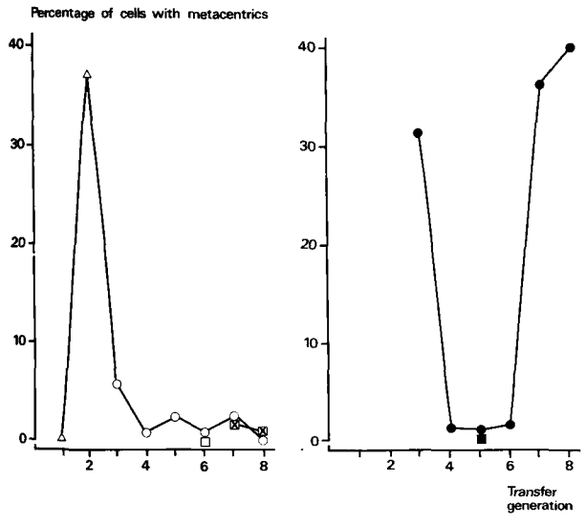

Fig. 2. Percentage of cells with one or more metacentric chromosomes. △ Fast series transfer generations B1–B2. ○ Fast series transfer generations B3–B8. □ Transfer generation B6 (Hi). ⊠ Transfer generations B7 (HiU) and B8 (HiU). ● Slow series transfer generations b3–b8. ■ Transfer generation b5 (Hi).

Table 1 *(cont.)*

59	60	61	62	63	64	65	80
3.2	2.4	4.0	5.6	8.8	4.8	0.8	
13.6	23.2	10.4	3.2	1.6	1.6		
6.4	50.4	4.8	1.6	1.6			
1.6	4.8	3.2	5.6	9.6	3.2	1.6	
0.8	8.0	4.8	20.0	18.4	5.6	1.6	
		0.8	12.0	12.8	0.8		
2.4	8.0	16.8	21.6	8.8	2.4		
4.0	8.8	12.8	14.4	4.0			
4.2	2.1	16.7	8.3	2.1			
2.0							
0.8							
0.8							
	1.6						0.8
1.6	0.8			0.8			
1.6	4.8	16.0	20.0	12.0	3.2	0.8	
0.8	2.4	10.4	23.2	3.2	2.4		
0.8	3.2	8.8	13.6	4.0	1.6		

formed from transfer generation b4 also to hyperimmunized recipients b5 (Hi) (Fig. 1). In the same way transplantation was carried out from transfer generation B5 to hyperimmunized mice B6 (Hi). From this generation two succeeding transplantations were performed, but now to untreated recipients B7 (HiU) and B8 (HiU).

Hyperimmunization assays. Suspensions from tumours of transfer generation b3 and B4, respectively, were separately prepared by pressing the freshly excised tissue through a stainless wire gauze into 2 ml saline solution. The suspension was cooled with ice cubes and irradiated with 150 Gy (15 000 R). Doses of 0.2 ml concentrated cell suspension were injected subcutaneously in both flanks of the mice. The injections were given three times at weekly intervals. One week after the last transfer of heavily irradiated cell material, separate transplantation was performed from a tumour of transfer generation b4 to both hyperimmunized (b5 (Hi)) and untreated mice (b5) and also from a tumour of transfer generation B5 to hyperimmunized (B6 (Hi)) and to untreated (B6) (Fig. 1). It was not considered necessary to estimate the number of cells transplanted, since the main purpose was to analyse the chromosome pattern and not the transplantation antigenicity. Extreme care was taken to choose tumour tissue from the same area and of a similar size as when transplanting to untreated hosts.

Chromosome analysis. The chromosome preparations were obtained according to the air-dry technique described in Part I. At the most, 5 successful tumours per transfer generation were examined and, if possible, 25 cells per tumour were analysed; altogether 2 148 cells. Cells suitable for chromosome counting were selected at magnification 200× and analysed at 1 000×. Only the percentile chromosome distribution per transfer generation is presented. No structural analysis or karyotyping was performed, primarily due to limited possibilities with the conventional staining method used.

Histologic examination. Sections of the tumour were fixed in Stieve's solution, embedded and stained according to the van Gieson method or with haematoxylin eosin. The definition of tumour classification has been presented previously (NILSSON & RÖNNBÄCK).

Results

Registration of chromosome abnormalities. The registration of abnormalities was limited to numeri-

Table 2

The variation in chromosome distribution often found between the tumours of a transfer generation is illustrated by the chromosome pattern obtained from the tumours of three B-generations. 25 cells per tumour were analysed

Transfer generation	Tumour No.	Growth period (days)	Size (cm^3)	Chromosome number														
				39	40	41	53	54	55	56	57	58	59	60	61	62	63	64	65
B1	1:1	16	0.78		19	2						1	1					2	
	1:2	17	0.96		14						1			3	2	3	2		
	1:3	20	0.59		19										1		4		1
	1:4	21	0.91		18											3	1	3	
	1:5	27	0.72		12						1	1	3	•	2	1	4	1	
B3	3:1	17	1.40	2	5						1	1	1	12	2		1		
	3:2	17	0.84		1						3	3	3	15					
	3:3	18	1.14		1		1						1	21	1				
	3:4	18	0.88		4							4	3	11	2		1		
	3:5	20	0.40	1	17									4	1	2			
B6	6:1	12	0.76		25														
	6:2	12	1.26		18										1	3	3		
	6:3	14	1.31		22											2	1		
	6:4	14	0.90		15											3	6	1	
	6:5	17	0.90		12											7	6		

cal chromosome deviations and the occurrence of metacentric chromosomes (Part I). Thus, numerous occasionally appearing structural rearrangements were not recorded.

The percentile chromosome distribution per transfer generation is recorded in Table 1. The variation in chromosome numbers between the tumours of a transfer generation is illustrated in Table 2. A diagram of the transplantation appears in Fig. 1. The percentage of cells with one or more metacentrics per generation is presented in Fig. 2. The mean size and the mean growth period of the tumours per generation are presented in Figs 3 and 4. Finally, Figs 5 and 6 show a 58-chromosome cell and a 62-chromosome cell, respectively.

Chromosome distribution. In the first transfer generation (B1), a predominating number of 40-chromosome cells (65.6%) and a wide distribution within the triploid region was observed (Part I). In the second generation (B2) the percentage of 40-chromosome cells had declined to 25.6 per cent while, instead, an increased number of cells was found within the triploid region. From this generation, transplantation was carried out both from a fast and from a slow growing tumour. By separate serial transplantation the two parallel transfer series (B and b) were established (Fig. 1). When comparing the chromosome distribution of transfer generations B3 and b3, generation B3 showed 50.4 per cent 60-chromosome cells, range 57 to 63 generation b3 a range of 55 to 63 and flat peaks at 57 and 61 (22.9 and 16.7%, respectively). In contrast to generation B3, only 2.1 per cent 60-chromosome cells were found. The percentage of 40-chromosome cells was 22.4 per cent (B3) and 33.3 per cent (b3). It should, however, be noticed that only 48 cells were counted from the b-generation. The subsequent B-generations (B4–B8) showed a rather high percentage of 40-chromosome cells (33.6–73.6%). Within the triploid region the 61- to 63-chromosome cells were the most frequently appearing types. In transfer generations b4 to b8, a different chromosomal progression was observed, in the sense that the only generation with a preponderance of 40-chromosome cells was b4 (59.0%). Also in contrast to the B-series, the b-series was characterized within the tripolid region by cells with continuously lower chromosome numbers. Peaks were found within the 54 to 57 region.

As from transfer generation b4, transplantation was performed also to hyperimmunized hosts; a generation designated b5 (Hi) was obtained. When comparing this generation with the corresponding untreated generation b5, rather similar chromosome pattern was found, but with the difference that the b-generation had peaks at 55 and 56 (18.4 and 44.0%, respectively) and 24.0 per cent 40-chromo-

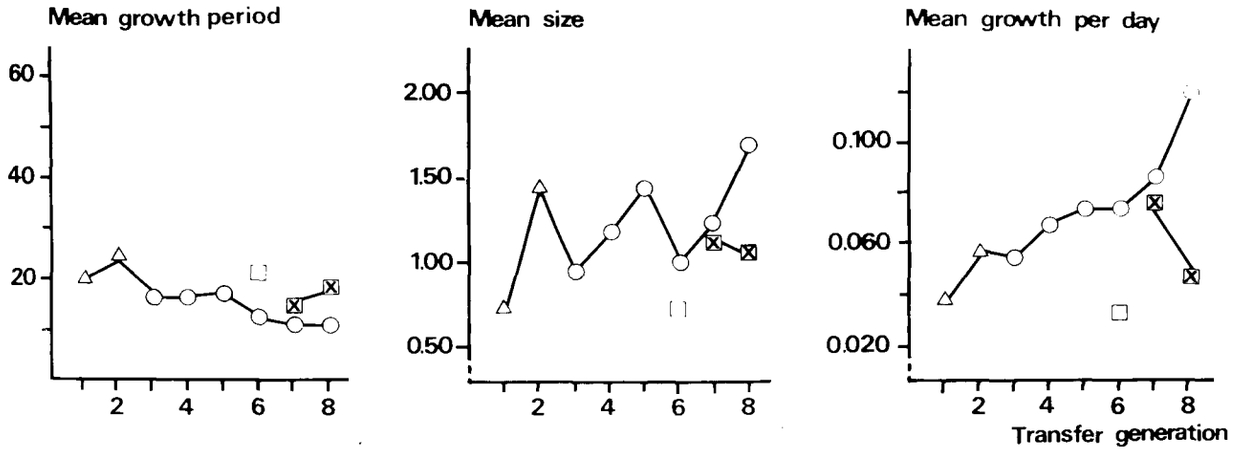

Fig. 3. Mean growth period (days), mean size (cm^3) and mean growth per day (cm^3) of the B-tumours per generation. △ Fast series transfer generations B1–B2. ○ Fast series transfer generations B3–B8. □ Transfer generation B6 (Hi). ⊠ Transfer generations B7 (HiU) and B8 (HiU).

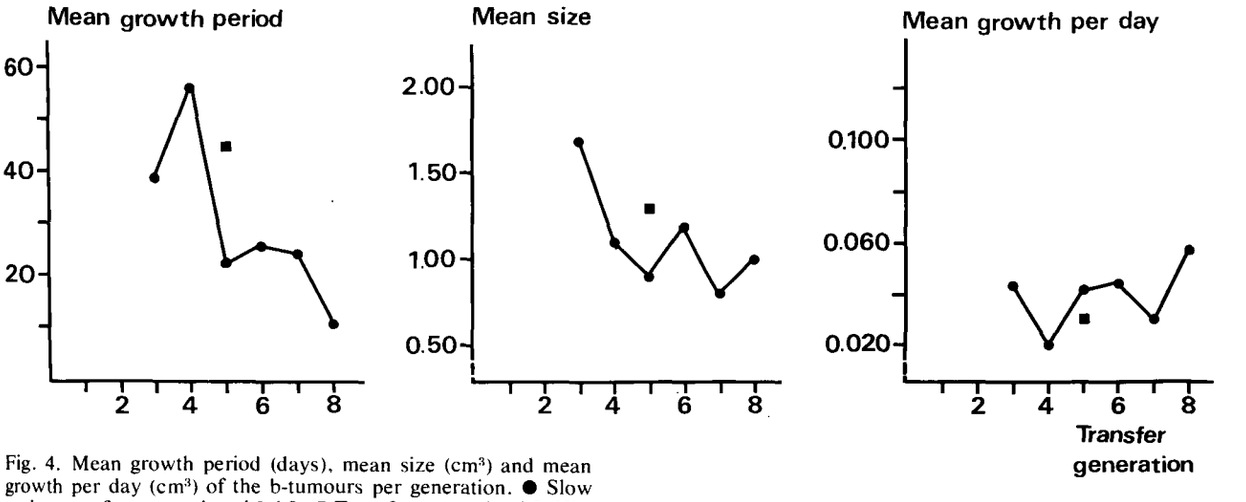

Fig. 4. Mean growth period (days), mean size (cm^3) and mean growth per day (cm^3) of the b-tumours per generation. ● Slow series transfer generations b3–b8. ■ Transfer generation b5 (Hi).

some cells, while generation b5 (Hi) displayed peaks at 56 and 57 (24.8 and 41.6%, respectively) and 16 per cent 40-chromosome cells. Transplantation was also performed from generation B5 to hyperimmunized recipients (B6 (Hi)). In generation B6 73.6 per cent 40-chromosome cells were found, in generation B6 (Hi) 36.8 per cent only. Furthermore, within the triploid region generation B6 displayed a narrow range of variation (61–64), whereas the tumours of the hyperimmunized hosts showed a wide distribution (56–64). From generation B6 (Hi) transplantation was carried out to untreated hosts. This generation, denominated B7 (HiU), showed in comparison with the preceding generation B6 (Hi) an increased number of 40-chromosome cells (56%). Within the triploid region, a narrower range of variation (59–64) was found. The parallel B7-generation, on the other hand, showed a reduced percentage of 40-chromosome cells (39.2%). Within the triploid region an almost similar range (58–64) as in B7 (Hi) was recorded. Finnaly, transfer generations B8 and B8 (HiU) had a rather similar chromosome pattern.

Mean outgrowth period of the tumours per transfer generation (Figs 3, 4) varied to some degree between the series. Of interest was a rather distinct variation between the B- and the b-series, and a prolonged outgrowth period when using hyperimmunized mice. The B-series was characterized by faster growing tumours with an outgrowth period

per generation ranging from 13.8 to 18.6 days, while the b-series displayed slower growing tumours with a mean value per generation ranging from 16.4 to 54.8 days. In generation b5 (Hi) a mean outgrowth period of 44 days was recorded, in the parallel generation b5 22 days. Also when transplanting from the B-series to hyperimmunized hosts B6 (Hi), a prolonged outgrowth period was observed in comparison with the untreated generation B6. The mean value for generation B6 (Hi) was 23.2 days and for generation B6 13.8 days only. However, when retransplanting from hyperimmunized to untreated hosts, once again faster growing tumours appeared. The mean outgrowth period of generation B7 (HiU) and the subsequent generation B8 (HiU) agreed well with the parallel generations B7 and B8. The mean values for the B (HiU)-generations were 14.8 and 18.0 days, respectively, and for the B-generations 14.0 and 14.2 days.

Metacentric chromosomes. The percentage of cells with one or more metacentrics per transfer generation is recorded in Fig. 2. It should be noticed that, besides generation B2 which showed 37 per cent cells with metacentrics, high values were also recorded for transfer generations b3 (31.2%), b7 (36.2%), and b8 (40%).

Histologic classification. The primary tumour B was classified as an osteosarcoma of predominantly mixed type containing cartilage components of varying degree of differentiation plus tumour bone and, in the peripheral regions, mainly cells of anaplastic type. Samples obtained from tumours of the B- and b-generations had continuously more anaplastic cells, but with the difference that the b-tumours also displayed a focal osteoid formation. Because of a failure in preparation no analysis was performed on the tumours of the hyperimmunized generation B6 (Hi). In tumours of generation b5 (Hi) a moderate bone formation was found indicating a higher level of cellular differentiation. Along the bone trabeculae the cells resembled osteoblasts and in other areas were still more anaplastic.

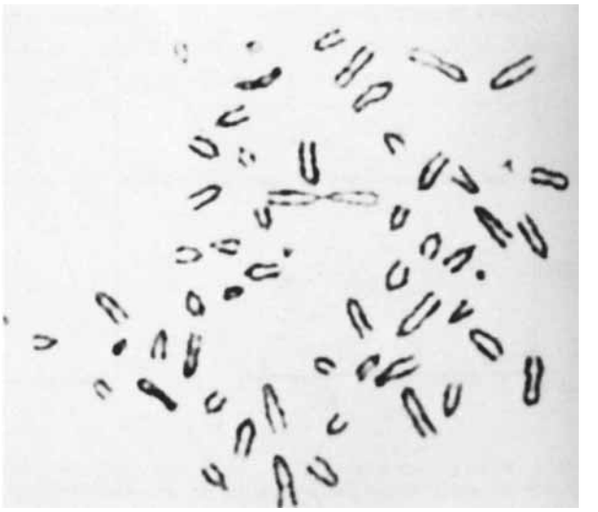

Fig. 5. A 58-chromosome cell from a tumour of transfer generation b5 (Hi).

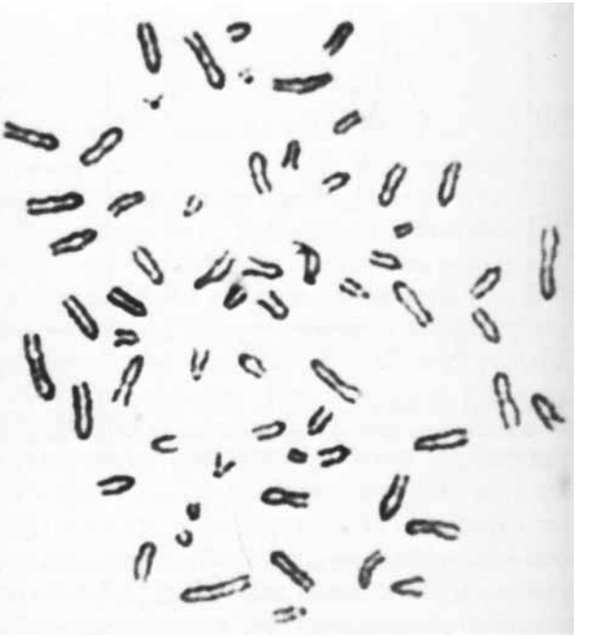

Fig. 6. A 62-chromosome cell from a tumour of transfer generation B5.

Conclusions

A primary aim of the present investigation was to test to what extent the selection of tumour used for transplantation may affect the chromosome pattern in succeeding transfer generations, but also to analyse whether tumour transplanted to hosts with a changed immunologic response would display a different chromosome pattern in comparison with tumour transplanted to untreated recipients. Another intention was to examine whether variation in outgrowth period could be observed, when separate transfer series were established from a slow and a fast growing tumour and when transplanting to hyper- and nonhyperimmunized recipients. The ages of the tumours used for transplantation were, except for transfer generation B2, selected so as to

be as representative as possible for the generations in order to avoid factors that may interfere with the evaluation of the results. Furthermore, the size of the peripheral tumour pieces used for transplantation was standardized to the utmost possible extent.

The B- and b-series displayed a different chromosomal progression (Table 1), and primarily within the triploid region. The B-series was thus characterized by a predominating number of 60- to 64-chromosome cells, while the b-series showed continuously lower chromosome numbers and with peaks at 54 to 57. Furthermore, the tumours of the B-series often displayed a higher frequency of 40-chromosome cells. However, even if these results indicate differences in chromosomal progression, they should not be considered as a typical variation of a slow and fast growing tumour series. It should also be realized that coincidental changes may have caused this variation, especially with regard to often noticeable differences between tumours of one and the same transfer generation and also between generations of a series. This circumstance may also explain why no direct effect was observed when transplanting tumours to hyperimmunized recipients, even if these tumours for instance displayed a smaller number of 40-chromosome cells than the tumours of the untreated hosts. Concerning the mean outgrowth period of the tumours per series, it was found that the mean value for the B-series was 16.0 days and for the b-series 29.0 days. However, in the latter series faster growing tumours appeared in the late generations. When transplanting to hyperimmunized recipients a prolonged outgrowth period was observed in comparison with the untreated parallel generations, but it was also noticed that, when retransplanting to untreated recipients, faster growing tumours were obtained. Thus, it cannot be ruled out that there may be a certain variation caused by difference in the immunologic response, but that this variation is not quite uniform, as similarities were found between untreated and some hyperimmunized recipients. Nevertheless, the present results should indicate the need for some caution when evaluating at least transplanted radiation-induced tumours.

SUMMARY

Highly inbred CBA mice were used. The registration of chromosome abnormalities was limited to numerical deviations and the occurrence of metacentric chromosomes. By separate serial transplantation from a ^{90}Sr-induced osteosarcoma two parallel transfer series (B and b) were established. From these series transplantation was also performed to hyperimmunized hosts B (Hi) and b (Hi). Besides differences in mean outgrowth period between B- and b-generations, a variation in chromosome pattern was observed. However, this variation should not be evaluated as a typical chromosomal progression of fast and slow growing tumour series.

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