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Some experimental studies in two sublines of the Yoshida sarcoma, with special consideration of the derivation of sublines¹

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The chromosome individuality of a neoplastic cell-population is not always permanent even in highly homogeneous and uniform populations. It has been shown that during long continued serial transfers, the chromosomes of the stem cells of a population generally undergo progressive changes in both number and structure from those of the time of its origin (Levan 1956a, b, Makino 1957a, b, Tonomura and Sasaki 1957, Makino and Sasaki 1958, Sasaki 1958). Further, it has been found that the stem-line chromosome pattern differs in different transfer lines, and that the numerical and structural changes of chromosomes take place in close association with the derivation of sublines from the stock tumor, though the mechanism of chromosome changes in relation to the development of new types of tumors has remained unknown (Makino and Sasaki 1958). With a purpose to analyse cytologically the evolutionary change in neoplastic cell-populations in connection with the derivation of sublines, some experimental studies were undertaken with special reference to morphological changes of the chromosomes and the consequent shifts in neoplastic properties in two sublines of the Yoshida sarcoma.

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Material and methods

In the present study use was made of two sublines of the Yoshida rat ascites tumor as material: they were referred to as sublines C and D in reference to the paper of Makino and Sasaki (1958). Subline D was derived from subline C by intraperitoneal transplantation of refrigerated tumor cells of the latter (Makino and Sasaki 1958, Sasaki 1958b). Each subline is generally characterized by its own specific stemline chromosome ideogram

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which is grossly distinguishable from the other by the presence of 3 or 4 V-shaped chromosomes of prominently large size: there are 3 V-shaped elements characterizing subline C, while 4 occur in subline D.

Eleven strains of rats weighing 60 to 120 grams were used for transplant experiments. Transplantation of the ascites tumor was performed according to the routine method using sharp-pointed sterile glass pipettes. For observation of the chromosomes, water-pretreatment squash technique exclusively was adopted (Makino 1957a).

Results

1. General features of sublines C and D

Before describing the cytological analysis, it is necessary to give some information on the general differences and likenesses between the two tumor lines considered here in respect to the disease types.

a. *Transplantability and survival range.* Sublines C and D of the Yoshida sarcoma have been maintained during about 150 generations, covering a period of about 2 years using rats of the following seven strains: Wistar (W/Ma), Wistar (wp), Wistar-Pacific, Wistar-King A, Gifu, Tokyo and Castle's black mutant $\times$ Wistar-King A. The data presented in this paper are based on the records of the above experiments. In addition, rats of four other strains, Long-Evans, Buffalo, Fischer and Albany, were used for the examination of the susceptibility to the sublines, but not for the serial transfer of tumors. The results are summarized in Table 1. In one of the author's previous papers (Sasaki 1959), the results were reported of transplant experiments which dealt with four sublines of the Yoshida sarcoma. In the present investigation, the data on lethal takes are based on death of the tumor-bearing animals caused by the growth of tumor cells.

In the experiments with the rats of the following eight strains: Wistar (W/Ma), Wistar (wp), Wistar-Pacific, Wistar-King A, Gifu, Tokyo, Castle's black mutant $\times$ Wistar-King A and Long-Evans, both tumor sublines showed a uniformly high transplantability giving 70 to 100 per cent lethal takes. Especially in rats of W/Ma, Gifu and Tokyo strains, the transplantability of the two sublines was very high being 95 to 100 per cent in lethal takes. In striking contrast to the above cases, condition differs in the rats of Fischer and Buffalo strains. Rats of Fischer strain were entirely nonsusceptible to subline C while subline D showed 80 per cent transplantability to rats of the same strain. In Fischer rats, however, the tumor cells of subline C showed active growth for about 5 days following inoculation; in some animals a considerable amount of ascitic fluid was accumulated in the peritoneal cavity with a slight invasion to tumor cells in visceral tissues, though after about 8 to 10 days following inoculation the tumor cells suddenly decreased showing degenerative changes and further 2 or 4 days later the tumor showed spontaneous regression without exception. At autopsy, after 3 to 4 months, no

visible tumorous growth was found in either peritoneal cavities or visceral tissues of those rats. Further, it is a remarkable fact that the transplantation rate and survival days of tumor-bearing animals somewhat differ either by the strains of rats or by tumor sublines used for experiment. The data are shown in Table 1. In Wistar strain (W/Ma), the two sublines showed a

Table 1. Transplantation rate and survival days of tumor-rats in two sublines of the Yoshida sarcoma observed in several rat strains

Tumor	Subline C		Subline D	
Transplant generation	1-147 (Aug. 1955 - Jun. 1957)		1-150 (Jan. 1956 - Jan. 1958)	
Strain of rats	Tumor takes (%)	Average survival (days)	Tumor takes (%)	Average survival (days)
Wistar (W/Ma)	72/74** (97%)	9.0	84/86 (98%)	10.9
Wistar (wp)	7/8 (88%)	11.4	8/10 (80%)	10.4
Wistar Pacific	4/5 (80%)	8.0	10/14 (71%)	16.1
Wistar-King A	11/13 (85%)	15.0	20/21 (95%)	13.9
Gifu	27/27 (100%)	8.5	37/37 (100%)	11.2
Tokyo	41/43 (95%)	8.7	10/10 (100%)	9.8
Castle's Black × Wistar-King A*	40/45 (89%)	10.2	42/46 (91%)	12.3
Long-Evans	8/10 (80%)	9.3	7/10 (70%)	15.6
Buffalo	2/6 (33%)	16.5	3/4 (75%)	10.7
Fischer	0/25 (0%)	—	20/25 (80%)	14.0
Albany	— —	—	3/3 (100%)	9.3

* This strain includes five strains, cbn, cbb, cbw, cbnh and cbbh, which were segregated from Castle's black mutant × Wistar-King A.

** The numerator shows the number of animals died of the tumor, and the denominator the number of animals received inoculation of the tumor.

Table 2. Survival of Wistar rats (W/Ma) with intraperitoneal transplants of the Yoshida sarcoma sublines C and D

Tumor lines	Number of rats died tumor																				Total	Mean life span in days
	Days after transplantation																					
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	21	24	28	32	44		
Subline C	—	7	20	16	11	7	3	3	2	—	2	—	—	—	—	—	—	—	1	—	72	9.0
Subline D	1	1	10	17	17	10	5	5	5	3	1	1	1	2	1	1	1	1	—	1	84	10.9

high transplantability in the experiments using a large number of animals in order to collect the data in detail. The data in Table 2 show that almost all tumor-animals died of disease 7 to 10 days following inoculation by either of the sublines, though there is a difference in survival range between the two sublines.

b. *Mitotic frequency.* The daily frequency of dividing cells including those at prophase, metaphase, anaphase and telophase was observed on the basis of 2000 tumor cells per day throughout the whole life span in two Wistar rats (W/Ma), one bearing subline C and the other subline D, with the inoculum of 0.05 cc of 4 day-old ascites in each.

Fig. 1. Graphical representation of daily frequencies of dividing cells in two sublines of the Yoshida sarcoma.

previously reported by Makino and Sasaki (1958), the modal chromosome number of subline C was found at 38–39 (76%) in the 10th to 26th transfer generations. In later generations, however, a shift occurred in modal number

The results indicate that the daily mitotic frequencies of tumor cells followed a similar pattern in the two sublines as shown in Fig. 1.

2. Chromosome pattern

The difference in transplantability existing between the two sublines is very striking as described above. Further interesting and pronounced differences between them seem to lie in their chromosome patterns. As

Table 3. Chromosome number distribution around the $2n$ mode in two sublines of the Yoshida sarcoma

Tumor	Transplant generation	Chromosome number								Total
		35	36	37	38	39	40	41	42	
Subline C	10–26 (Oct. 1955–Jan. 1956)	—	5 (2.8%)	5 (2.8%)	76 (42.2%)	61 (33.9%)	29 (16.1%)	3 (1.7%)	1 (0.5%)	180 (100%)
	91 (Oct. 1956)	—	1 (1.6%)	—	3 (4.9%)	46 (74.2%)	10 (16.1%)	—	2 (3.2%)	62 (100%)
	175 (Oct. 1957)	—	1 (4.8%)	—	1 (4.8%)	16 (76.2%)	3 (14.2%)	—	—	21 (100%)
Subline D	13–27 (Mar. 1956–Jun. 1957)	1 (0.7%)	5 (3.6%)	8 (5.8%)	21 (15.1%)	78 (56.1%)	23 (16.5%)	2 (1.5%)	1 (0.7%)	139 (100%)
	54 (Oct. 1956)	—	1 (1.5%)	1 (1.5%)	4 (6.0%)	52 (77.6%)	9 (13.4%)	—	—	67 (100%)
	183 (Jul. 1958)	—	—	1 (1.2%)	5 (6.0%)	68 (82.0%)	5 (6.0%)	2 (2.4%)	2 (2.4%)	83 (100%)

towards 39 in the 91st (74%) and in the 175th (76%) generations; the data are given in Table 3. In subline D, the modal number was 40 (51%) in the second to fifth generations, while it was found at 39 in later generations (Table 3, See also Table 2 in Makino and Sasaki 1958, p. 468). The frequency of occurrence of the stem-cells which have the modal number at 39 increased with the passage of transfer generations, giving 56% for the 13th to 27th generation, 76% for the 54th generation and 82% for the 183rd generation, respectively. It is thus evident that the two sublines here considered are identical in the mode of the stemline chromosome number in later transfer generations. Irrespective of the possession of the same number of chromosomes, however, the stem-cells of the two sublines are clearly distinguishable by their specific chromosome ideograms. The stem-cells of subline C are grossly characterized by the presence of three V-shaped chromosomes of remarkably large size, while subline D contains four such V-shaped elements (Figs. 21-22). In early transfer generations, almost all cells lying in the diploid range

Table 4. Frequency distributions of 2V-, 3V- and 4V-type cells in the Yoshida sarcoma sublines C and D

Tumor	Transplant generation	2V-type cells	3V-type cells	4V-type cells	Total no. of cells observed
Subline C	10-46	1	195	—	196
	91	1	127	—	128
	175	—	72	—	72
	215	44	104	2	150
Subline D	2-27	3	1	225	229
	54	1	—	116	117
	183	2	8	180	190

were of 3V-type in subline C, while they were 4V-type cells in subline D. Table 4 shows the frequency distribution of 2V-, 3V- and 4V-type cells in the two sublines. Subline C in the 10th to 175th transfer generations showed exclusively 3V-type stem-cells without showing 4V-type cells at all, though there did occur two 2V-type cells. In the samples of subline C obtained at the 215th generation, however, a considerable number of 2V-type cells being about 30% in frequency, were found to occur together with a few 4V-type cells (2 out of 150 cells observed). In subline D, 4V-type cells showed a predominant occurrence in samples taken from an early to the 54th generation, 2V- or 3V-type cells being very rare in occurrence. But in the 183rd generation, 2V- and 3V-type cells made their appearance in a small number of samples.

Based on the results of the above cytological observations, the following interpretations may be possible for the derivation of subline D from subline C, showing either that the reserved 4V-type cells occurring in the cell-population of subline C might surpass under certain environmental changes the

3V-type cells in their functional activity, or that certain mutational variants from the 3V-type stem-cells of subline C may come to possess metabolic superiority over their competitors giving rise to a new stemline. In order to obtain any data in support of one of the above two interpretations, comparative ideogram analysis was undertaken in the 3V-type cells of subline C

Fig. 2-7. Ideogram analyses of two sublines of the Yoshida sarcoma. 2-3, two 3V-type cells of subline C. 4-6, three 3V-type cells of subline D. 7, a 2V-type cell of subline D.

and in the 3V-type cells occurring in subline D, along with the comparison between the 4V-type cells of subline D and the cells of the same type which rarely occur in subline C. In addition, the ideograms of the 2V-type cells occurring in both sublines were also studied by way of comparison. The observations were made based on twelve reliable metaphase plates; in every

case, every one of 39 elements showed very clearly its own characteristic centromeric structure. To facilitate a general comparison between the ideograms of different lines, the chromosomes were divided after analysis into three morphological classes, V-, J- and rod-shaped ones, and were arranged in each cell in a tentative order of size (Figs. 2-13). The stem-line ideogram

Figs. 8-13. Ideogram analyses of two sublines of the Yoshida sarcoma. 8-10, three 4V-type cells of subline D. 11, a 4V-type cell of subline C. 12-13, two 2V-type cells of subline C.

of 3V-type cells in subline C, as shown in Figures 2 and 3 on the basis of analysis of two cells, consists of 14V's+13J's+12R's. However, the 3V-type cells in subline D in respect to distribution of the three chromosome types are not identical with the 3V-type cells in subline C. Furthermore, a considerable variation in distribution of the three types was observed in three

3V-type cells from subline D here studied: the chromosome formula is represented by $13V's+15J's+11R's$, $13V's+14J's+12R's$ and $11V's+17J's+11R's$, respectively in those three cells (Figs. 4-6). The same situation was found in comparison between the 4V-type cells from subline D and C. The stemline ideogram of the 4V-type cells in subline D is formulated as $13V's+12J's+14R's$ (Figs. 8-10), while a 4V-type cell from subline C is represented by $15V's+12J's+12R's$ (Fig. 11). A difference of similar nature was detectable again between the 2V-type cells of both sublimes (Figs. 7, 12, 13). Figures 12 and 13 show the chromosome complex of the 2V-type cells from subline C obtained in the 215th generation where about 30 per cent of cells observed were 2V-type. In this case no difference in distribution of the three chromosome types occurs between two plates under study (Figs. 12, 13), each being formulated as $12V's+16J's+11R's$. Table 5 summarizes the above data.

Table 5. Ideogram analysis of the Yoshida sarcoma sublimes C and D for three morphological chromosome classes

Tumor	Type	Figure	Chromosome distribution on three morphological classes			
			V	J	R	Total
Subline C	2V	12	12	16	11	39
	2V	13	12	16	11	39
	3V	2	14	13	12	39
	3V	3	14	13	12	39
	4V	11	15	12	12	39
Subline D	2V	7	9	13	17	39
	3V	4	13	15	11	39
	3V	5	13	14	12	39
	3V	6	11	17	11	39
	4V	8	13	12	14	39
	4V	9	13	12	14	39
	4V	10	13	12	14	39

It is evident from the results of the above observations that 4V-type cells of subline D were not produced as a result of a mere selection of masked 4V-type cells of subline C. Further it is shown that 4V-type cells of subline D, and 3V-type cells of subline C, have in each the characteristic chromosome constitution of a stem-line, that 3V-type cells of subline D are not identical in chromosome constitution with the 3V-type stem-cells of subline C, and further that the chromosome constitution of 4V-type cells of subline C differs from that of 4V-type stem-cells of subline D. It is therefore apparent that the 3V-type cells of subline D may be produced by the mutational change of 4V-type cells of that subline and that the 4V-type cells of subline C may be changed from the 3V-type stem-cells of that subline, while the 2V-type

cells of subline C found in the 215th generation may be a new stemline, since it shows a relatively high frequency in occurrence with its own definite karyotype.

3. Double inoculation experiments

If the 4V-type cells of subline D may have arisen as a result of variation from the stem-cells of subline C, the 4V-type cells would overcome the 3V-type cells in double inoculation with tumor cells from the two sublines into the same host. In order to examine that assumption, double inoculation experiments were undertaken using the tumor materials as follows: the samples for subline C were obtained during the period from the 91st to 145th transplant generations and for subline D from the 54th to 104th generations, since in the above samples 3V-type cells in subline C and 4V-type cells in subline D had maintained almost pure population in each (Table 4).

a. *Simultaneous inoculation with the two sublines in the same host.*

About 0.05 cc of 3 day-old ascites from both tumor lines were inoculated simultaneously in the peritoneal cavity of the same host. Nine rats of W/Ma strain were given simultaneous inoculation, and the daily frequency of 3V- and 4V-type cells was observed in each on the basis of about 40 to 200 metaphase cells in the mixed cell-population formed by the

Fig. 14. Daily frequencies of 3V-type and 4V-type cells, following double inoculation with the Yoshida sarcoma sublines C and D.

two different sublines. As shown in Figure 14, on the first day after inoculation, the frequency of the 3V-type cells was somewhat higher than that of the 4V-type cells. On the second day, both cells gave approximately similar counts. From the third day on, the 3V-type cells gradually increased in number. In some samples, however, even on the first or second day after inoculation, the 3V-type cells were predominant with a showing of over 80 per cent. On the fifth day after the simultaneous inoculation, the tumor ascites was further inoculated into a new rat of the same strain, thus at five day intervals three series of successive transplantations were made for seven transfer generations using W/Ma rats. The frequencies of 3V- and 4V-type cells were observed on the second to fifth day after each inoculation based on more than 100 metaphase cells. An example from the results of the above three series of experiments is shown in Figure 15. The percentage of 4V-type cells decreased gradually with the accretion of transplant gener-

ations. At the third transplant generation, sample of the tumor ascites of the mixed cell-population characterized by a predominancy of 3V-type cells was inoculated into a rat of Fischer strain. This rat died of tumor on the

Fig. 15. Frequency distributions of 3V- and 4V-type cells following the simultaneous inoculation with two sublines of the Yoshida sarcoma and those of in seven successive transfers at 5 day intervals. The frequencies of the 3V- and 4V-type cells were obtained in samples on the 2nd to 5th days after each inoculation, based on over 100 metaphase cells.

preparations sampled one day after inoculation of subline D, metaphase cells encountered under microscope were all of 3V-type cells. The samples obtained 3 days after inoculation of subline D, however, contained a small number of 4V-type cells, which thereafter showed decrease in number in the samples obtained on the 5th day after the second inoculation (Fig. 16 A). A similar situation was observed in another experiment which was carried out under the same experimental scheme as above.

c. *Inoculation of subline C in animals already having subline D.* The tumor ascites of subline D, about 0.05 cc, was injected in W/Ma rat, and

10th day after inoculation. This seems to be the natural sequence to show that the 4V-type cells occurring in the mixed cell-population are nothing other than the descendants of subline D, since the previous experiment (Sasaki 1959) showed that subline C did not yield lethal transplantability in Fischer rat at all.

b. *Inoculation of subline D in animals already having subline C.* Samples from subline C, 0.05 cc in amount, were injected in a W/Ma rat, and 2 days later 0.05 cc of subline D ascites was inoculated in the same host. In the following procedure the per cent frequency of 3V- and 4V-type cells was calculated on the basis of 20 to 100 clearly analyzable metaphase plates in every sampling (Figs. 16-20). In

Fig. 16. Frequency distributions of 3V- and 4V-type cells following double inoculations with two sublines of the Yoshida sarcoma. A, inoculation of subline D in pre-existence of subline C. B, inoculation of subline C in pre-existence of subline D.

2 days later the same host received an injection of 0.05 cc of subline C. The samples obtained 1 day after inoculation of subline C contained a small number of 3V-type cells. From the third day on, the 3V-type cells increased gradually in number (Fig. 16 B).

From the results of the above three experiments of double inoculation, the conclusions may be reasonable that both sublines can survive under the condition of co-existence forming a mixed cell-population in the peritoneal cavity of the same host, and that subline C has a tendency to surpass subline D in the mixed cell-population. It is then evident that the data obtained here do not fulfil the assumption proposed by the author.

d. *Cell population analysis of invading tumor nodules.* In the peritoneal cavity of animals that died of the Yoshida sarcoma, tumor invasion is rather common in visceral tissues. In the two sublines here considered, solid tumor nodules were formed in the omentum, mesentery, retroperitoneal tissues in the perirenal region and ovary or testis. Simultaneous inoculation with both sublines, 0.05 cc in each, was undertaken using rats of W/Ma and Fischer strains. On the fifth, sixth and seventh day after the simultaneous inoculation, animals were sacrificed with the pre-treatment of colchicine at 10γ for 8 hours. The tumor

nodules in omentum and retroperitoneal tissue were cut into small pieces, transferred to distilled water for about 10 minutes, and then subjected to acetic dahlia squash technique for the study of chromosomes. The chromosomes of tumor cells in ascitic fluid were observed in parallel for comparison. The results are as shown in Figure 17. In W/Ma rats, 3V-type cells form

Fig. 17. Frequency distributions of 3V- and 4V-type cells in invaded tumor nodules and in ascitic fluid of rats, following simultaneous inoculations with the two sublines of the Yoshida sarcoma. As: ascites, Om: omentum, Re: retroperitoneal tissue.

Fig. 18. Frequency distributions of 3V- and 4V-type cells in the mixed cell-population with two sublines of the Yoshida sarcoma, prior to and after the cold storage.

a dominant population in two solid phases of omentum and retroperitoneal tissue as well as in the ascitic phase, while in Fischer rats, 4V-type cells were predominant in the solid phase, whereas in the ascitic phase 3V-type

Fig. 19. Frequency distributions of 3V- and 4V-type cells in the mixed cell-population with two sublines of the Yoshida sarcoma, before and after the TEM treatment.

cells were superior to the latter. The different power of invasion in the two types of tumor cells between Wistar and Fischer rats may probably have connection with the fact that transplantability of the two sublines to Fischer rats is dissimilar.

e. *Mixed cell-population under some selective circumstance.* The following experiments were aimed at ascertaining whether a certain unfavorable environment might act as selective force in affecting less damage to the 4V-type cells than to the 3V-type cells in the mixed cell-population produced by the simultaneous inoculation of the two sublines. Cold storage was chosen at first as a selective factor, since subline D was originally derived from subline C through the inoculation of refrigerated tumor cells of subline C. On the second day after the simultaneous inoculation of the two sublines, about 0.1 cc of the tumor ascites was sealed into sterilized glass pipetted which were stored in the refrigerator at -2°C for six days. Then the refrigerated tumor ascites was inoculated in the peritoneal cavity of new rats. The experimental conditions were controlled to be nearly identical to those in the case of induction of subline D. Two rats (W/Ma) which received this injection developed ascites tumors and died of disease on the 16th and 26th day, respectively, after the inoculation of the tumor. Following inoculation of refrigerated tumor cells and before death of the hosts, the tumors were sampled for the study of chromosomes on the 11th, 13th and 15th day in one of the two rats, and on the 16th, 20th, and 24th

Fig. 20. Frequency distributions of 3V- and 4V-type cells in the mixed cell-population with two sublines of the Yoshida sarcoma, before and after the podophyllotoxin treatment.

day in the other rat. In both cases, the cold storage did not seem to have acted as an anticipated selective force upon the mixed cell-population; since even after the cold storage, the 4V-type cell-population did not surpass the 3V-type cell-population. The picture may be well understood by reference to Figure 18.

Next, two kinds of chemicals, TEM (triethylene-melamine) and podophyllotoxin, known as carcinostatic agents, were adopted for test as possible selective forces. A rat (W/Ma) was given an intraperitoneal injection of TEM

Figs. 21-26. Photomicrographs of metaphase chromosomes in two sublines of the Yoshida sarcoma. $\times 1000$. 21, a 3V-type cell of subline C. 22, a 4V-type cell of subline D. Figs. 21 and 22 correspond to Figs. 2 and 10, respectively. 23, a 2V-type cell of subline C. 24, a 3V-type cell of subline D. 25, a 2V-type cell of subline D. 26, a 4V-type cell of subline C. Figs. 23, 24, 25, and 26 correspond to Figs. 13, 5, 7, and 11, respectively. Arrows indicate the large V-shaped chromosomes.

(0.2 mg/kg) on the 2nd day following the simultaneous inoculation of the two sublines. Within 3 days after the injection, almost all tumor cells in the ascites were damaged by the chemicals, but on the fifth day following the injection, regrowth of the tumor occurred in the treated animals. On the 6th, 8th and 10th days after the injection of TEM, chromosome analysis of tumor cells was undertaken. The samples obtained at the 2nd day after the simultaneous inoculation contained 3V- and 4V-type cells in about equal number, while on the 6th day after the injection, nearly 60 per cent of the

cells were the 3V-type. Thereafter the 3V-type cells increased in number gradually on the 8th and 10th days after injection (Fig. 19).

In the following experiments, the mixed cell-population based on the two sublines was exposed to podophyllotoxin (10 mg/kg). Podophyllotoxin at this dosage displayed rapid and severe damaging action against ascites tumor cells; within six hours after the injection of this chemicals almost all cells in the ascites were affected and disintegrated by this drug. But at 24 hours after injection, tumor regrowth initiated and dividing cells re-appeared in the ascites. Chromosome analyses were made at 24, 48 and 72 hours after the injection of the drug based on 40 to 60 metaphase plates in every sampling. On the 2nd day following the simultaneous inoculation, just before the podophyllotoxin injection, the 3V-type cells were found about 60 per cent in the mixed cell-population. But in the samples obtained at 24, 48 and 72 hours after injection, the 3V-type cells were reduced in number to from 28 to 33 per cent (Fig. 20).

From the above three experiments, the suggestion may be made that though the 3V-type cells usually surpass the 4V-type cells in the mixed cell-population, the latter cells tend to overcome the 3V-type cells under the influence of some selective pressure.

Discussion

The mosaic concept of the neoplastic cell-population has been suggested and discussed by Hauschka (1952, 1953a, 1957) based on many morphological, physiological and immunogenetic observations. The single-cell isolation experiment seems to be useful for the cytological interpretation of this problem, since the clonal derivatives established by single-cell inoculations of the Ehrlich and Krebs-2 mouse tumors have shown different modes of chromosome number (Hauschka 1953a, b, 1957, Levan 1956a, Hauschka and Levan 1958). Kaziwara (1954) has established stable polyploid sublines from a hyper-diploid Ehrlich ascites tumor, and discussed the mechanisms of shifts in ploidy from the viewpoint of immunoselection. According to the view of mosaicism, in the case of the Yoshida sarcoma unfavorable environment such as cold storage seems to act as a selective force in respect to a few masked 4V-type cells occurring in subline C. But detailed ideogram analyses between the two sublines have made it clear that the chromosome complements of 4V-type cells found in subline C are different from those in subline D. The above findings seem to show that the 4V-type cells rarely occurring in the population of subline C are mutational variants of the 3V-type stem-cells. The same can be said with respect to the 3V-type cells (or 2V-type cells) found in subline D. But the 2V-type cells, which appeared with a comparatively high percentage (30%) in the later transfer generations of subline C, seem to form a stem-line of this tumor; they are entirely identical in chromosome constitution with each other.

Graff *et al.* (1952) studied the growth of Carcinoma 755 and Sarcoma 180 transplanted in the same host and reported that Sarcoma 180 inhibited the growth of Carcinoma 755. Reciprocal influence of a similar nature between two tumors after double implantation in the same host has been reported to exist between the Yoshida sarcoma and the ascites hepatoma of rats (Sato 1952), and between the former and the p-toluyldiamine sarcoma of rats (Isaka 1952). Makino and Tonomura (1955) have reported that the tumor cells from two different rat tumors, the MTK-sarcoma II and Takeda sarcoma, when inoculated in the same host, affect each other: there is a decrease of the mitotic rate and striking damage to tumor cells with subsequent retardation of tumor growth. In the double inoculation experiments with two sublines of the Yoshida sarcoma, no such destructive effect as above was observed: there followed a co-existence of the two sublines without any visible damage to tumor cells in the same host, though subline C was generally predominant over subline D in the mixed cell-population, so far as the metaphasic cells are concerned. A similar situation was found by Makino and Kanô (1955) to occur in two single-cell clone of the Hirosaki sarcoma. Recently, Hansen-Melander (1958) established two different single-cell clones from a hyper-diploid mouse tumor, Lundschtitz tumor, which two clones showed a different chromosome patterns from the original tumor as well as from each other. She made some double inoculation experiments with the two clones and the original tumor to compare the competitive capacity between the two clones and the mother tumors, and reported that in every experiment the newly established single-cell clone surpassed the original tumor in activity, with a strong suggestion that the two clones have an improved competitive capacity. The two sublines (C and D) here considered showed certain differences and likenesses between them in their transplantability and growth rate, as well as in the chromosome pattern, but the differences in property between the two sublines seems not greater than those existent between the MTK-sarcoma II and Takeda sarcoma. Interesting is the fact that sublines C and D are dissimilar in respect to their immune response (Matsumoto 1959), and to transplantability (Sasaki 1959). Probably these two sublines have different responses to certain environmental factors such as host environment, drug action, and cold storage. Based on the results of the present selection experiments in the mixed cell-population, the conclusion may be possible that subline C which generally surpasses subline D in the mixed cell-population, may be overcome by subline D under the influence of some selective pressure, with, in the extreme case, the possibility of a complete selection.

The results of comparative ideogram analyses between the two sublines of the Yoshida sarcoma, double inoculation experiments with the two sublines, and the selection experiments on the mixed cell-population seem to permit the following conclusion with regard to the mechanisms of the rise and development of tumor sublines: once tumor have developed and undergone serial transfers, many mutational changes including structural alterations of chromosomes as well as gene mutation take place continuously in their stemline cells. Some of these random variants may acquire a self-perpetuating capacity in succeeding generations. The population equilibrium may be disturbed in more or less degree by the occurrence of such mutational variants. These changes may be minor or they may be major ones; some of these viable variants increase in number with the accumulation of transfer generations and come to possess competitive ability necessary to enable survival as new stemline cells. Further, a selective pressure such as that of hostile environment or experimental factors seems to play an important role in the derivation of establishment of new tumor types. Thus, the neoplastic population as a whole undergoes continuous changes to adapt to environments

resulting in the formation of a new stem-line (or -lines) of tumor cells during prolonged serial transfers of the same tumor, or of different lines.

Summary

Some experimental and cytological studies were undertaken in the hope of making clear the mechanism of the rise and further development of sublines in the neoplastic cell-population. Use was made of two sublines, C and D, of the Yoshida sarcoma.

1. The transplantability and life span of tumor-bearing animals differ by rat strains or by tumor sublines. Subline C was wholly non-transplantable to Fischer rats, while subline D showed a transplantability at 80 per cent lethal takes to the same strain of rats.

2. The two sublines differ in their chromosome pattern. The modal chromosome number showed a shift with an increase in frequency of the stem-cells having the modal number of 39 with the accretion of transfer generations in both sublines.

The stemline cells of both tumor lines are clearly distinguishable from each other by their specific ideograms: the stem-cells of subline C have three V-shaped chromosomes of outstandingly large size thereby differing from subline D which contains four such V-shaped elements.

The 4V-type cells rarely occur in subline C, and differ in chromosome constitution from those in subline D. The same situation was found to occur in the 3V-type and 2V-type cells occurring in subline D.

The 2V-type cells occur in subline C at 215th generation, and possess a definite chromosome constitution forming a stemline of cells.

3. Double inoculation experiments with the two sublines produced results showing that there occurs co-existence of the two sublines in the same host without any visible damage to the tumor cells. The 4V-type cells of subline D are generally outnumbered by the 3V-type cells of subline C in the mixed cell-population. The results of selection experiments suggest that though the 3V-type cells usually surpass the 4V-type cells in the mixed cell-population the latter cells tend to displace the 3V-type cells under the influence of a certain selective pressure.

On the basis of the above described experiments and cytological observations, the possible mechanism of the rise and development of tumor sublines were discussed.

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