Cytological Studies of Tumors, XVI. Cytological Differences of MTK-sarcoma II and Takeda Sarcoma, with Preliminary Experiments on Double Inoculation with the Two Tumors[1)]

By

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(With 24 Text-figures)

At the suggestion of Professor Makino that various tumors may affect a host in different ways and that two different tumors in the same host might influence each other, the present author has undertaken a study to inquire into the subject from the cytological standpoint.

The study has been aimed originally by the author with the cooperation of Dr. Makino, to ascertain the reciprocal action occurring between two different ascites tumors of rats after double inoculation. For a study of this nature the ascites tumors seem to be favorable as material, because two kinds of tumor cells might exist after peritoneal injection in close contact with one another in the same body cavity. Further the developmental conditions of the tumor cells after injection and the process of their division can easily be traced in a single tumor-bearing animal at any time desired. Ease in both transplantation and cytological sampling is also well suited for the purpose of this study.

The Takeda sarcoma and MTK-sarcoma II, both developing in rats, were chosen as material, since these two ascites tumors are clearly distinguishable from each other in many cytological characters as well as in the type of the disease. The present paper describes, mainly, the essential differences seen in the above named two tumors, in preparation for further experiments, with preliminary notes on the reciprocal influence of these tumors in the same host following double transplantation.

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Material and method

The MTK-sarcoma II was induced in the course of hepatoma-producing-experiments by application of azo dyes in a Wistar rat by Tanaka and Kanô (1951). According to them, the rat received the administration of o-aminoazotoluene for 312 days, and then was fed on p-dimethylaminoazobenzene for 103 days. Serial transfers of this tumor have been continued for 201 generations (July, 1954).

The Takeda sarcoma developed originally from the transplantation of a spontaneous solid tumor of unknown origin found on the breast of a white rat (Takeda et al. 1952). According to them, some crushed tissue pieces prepared from this solid tumor were inoculated in the pertioneal cavities of several white rats. Some of them so treated produced in their peritoneal cavities a considerable amount of ascites which was a suspension of tumor cells. Successive transmissions of this ascites tumor have been made from rat to rat.

Cytological observations were based on the smear preparations of the tumors, stained by different methods according to the respective purposes. The stains employed were acetic dahlia, acetic gentianviolet, Giemsa, janus green, neutral red, toluidine blue, Sudan-III and basic fuchsin after Feulgen.

General characteristics of MTK-sarcoma II and Takeda sarcoma under comparison

In the following, the differences in both cytological and general characteristics between MTK-sarcoma II and Takeda sarcoma by which the two are distinguishable from each other are described under comparison.

1. *Tumor cells :* Generally speaking, the tumor cells are larger in Takeda sarcoma than in MTK-sarcoma II. The nuclei are oval or kidney-shaped and situated on one side of the cytoplasm in MTK-sarcoma II, while Takeda sarcoma cells contain comparatively larger nuclei of oval shape, with irregularly scattered chromatin material. The number of nucleoli is one or two in MTK-sarcoma II, but commonly three, sometimes five to six, in Takeda sarcoma.

2. *Azur granules :* The tumor cells of MTK-sarcoma II contain distinct azurophilic granules in the cytoplasm being arranged in a rosette form, while those of Takeda sarcoma show no such granules as above appearing in a rosette arrangement. Commonly, the cytoplasmic granules are very few in occurrence in the latter tumor cells.

3. *Janus-green granules :* The cytoplasmic granules demonstrated by janus green are generally visible in tumor cells of both tumors. Occasionally Takeda sarcoma cells show condensed granules of unknown nature diffusely stained with the same dye.

4. *Neutral red granules :* MTK-sarcoma II cells are characterized by

cytoplasmic granules stained with neutral red, but Takeda sarcoma cells never show any granules of similar nature, except some generating cells which contain indefinite bodies deeply stained with neutral red.

5. *Lipid granules:* Generally, the cells of MTK-sarcoma II show no lipid granules as demonstrated by Sudan-III in their cytoplasm, but in the latter part of the transplant generation lipid granules make their occasional appearance. In Takeda sarcoma, however, the tumor cells generally offer lipid granules detected by the same stain at any time observed.

6. *Feulgen's nuclear reaction:* The chromatic substance generally reacts to Feulgen stain more intensely in Takeda sarcoma cells than in MTK-sacroma II cells.

7. *Metachromatic granules:* The cytoplasmic granules showing metachromasy are very remarkable in occurrence in MTK-sarcoma II cells, while they are rather invisible in Takeda sarcoma cells.

8. *Transplantability:* The MTK-sarcoma II is highly sensitive to Wistar rats, being more than 90 percent in transplantability. But the rats of the same strain are less susceptible to Takeda sarcoma.

9. *Life span of tumor-bearing rats:* The whole life span extending from the day of transplantation of the tumor to the death of the host is shorter in Takeda sarcoma than in MTK-sarcoma II. The average life days are 7.7 in the former tumor and 9.2 in the latter.

10. *Intraperitoneal cellular reaction:* The two tumors considerably differ in cellular reaction as seen in the peritoneal cavity of the host after transplantation. The picture may be well understood by referring to Figures 1 and 2.

11. *Mitotic rate in a transplant generation:* Daily observations of the mitotic rate were carried out through the whole life span of tumor-bearing rats in comparison between Takeda sarcoma and MTK-sarcoma II. Results indicate that Takeda sarcoma seems to be higher in mitotic frequency than MTK-sarcoma II. In the Takeda sarcoma the mitotic rate strikingly increases during the early part of a transplant generation and decreases suddenly towards the latter part. In MTK-sarcoma II the decrease of the mitotic rate in the latter part is rather gradual. The evidence is clearly shown in Figure 3 which indicates the comparative mitotic rate between the two tumors.

12. *Chromosome number:* The range in variation of the chromosome number is rather narrower in MTK-sarcoma II than in Takeda sarcoma. The number of chromosomes in the former fluctuates between 38 and 42, with the modal number of 40. In Takeda sarcoma the chromosome number shows a wide range from 37 to 163, with the modal values at from 70 to 90. The most frequent number is 84 which corresponds to the tetraploid number of rats. The numbers vary around 84 upward and downward in a quite gradual way. Details are shown in Figures 4 and 5. It is evident from the above results that MTK-sarcoma II

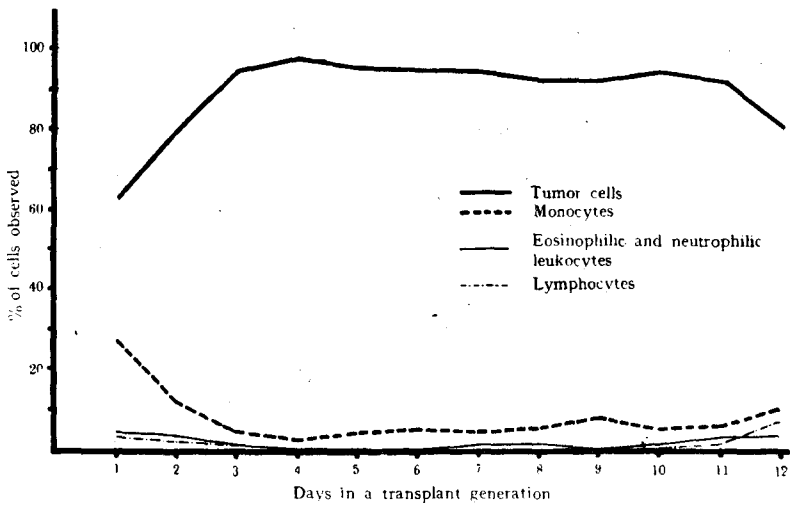


Fig. 1. Graphical representations illustrating the daily frequencies of tumor cells, monocytes, eosinophilic and neutrophilic leukocytes and lymphocytes in a transplant generation of MTK-sarcoma II.

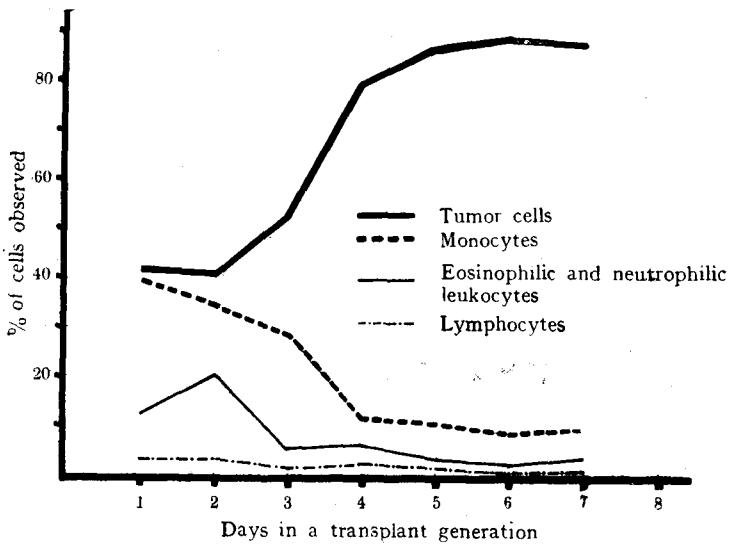


Fig. 2. Graphical representations illustrating the daily frequencies of tumor cells, monocytes, eosinophilic and neutrophilic leukocytes and lymphocytes in a transplant generation of Takeda sarcoma.

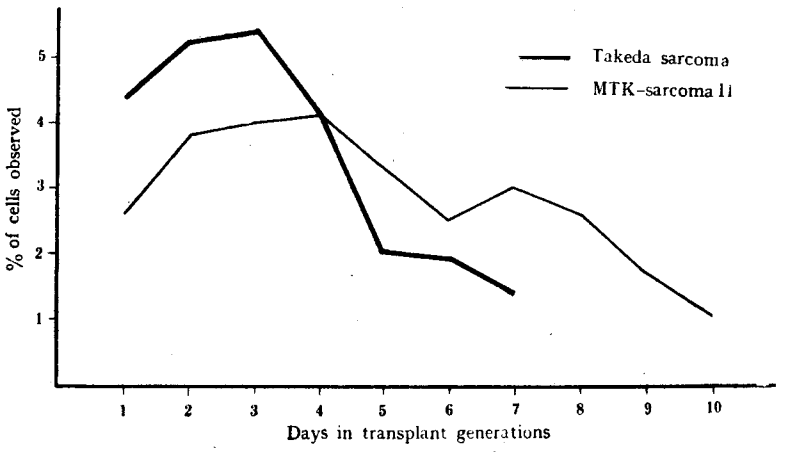


Fig. 3. Graphical representations of the daily frequency of mitotic cells in a transplant generation of Takeda sarcoma, in comparison with that of MTK-sarcoma II.

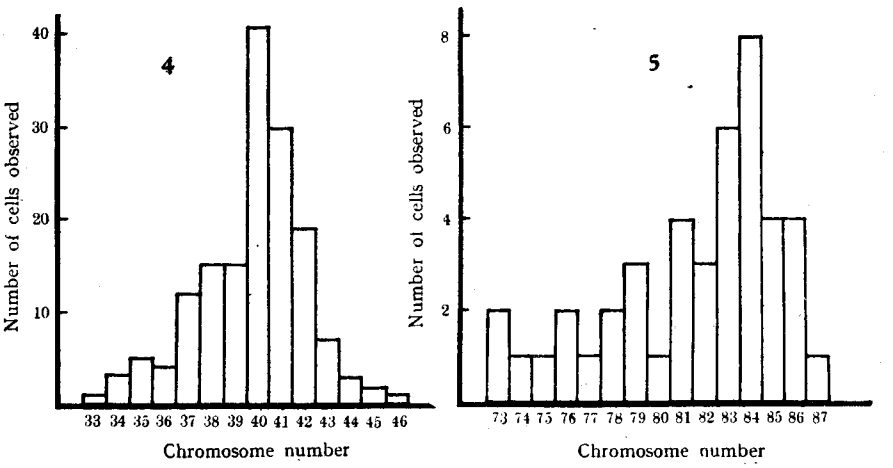
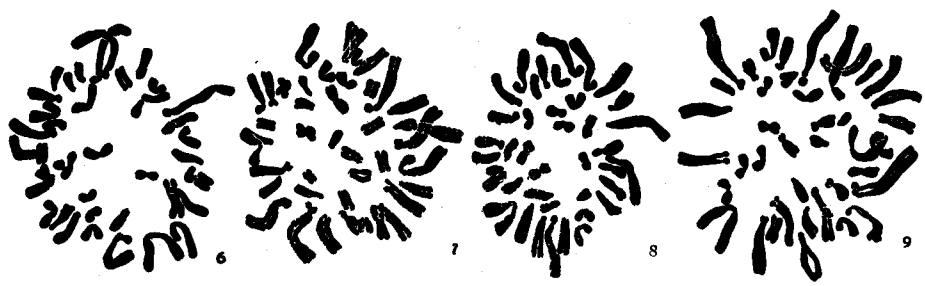


Fig. 4. Diagram showing the variation of chromosome numbers and their frequency distributions in MTK-sarcoma II. From the results of the daily observations throughout a transplant generation of a tumor rat.

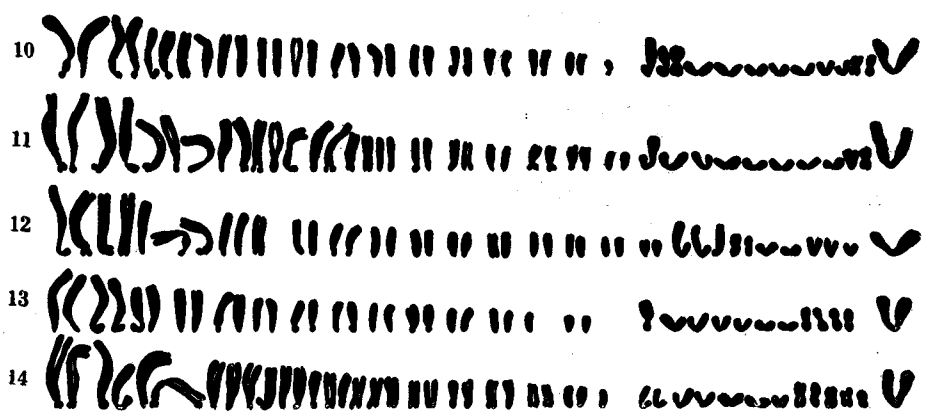
Fig. 5. Diagram showing the variation of chromosome numbers and their frequency distributions in Takeda sarcoma. From the results of the daliy observations throughout a transplant generation of a tumor rat.

is characterized by the subdiploid tumor cells, while Takeda sarcoma is a subtetraploid tumor provided with stemline-cells of a subtetraploid complex.

13. *Chromosome morphology :* Morphological analysis of the chromosomes of MTK-sarcoma II was made by Makino and Kanô (1953). According to them the chromosome complex of this tumor is provided with two distinct groups ; one consists of rod-shaped elements ranging from 28 to 30 in number and the other comprises V- and J-shaped elements of varying sizes, about 10 to 12 in number. In addition to these chromosomes there is a constant occurrence of a prominent V-shaped chromosome of outstandingly large size. The results of the present author's study are in a fair coincidence with those of Makino and Kanô (1953) as above. Examples are shown in Figures 6 to 14.



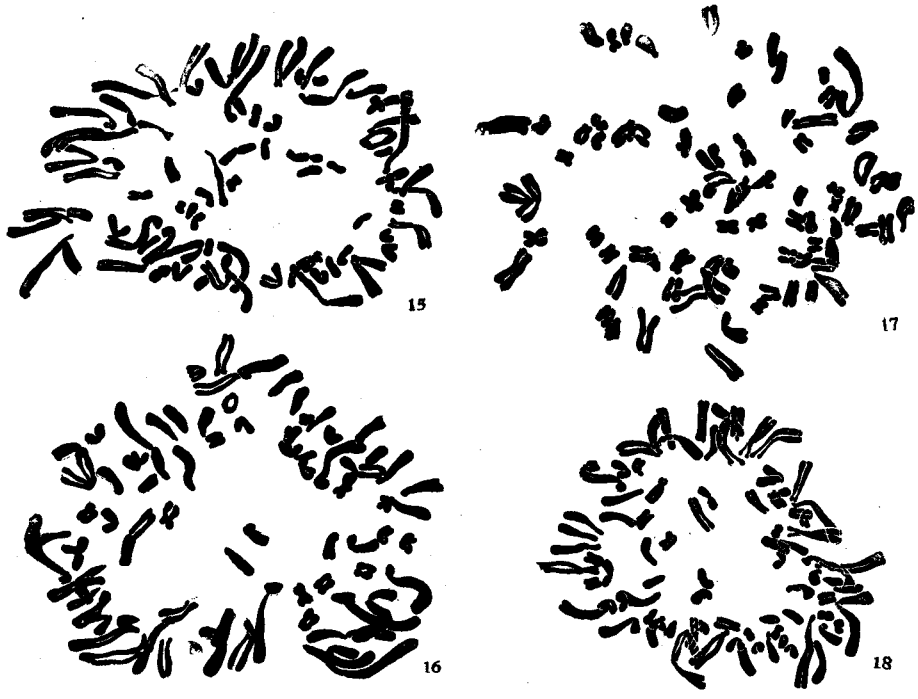
Figs. 6–9. Chromosomes of the tumor stem-cells of MTK-sarcoma II. Camera-lucida drawings, ca. × 1500. Fig. 6 ; 42 chromosomes. Fig. 7 ; 43 chromosomes. Fig. 8 ; 41 chromosomes. Fig. 9 ; 41 chromsomes.



Figs. 10–14. Serial alignments of chromosomes of tumor cells of MTK-sarcoma II. In each, 28 to 30 elements are rod-shaped and 11 to 13 are V- and J-shaped.

Chromosome study of Takeda sarcoma cells was made by Yosida (1954) and by the present author who reported that Takeda sarcoma is characterized by

subtetraploid stemline-cells consisting of certain numbers of rod-, V- and J-shaped chromosomes of varying sizes. Closer investigation by the present author reveals that the subtetraploid tumor cells of this sarcoma comprise from 57 to 60 rod-shaped chromosomes and from 23 to 25 V- and J-shaped ones (Figs. 15–23). Among the latter group of chromosomes the occurrence of a prominent J-shaped chromosome of outstandingly large size, with frequent occurrence of large V-shaped elements, one or sometimes two in number, is very remarkable. It is thus apparant that a striking difference exists between the chromosomes of Takeda sarcoma and those of MTK-sarcoma II.



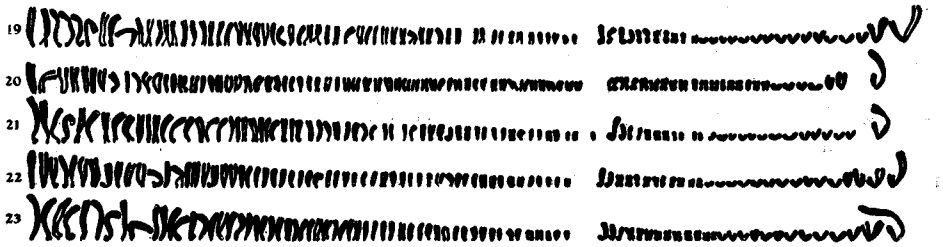
Figs. 15–18. Chromosomes of the tumor stem-cells of Takeda sarcoma. Camera-lucida drawings, ca. × 1500. Fig. 15 ; 84 chromosomes. Fig. 16 ; 83 chromosomes. Fig. 17 ; 82 chromosomes. Fig. 18 ; 84 chromosomes.

Results of simultaneous inoculation with two different tumors in the same host

The following experiments were undertaken to investigate the reciprocal influence of two different tumors in the same host, with MTK-sarcoma II and Takeda sarcoma as material. The sample of the MTK-sarcoma II was obtained

from tumor-bearing rats on the 4th day (or sometimes on the 5th day)[1] after transplantation, while in Takeda sarcoma the tumor was sampled from the host on the 3rd day[2] after transplantation. Both ascites tumors thus sampled were injected simultaneously, 0.05 cc in volume for each, in the peritoneal cavity of healthy rats. The results of experiments derived from 18 experimental animals are described as follows :

Following double inoculation of two tumors, many tumor cells introduced in the peritoneal cavity of the new host underwent degeneration. About 24 hours after inoculation, the tumor cells began their proliferation. There were many cells in process of division. The number of mitotic cells increased with the passage of time. The preparations sampled at this period showed the tumor cells of both kinds in process of division, with about 40 chromosomes for MTK-sarcoma II and about 80 chromosomes for Takeda sarcoma. It was assumed therefrom that the tumor cells of both tumors started their multiplication in the same host within one day after inoculation. In order to estimate the activity of the tumor cells, the number of metaphasic cells was calculated on the basis of the study of 200 cells per day through a whole transplant generation. The results are as shown in



Figs. 19–23. Serial alignments of chromosomes of tumor cells of Takeda sarcoma. In each, 58 to 60 elements are rod-shaped and 23 to 25 are V- and J-shaped.

Table 1. It is evident therefrom that on the first day after inoculation the frequency of Takeda sarcoma cells characterized by approximately 80 chromosomes is apparently higher than that of MTK-sarcoma II having about 40 chromosomes. On the second day, the relative percentage in occurrence of both types of tumor cells showed approximately similar value. On the third day after inoculation, the tumor cells with about 40 chromosomes were found showing a remarkable increase in number. Then, their increase continued further with the passage of time. In preparations sampled on the 5th day, there was no occurrence in the Takeda sarcoma cells, so far as the metaphase observations were concerned.

On the other hand, the mitotic rate was observed in tumor cells of both

1), 2) At these times the tumor cells showed the most active multiplication in both tumors.

kinds with daily material through the whole transplant generation. As shown in Figure 24, the mitotic rate showed as a whole a sudden decrease on the 3rd day after inoculation. At this time large numbers of the Takeda sarcoma cells in the samples were found to have been damaged. The ascites was considerably

Table 1. Daily frequencies of diploid, tetraploid and aneuploid tumor cells at metaphase occurring in the tumor after simultaneous double inoculation.

Days after trans-plantation	Subdiploid cells at metaphase		Subtetraploid cells at metaphase		Aneuploid cells at metaphase		Total number of metaphase cells observed
	Number obs.	%	Numbsr obs.	%	Number obs.	%	
1	83	39.3	122	57.4	6	2.8	211
2	121	54.5	94	42.3	7	3.1	222
3	148	65.2	74	32.6	5	2.2	227
4	174	77.7	48	21 4	2	0.9	224
5	204	97.1	2	1 0	4	1.9	210
6	185	100.0	0	0	0	0	185
7	207	100.0	0	0	0	0	207
8	198	100.0	0	0	0	0	198
9	184	97.4	0	0	5	2.6	189
10	103	92.8	0	0	8	2.2	111

reduced. It seems very probable that the reduction in the tumor ascites was due to the result of the inhibition in growth of the tumor through the reciprocal action between MTK-sarcoma II and Takeda sarcoma. In the preparations obtained by 7 days following inoculation, most of the debris from degenerating cells had disappeared from the ascites, but there were present a certain number of tumor

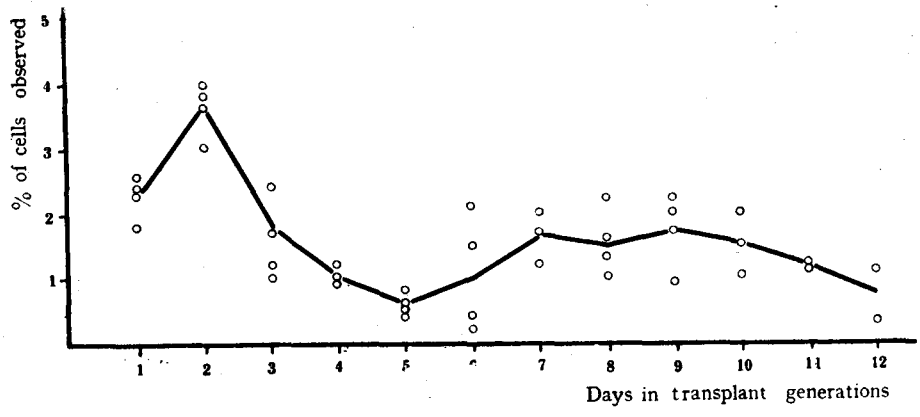


Fig. 24. Graphical representation of the daily mitotic rates in the simultaneous double inoculation with MTK-sarcoma II and Takeda sarcoma, observed through trnasplant generations. Data were based on four experimental animals.

cells of remarkably small size. They appeared in active division through the 7th to 8th day after inoculation. In division they showed a subdiploid chromosome complex, 40 or thereabouts in chromosome number. Based on such characteristic features of chromosomes as above there is no doubt but that these tumor cells are all derivatives from MTK-sarcoma II. The increase in the number of tumor cells caused an accumulation of tumor ascites followed by subsequent regrowth of the tumor. By 8 to 9 days after inoculation, regrowth of the tumor had been attained again in every experimental animal with remarkable accumulation of tumor ascites. The average of the life span of these experimental animals was found to be 11 days ; the maximum life span was 17 days.

Some experimental animals showed, at autopsy, the formation of solid tumors in certain peritoneal tissues. These solid tumors were crushed and injected into the peritoneal cavities of new rats. Every rat which received this injection developed MTK-sarcoma II.

Based on the above findings it can be concluded that the growth of Takeda sarcoma is inhibited by the co-existence of MTK-sarcoma II in the same host. Recently Graff et al. (1952) reported that Sarcoma 180 inhibited the growth of Carcinoma 755 at the simultaneous implantation in the same host, and suggested the possibility that Sarcoma 180 elaborates a substance which inhibits the growth of Carcinoma 755. Satoh (1952) observed that Yoshida sarcoma inhibited the growth of the ascites hepatoma in mixed transplantation. It seems to the author that, in the present case, the antagonistic influences of two types of tumor cells should be taken into consideration for the growth inhibition of tumors.

Detailed quantitative data on double inoculation together with technical procedure and discussion will be published elsewhere in the joint name with Dr. Makino in the near future.

Summary

The present paper described the cytological differences, as well as those of some other characters, existing between MTK-sarcoma II and Takeda sarcoma, with preliminary notes on the reciprocal influence of these two different tumors in the same host after simultaneous inoculation. The results indicated that the growth of Takeda sarcoma was inhibited by the co-existence of MTK-sarcoma II in the same host.

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