Cytological Studies of Tumors, XXXIII. A Study of the Chromosomes and Transplantability in a Mammary Tumor of CBA Mice

By

Yasumoto Kikuchi

(Zoological Institute, Hokkaido University)

(With 13 Text-figures)

The relationship between the transplantability and chromosomal conditions of a tumor is a subject which attracts attention in the field of cancer cytology. Recently, particular interest has increasingly been taken in immunogenetics in relation to tumor transplantation, and in the chromosomal pattern of the tumor in connection with homo- and hetero-transplantations (Hauschka and Levan 1953, Hauschka 1953, Kaziwara 1954, Sachs and Gallily 1955, 1956).

During the past several years, chromosome cytology of ascites tumor of rats and mice has established the reality of the stem-cell hypothesis. The genetic features of each tumor take the form of characteristic chromosome-number mode(s) and chromosome pattern(s) of tumor cells forming a stem-line (or -lines) which has maintained a certain definite constancy during serial transfers (Makino 1957a, b). Since the genetic mechanisms of the tumor cells are reflected in numerical and structural changes of the chromosomes, it seems very important to analyse the stem-line chromosomes of tumors in reference to their general characteristics such as transplantability, growth pattern and duration of survival.

The present study deals with the chromosome condition in relation to transplantability in CBA tumor-I which spontaneously developed in a CBA mouse, and in a subline of that tumor (CBA tumor-I-i).

The author wishes to express his sincere gratitude to Professor Sajiro Makino for his direction and improvement of the manuscript. Further the author is greatly indebted to Dr. Motomichi Sasaki for his technical direction with valuable advice and for placing some unpublished data at the auther's disposal. Also many thanks are due to Drs. Tatsuya Tanaka, Akira Tonomura, Akiko Sato, Takao Matsumoto and Akio Awa for their valuable advice and criticism. Thanks are extended to Dr. H. Kobayashi, Pathology Department, Medical School, for pathological examinations of the specimens used.

Tumors used and cytological methods

The CBA tumor-I here under consideration is one of the spontaneous mammary adenocarcinomas originally developed in a purely inbred mouse of CBA

Contribution No. 492 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Supported by a grant-in-aid for Fundamental Scientific Research from the Ministry of Education (No. 9208 in 1960).

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool., 14, 1960.

strain. Out of 40 female CBA mice, 13 animals aged 4 to 15 months developed spontaneous mammary tumors. Thus, the incidence of the mammary tumor in CBA mice was found to be 32.5 per cent. They are referred to as CBA tumor-I to -XIII in the following descriptions (Table 1). CBA tumor-I, -III and -VII

Table 1. Tumor developed in mice of CBA strain maintained in the Makino Laboratory

Tumors	Date of development	Months of age	Diagnosis
CBA tumor-I	April '58	14	Mammary adenocarcinoma
CBA tumor-II	May '58	5	(Undecided)
CBA tumor-III	Dec. '58	12	Mammary adenocarcinoma
CBA tumor-IV	Jan. '59	10	(Undecided)
CBA tnmor-V	April '59	14	Mammary carcinoma
CBA tumor-VI	May '59	11	Mammary carcinoma
CBA tumor-VII	June '59	15	Mammary adenocarcinoma
CBA tumor-VIII	July '59	13	Mammary carcinoma
CBA tumor-IX	Aug. '59	14	(Undecided)
CBA tumor-X	Oct. '59	11	〃
CBA tumor-XI	April '60	7	〃
CBA tumor-XII	July '60	7	〃
CBA tumor-XIII	Aug. '60	4	〃

were diagnosed as mammary adenocarcinomas (Fig. 8), while the CBA tumor-V, -VI and -VIII as mammary carcinomas (Fig. 9). No histological exmainations have been made on CBA tumor-II, -IV, -IX, -X, -XI, -XII and -XIII. From amongst the above 13, only CBA tumor-I was successfully transferred from mouse to mouse during about 2 years since its origin in April 1958 for over 35 generations. At the 6th transfer generation of CBA tumor-I, one subline-tumor was developed from the original line and transferred intraperitoneally in mice of the same strain for over 30 generations. This subline is designated as CBA tumor-I-i in the following descriptions. Mice which had received the intraperitoneal inoculation of tumor cells of this subline developed solid tumor nodules in such of their visceral tissues as mesentery, retroperitoneal tissues, omentum, liver and ovary. About 7 to 10 days after inoculation, however, a few tumor cells were found alive in the ascites fluid. In view of the above facts, CBA tumor-I-i may probably not be of a complete ascites type.

The transplantability of CBA tumor-I was examined with the following 13 different strains of mice : A/He, AKR, CBA, C3H/He, C57BL/6, DBA, dd, DM, EM-ag, EM-bl, MT, NH and Swiss-albino. For CBA tumor-I-i, use was made exclusively of CBA mice. More than 550 mice of both sexes, aged 2 to 5 months were used in the transplantation experiments. The tumor tissues were removed from tumor-bearing mice and minced into small pieces. About 0.2 cc of the tissue suspension thus made was inoculated either subcutaneously or intraperitoneally. Histological observations were based on tumor tissues fixed with Bouin's solution and stained with hematoxylin and eosin. The preparations for chromosomal study were made according to a water pretreatment squash method with acetic dahlia (Makino

1957b). The normal somatic chromosomes were also investigated in cells in tissue culture with the water pretreatment squash method.

Results of observations

1. Transplantability of CBA tumor-I and its subline -I-i

In Table 2 are summarized the results of transplantation experiments with CBA tumor-I on several different strains of mice. A total of 149 CBA mice of both sexes were employed in the experiment. Both CBA tumor-I and -I-i showed 100 per cent lethal transplantability to mice of CBA strain. The mice of EM-ag and C3H/He strains were highly susceptible to CBA tumor-I showing 93.3 and 85.7 per cent lethal takes, respectively. CBA tumor-I was not transplantable (0 per cent) to the following 5 strains of mice : DBA, DM, EM-bl, NH and Swiss-albino. Low susceptibility (3.2 to 5.3 per cent) was observed in animals from A/He, AKR, C57BL/6, dd and MT strains. In the latter most tumor cells showed

Table 2. Transplantability of CBA tumor-I in mice of various strains

Strains	Number of mice observed	Number of mice died of the tumor	Per cent of lethal takes
CBA*	106	106	100 %
EM-ag	15	14	93.3%
C3H/He	7	6	85.7%
MT	19	1	5.3%
dd	20	1	5.0%
A/He	29	1	3.4%
C57BL/6	91	3	3.3%
AKR	62	2	3.2%
DBA	7	0	0 %
DM	40	0	0 %
EM-bl	52	0	0 %
NH	8	0	0 %
Swissalbino	50	0	0 %

* In CBA mice, the transplantability of CBA tumor-I-i examined: 100 per cent lethal transplantability was obtained on the basis of 43 experimental animals.

Table 3. Survival days of CBA mice bearing CBA tumor-I and -I-i

Tumor lines	Number of mice dead of tumor					Total	Mean life span in days
	Days after transplantation						
	10-20	21-30	31-40	41-50	51<		
CBA tumor-I	4	16	12	4	1	37	29.5
CBA tumor-I-i	0	7	3	3	2	15	35.1

inactive growth. On the basis of the above data, the conclusion seems justifiable that the mice of CBA, C3H/He and EM-ag are highly susceptible to CBA tumor-I, whereas A/He, AKR, C57BL/6, DBA, dd, DM, EM-b1, MT, NH and Swiss-albino are resistant to that tumor in more or less degree.

The life span of CBA mice bearing CBA tumor-I was 29.5 days on the average, showing a range from 2 to 8 weeks. In the mice bearing CBA tumor-I-i, average survival days were 35.1 ranging from 2 to 10 weeks (Table 3). Generally, survival time of resistant mice, such as A/He, AKR, C57BL/6, dd and MT was comparatively longer than that of CBA. But C3H/He and EM-ag mice, which were highly susceptible to CBA tumor-I, showed a life span similar to that of the mother strain.

Table 4. Chromosome-number distribution in

Tumor	Transplant generation	Chromosome					
		38	39	40	41	42	43
CBA tumor-I	12-23	1	3	209	3	2	—
	(Jan.'59-Oct.'59)	0.4%	1.3%	90.1%	1.3%	0.9%	
	25-34	1	2	54	2	—	1
	(Nov.'59-Aug.'60)	1.4%	2.8%	73.5%	2.8%		1.4%
CBA tumor-I-i	11-29	1	4	277	3	4	2
	(Jan.'59-Aug.'60)	0.3%	1.3%	90.8%	1.0%	1.3%	0.7%

2. The chromosome conditions of CBA tumor-I and its subline -I-i

Chromosome number: The chromosome counting was made in clearly delineated reliable metaphase plates in the samples taken from both tumor lines at appropriate generations of serial transfers. The count was based on over 600 cells in total. As shown in Table 4, the chromosome number of CBA tumor-I varied within a rather wide range from 38 to 84, with a mode at 40 (90.1 per cent). A small but distinct peak of values was also found at 80 (3.0 per cent). Up to the 23rd serial transfer generation the cells having 40 chromosomes showed very high frequency of occurrence providing over 90 per cent. Then they showed a sudden decrease in number in later transfer generations showing 73.5 per cent.

In CBA tumor-I-i, the variation of chromosome number ranged from 38 to 83, with a modal number at 40 (90.8 per cent). In addition, there was another mode at 80 (2.7 per cent). In this tumor, a very high frequency was maintained in cells having 40 chromosomes up to late transfer generations (Table 4).

The evidence presented seems to indicate that the two tumors here considered are of diploid type, since they showed a stem-line number at 40 which persisted rather stably during serial transfers.

In order to learn the ploidy pattern in CBA tumor-I and -I-i, a rough counting of the chromosome number was made in several transfer generations, on the basis of 100 metaphase cells in each. The results are given in Table 5. The original tumor of CBA tumor-I showed diploid tumor cells at about 100 per cent. In CBA tumor-I, however, the diploid cells were 94.5 per cent on the average with a range from 93 to 97 per cent during the generations from 12 to 23, but they decreased to 76.3 per cent ranging from 72 to 78 per cent in later transfer generations. On the other hand, CBA tumor-I-i showed a relatively high modality of diploid cells which remained without change throughout serial transfers, giving an average value of 94.6 per cent (92 to 99 per cent).

CBA tumor-I and -I-i, based on exact counting

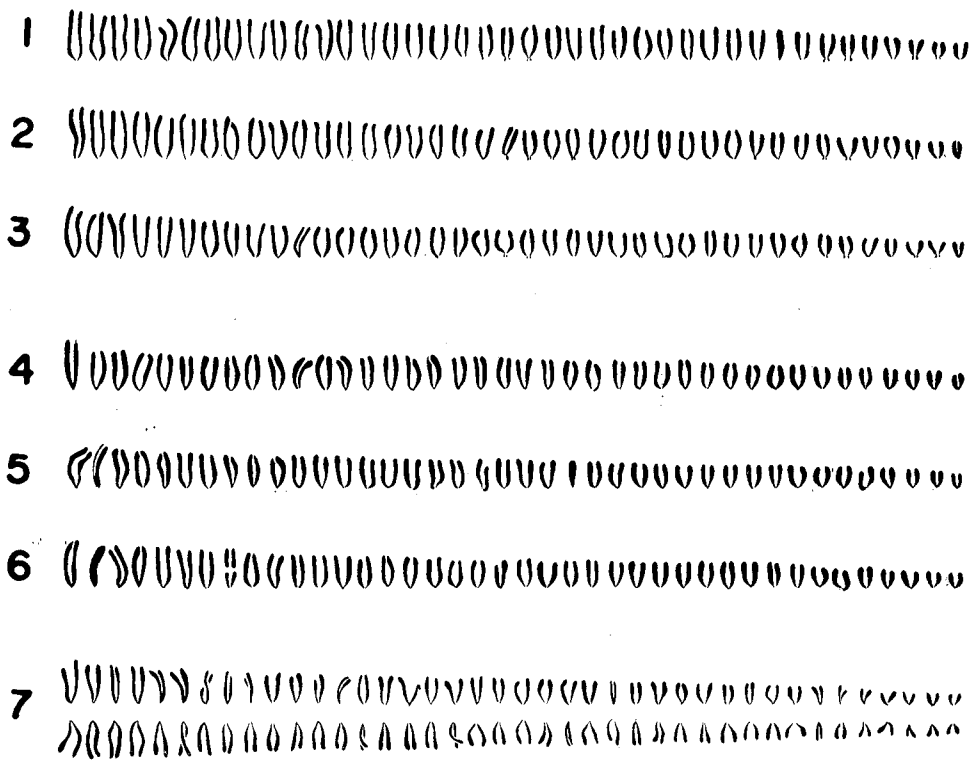
number									Total
77	78	79	80	81	82	83	84	100<	
—	2	1	7	—	2	1	1	—	232
	0.9%	0.4%	3.0%		0.9%	0.4%	0.4%		100%
1	—	1	9	1	—	—	—	1	72
1.4%		1.4%	12.5%	1.4%				1.4%	100%
—	1	1	8	2	1	1	—	—	305
	0.3%	0.3%	2.7%	0.7%	0.3%	0.3%			100%

Table 5. Frequency distribution of diploid cells in CBA tumor-I and -I-i during several transfers, based on rough chromosome counting

Tumor	Generation																						
	Primary tumor	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
CBA tumor-I	100	—	93	95	94	95	95	93	95	94	96	97	96	94	—	78	79	75	76	77	76	78	78	72	74
CBA tumor-I-i	—	98	99	96	98	96	94	95	96	95	94	95	94	94	92	94	91	93	92	92	—	—	—	—	—

Chromosome morphology: Stem-line idiograms in the two lines of CBA tumor were analyzed on the basis of five metaphasic cells having 40 chromosomes (Figs. 10, 11). Three out of the five idiograms here analyzed are illustrated in Figures 4 to 6. Each cells with 40 chromosomes is characterized by containing 40 acrocentric chromosomes. Thus, the stem-line chromosome of this tumor, are highly like those of normal somatic cells of mice both numerically and morphologically.

The chromosomes of normal tissue cells were analyzed in the tissue culture specimen from a new-born mouse (Fig. 12). Three representative idiograms are



Figs. 1–7. Idiogram analyses of CBA tumor-I, CBA tumor-I-i and normal mouse tissue. 1–3, normal somatic cells of a mouse, from lung culture (40 chromosomes). 4–5, CBA tumor-I-i (40 chromosomes). 6, CBA tumor-I (40 chromosomes). 7, a tetraploid cell from CBA tumor-I (80 chromosomes).

Table 6. Chromosome length (in μ) measured in five metaphase cells from the normal tissue and from CBA tumor-I

Material	Chromosome	Metaphase cell					Average
		1	2	3	4	5	
Somatic cells	Shortest	1.1	1.2	1.6	1.4	1.2	1.3
	Longest	3.9	2.9	4.2	4.2	3.4	3.7
	Average	2.5	2.1	2.9	2.8	3.4	2.5
CBA tumor-I	Shortest	1.6	1.3	1.2	1.4	1.3	1.4
	Longest	4.2	3.6	3.7	3.9	3.7	3.8
	Average	2.8	2.5	2.5	2.6	2.4	2.6

shown in Figures 1 to 3. Then, the length of the chromosomes was measured in cells of the normal mouse and CBA tumor-I by way of comparison. The length of the longest chromosome, and of the shortest one, together with the average length of the total diploid complement are presented in Table 6. The chromosomes of CBA tumor-I varied in length from 4.2 to 1.2 μ while those of the normal tissue showed a variation from 4.2 to 1.1μ. The quantitative data thus presented reveal a close similarity of chromosomes between CBA tumor-I and the normal tissue though the similarity may be superficial.

A tetraploid cell with 80 chromosomes was also analyzed and illustrated in Figure 7. It was shown that morphologically the tetraploid cell resulted from the duplication of a diploid one.

Aneuploid cells were observed in some samples. Some of those cells contained a few sub-metacentric chromosomes (Fig. 13). No such elements were found to occur in the stem-line cells with 40 chromosomes.

In conclusion, it may be said that there is no visible difference between the chromosomes of the normal tissue and CBA tumor-I, so far as number and morphology are concerned.

Discussion

Spontaneous mammary tumors occurring in mice of CBA strain were studied by Strong (1936, 1937, 1938a, b) and Bittner and Little (1937). According to them CBA strain is divided into two sub-lines, low- and high-mammary tumor sublines. The incidence of spontaneous mammary tumors in low-tumor strains vary from 2.8 to 4.8 per cent, while those of the high-tumor strains change from 13.5 to 22.2 per cent. The incidence of the mammary tumor observed in mice of CBA strain by the present study was found to be 32.5 per cent. It is then apparent that CBA strain used in the present study seems to be of a line of the high mammary tumor strain.

Both CBA tumor-I and -I-i which originated from a mouse of CBA strain, were highly transplantable to CBA mice from which the tumor originated. CBA tumor-I showed a low or negative transplantability to mice of other strains except C3H/He and EM-ag. It is of much interest to note that EM-ag mice showed higher susceptibility to CBA tumor-I. But, EM-bl of the same origin (Sato 1957) showed no susceptibility to that tumor. Some experiments are now in progress on the transplantability of CBA tumor-I with F_1, F_2 and back cross hybrid mice produced between the resistant and susceptible strains. Details will be published elsewhere in the near future.

It had been shown that transplantable diploid mouse tumors always contain a small population of polyploid cells. In a hyperdiploid Ehlrich ascites carcinoma, Kaziwara (1954) reported that polyploid sublines characterized by polyploid cells at 60 to 95 per cent were produced experimentally from the stock tumor, and that the chromosomes of polyploid cells represented an exact duplication of a diploid set. Hauschka (1953) reported that a

spontaneous shift from diploid to near-tetraploid occurred in an ascites tumor TA3 of mice during serial transplantation. Further, in several mouse tumors, Hauschka and Levan (1953) reported that the polyploid tumor lines occurred from diploid tumors, and that the diploid tumors were completely strain-specific while the tetraploid tumors were non-specific. As mentioned above, the CBA tumor here studied is apparently of diploid type and the strain specificity of this tumor is not rigidly fixed. Mention should be made here that the three mouse strains, CBA, C3H/He and EM-ag, which are highly susceptible to CBA tumor-I are of the same origin, while mice of EM-bl strain which is akin to EM-ag were wholly nonsusceptible to the same tumor.

It has been demonstrated that the chromosomes of the Yoshida rat sarcoma differ in shape and/or size from those of the normal tissue cells of rats (Tjio and Levan 1956, Makino and Sasaki 1958). Levan (1956) reported that three types of TA3 mouse ascites tumors were characterized by near-diploid chromosomes which were nearly identical with the normal mouse chromosomes, while two hypotetraploid tumors had chromosomes highly different from the normal chromosomes. Tjio and Östergren (1958) working with mammary tumors in milk virus strain of mice reported that the chromosomes of primary tumors are mostly akin to those of the normal mouse. Further, Hellström (1959) has observed that some of the mammary carcinomas induced by methylcholanthrene contained cells with 40 chromosomes as their dominating stem-line : he has stated that great structural or numerical chromosome changes are not neccessary for the development of all tumors.

A comparative idiogram analysis between CBA tumors (-I and -I-i) and the normal mouse tissue revealed that the stem-line chromosomes of these two tumor lines were fairly similar to the somatic chromosomes in general morphology, particularly in respect to number and length. It should be mentioned that the morphological similarity of the chromosomes does not always mean their structural similarity, because undetectable or internal structural changes might have occurred in the chromosomes of tumor cells in the course of their development in relation to their malignant changes.

Summary

The present study deals with chromosome conditions and transplantability in two lines (tumor-I and tumor-I-i) of CBA tumor of the mouse, spontaneous in origin.

Mice of the original CBA strain showed a very high susceptibility to two lines of CBA tumor giving 100 per cent lethal takes. The mice of C3H/He and EM-ag strains were highly susceptible to CBA tumor-I at about 90 per cent. In the mice of the following 10 strains, A/He, AKR, C57BL/6, DBA, dd, DM, EM-bl, MT, NH and Swiss-albino, the transplantation rate of the same tumor was very low showing 0 to 5.3 per cent takes.

CBA tumor-I and -I-i were found to be characterized by stem-cells provided with 40 acrocentric chromosomes, with a frequency distribution at about 90 per cent. There was observed a superficial similarity in the chromosomes between the two lines of tumors here considered and those of the normal mouse tissue.

References

Bittner, J.J. and C.C. Little 1937. The transmission of breast and lung cancer in mice. Jour. Hered. 28 : 117.

Hauschka, T.S. 1953. Cell population studies in mouse ascites tumor. Trans. New York Acad. Sci. Ser. II. 16 : 64–73.

———— and A. Levan 1953. Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. Exptl. Cell Reseach 4 : 457–467.

Hellström, K.E. 1959. Chromosomal studies on primary methylcholanthrene induced sarcomas in the mouse. J. Nat. Cancer Inst. 23 : 1019–1033.

Kaziwara, K. 1954. Derivation of stable polyploid sublines from hyperdiploid Ehlrich ascites carcinoma. Cancer Res. 14 : 795–801.

Levan, A. 1956. The significance of polyploid for the evolution of mouse tumor. Exptl. Cell Reseach 11 : 613–629.

Makino, S. 1957a. The concept of stem line-cells as progenitors of a neoplastic population. Proc. Intern. Genet. Symp. 1956 : 177–181.

———— 1957b. The chromosome cytology of the ascites tumors of rat, with special reference to the concept of the stemline cell. Intern. Rev. Cytol. 4 : 25–84.

———— and M. Sasaki 1958. Cytological studies of tumors, XXI. A comparative idiogram studies of the Yoshida sarcoma and its subline derivatives. J. Nat. Cancer Inst. 20 : 465–487.

Sachs, L. and R. Gallily 1955. The chromosomes and transplantability of tumors. I. Fundamental chromosome numbers and strain specificity in ascites tumors. J. Nat. Cancer Inst. 15 : 1267–1289.

———— 1956. The chromosomes and transplantability of tumors. II. Chromosome duplication and the loss of strain specificity in solid tumors. J. Nat. Cancer Inst. 16 : 803–841.

Sato, A. 1957. The separation of sublines of EM-mouse concerned with cort color. Misc. Rep. Yamashina Inst. Ornithol. Zool. 11 : 36–39.

Strong, L.C. 1936. Production of the CBA strain of inbred mice. Long life associated with low tumor incidence. Brit. Jour. Exp. Path. 17 : 60.

———— 1937. The incidence of spontaneous tumors on a "mixed oatmeal diet". Gann 31 : 13.

———— 1938a. The incidence of spontaneous tumors of mice of the CBA strain after a change of diet. Amer. J. Cancer 32 : 80–84.

———— 1938b. The incidence of spontaneous tumors in female mice (breeder) of the CBA strain. Amer. J. Cancer 32 : 85–89.

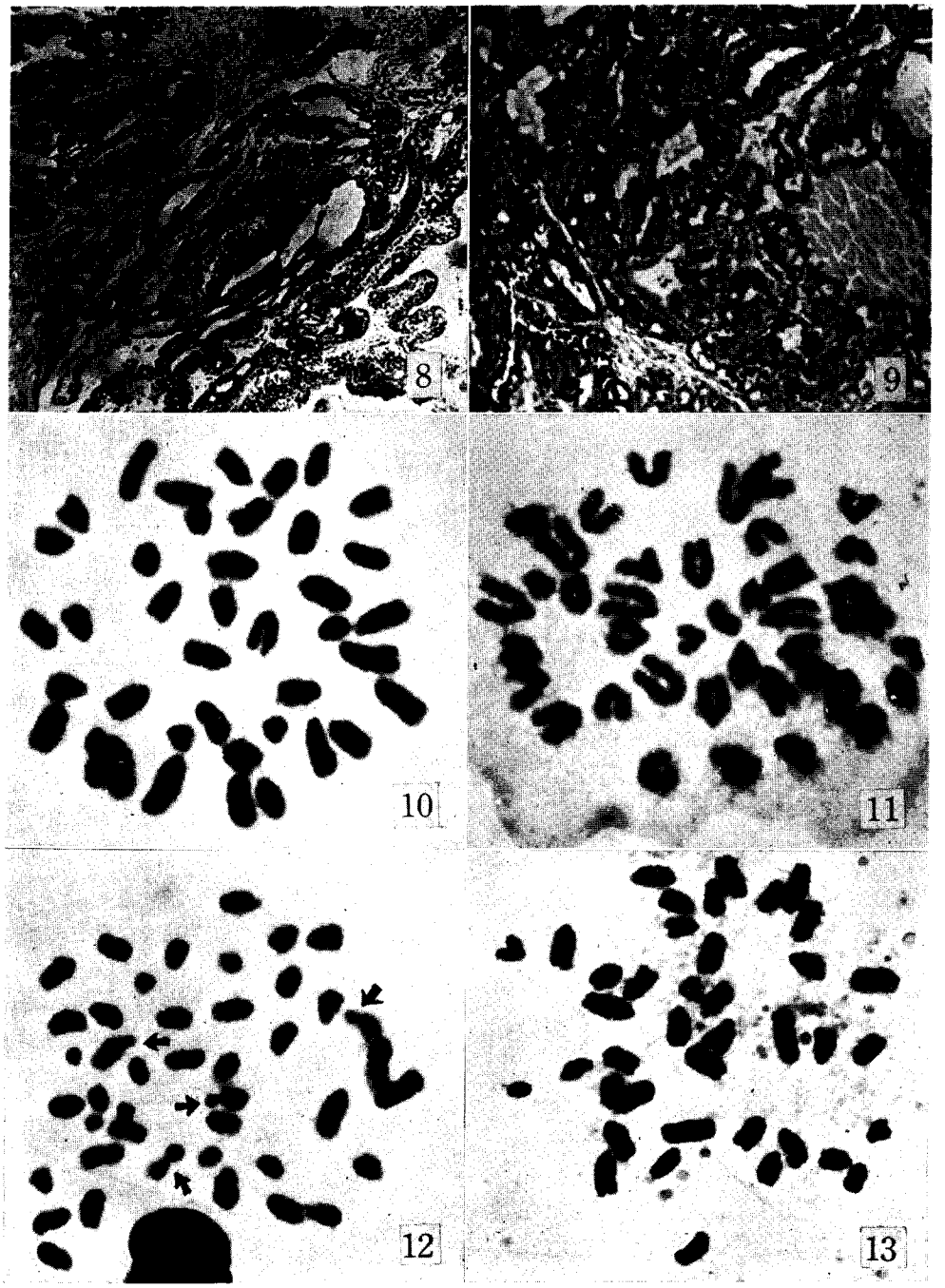
Tjio, J.H. and A. Levan 1956. Comparative idiogram analysis of the rat and the Yoshida rat sarcoma. Hereditas 42 : 218–234.

Tjio, J.H. and G. Östergren 1958. The chromosomes of primary mammary carcinomas in milk virus strain of the mouse. Hereditas 44 : 451–465.

Explanation of Plate X

Figs. 8–9. Photomicrographs of histological pictures of spontaneous mammary tumors found in CBA mice. 8, mammary adenocarcinoma from CBA tumor-I, × 150. 9, mammary carcinoma form CBA tumor-V, × 150.

Figs. 10–13. Photomicrographs of metaphase chromosomes of CBA tumor-I, CBA tumor-I-i and normal tissue, × 1800. 10, a stem-cell of CBA tumor-I showing 40 chromosomes. 11, a stem-cell of CBA tumor-I-i showing 40 chromosomes. 12, an aneuploid cell of CBA tumor-I-i showing 43 chromosomes. (Arrows indicate submetacentric chromosomes.) 13, a normal somatic cell of a mouse, showing 40 chromosomes.



Y. *Kikuchi*: *Chromosomes and Transplantability in a Mammary Mouse Tumor*