



HOKKAIDO UNIVERSITY

| | |
|------------------|---|
| Title | Cytological Effect of Chemicals on Tumors, XII. : A Chromosome Study in a Human Gastric Tumor following Radioactive Colloid Gold (Au198) Treatment (With 2 Tables, 8 Text-figures and 2 Plates) |
| Author(s) | SASAKI, Masao S. |
| Citation | 北海道大學理學部紀要, 14(4), 566-575 |
| Issue Date | 1961-12 |
| Doc URL | https://hdl.handle.net/2115/27336 |
| Type | departmental bulletin paper |
| File Information | 14(4)_P566-575.pdf |



**Cytological Effect of Chemicals on Tumors, XII. A
Chromosome Study in a Human Gastric Tumor
following Radioactive Colloid Gold (Au¹⁹⁸)
Treatment^{1),2)}**

By
Masao S. Sasaki

Zoological Institute, Hokkaido University
(With 2 Tables, 8 Text-figures and 2 Plates)

Recent investigations with the application of modern cytological techniques have revealed that in human tumors there occurs a stem-line (or -lines) of tumor cells, which have metabolic superiority and contribute principally to the neoplastic growth, quite comparable to those found in transplantable rat and mouse tumors (Hsu 1954, Levan 1956a, Hansen-Melander *et al.* 1956, Manna 1957, Wakabayashi and Ishihara 1958, Awano and Tuda 1959, Ishihara 1959, Makino *et al.* 1959, Tabata 1959, Tonomura 1959a, b, 1960). It is thus remarkable that the reported evidence in human tumors supports the stemline hypothesis established for animal tumors. Recently Ising and Levan (1957) attempted to discover any chromosome conditions particular to the pathological features in some human tumors. Makino *et al.* (1959) investigated the chromosome conditions in thirty human primary tumors with special consideration of the stem-line idiograms in relation to the types of the tumors. A good deal of interest has increasingly been aroused in the medical field with particular attention directed towards the genetic constitution and clinical and pathological properties of human tumors.

The present paper deals with a study of the chromosome condition in a human tumor which was characterized by two stem-lines of tumor cells, with special reference to certain functional responses of tumor cells to a radio-therapeutic treatment.

It is author's pleasant duty to express his sincere gratitude to Professor Sajiro Makino for his guidance and reading through of this manuscript. Further thanks are extended to Drs. Akira Tonomura and Motomichi Sasaki for their valuable advices, and to Dr. Soichiro Koiwai for supplying the material used in this study.

Material and method : The patient was a man aged 37 years, with the career of a coal-miner. The tumor was originally found in his stomach by physical examination at

1) Contribution No. 525 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) This work was supported by the sponsorship of the U.S. D.A. through its Far East Research Office (DA-92-557-FEC-34462), and by a grant to S. Makino from the Damon Runyon Memorial Fund for Cancer Research (DRG-563A).

the Sapporo Medical College. The tumor growth was active and produced a rich serous fluid in his peritoneal cavity. There were a large number of tumor cells in his peritoneal exudate. During a period from the 22nd October, 1959 to the 19th June, 1960, the patient was placed under radiotherapeutic treatment (Table 1). For the present study, five samplings were made before and after the administration of radioactive colloid gold, Au^{198} . The Au^{198} which emits radiation of low penetrating power has long been known to be effective to certain human tumors. The 1st sampling of the peritoneal fluid was made about thirty minutes before the intraperitoneal administration of $50\mu\text{c}$ of Au^{198} , and the 2nd, 3rd, 4th and 5th samplings were obtained 17, 23, 37 and 40 days after the administration. In each sample, tumor cells floating in the peritoneal fluid were collected by means of centrifugal selection at 1000 r.p.m. for 10 minutes. The slides for chromosome study were prepared according to the water pretreatment technique, in combination with the acetic dahlia squash method (Makino 1957).

Table 1. Data on radio-therapies and samplings.

| Date | Radio-therapies | Doses | Samplings |
|-----------------|-----------------|-----------------|--------------|
| Oct., 22, 1959 | Co^{60} | 6,000r | |
| Feb., 12, 1960 | X-ray | 2,600r | |
| March, 1, 1960 | Co^{60} | 3,300r | |
| April, 7, 1960 | X-ray | 3,200r | |
| April, 15, 1960 | Co^{60} | 3,000r | |
| April, 27, 1960 | | | 1st sampling |
| April, 27, 1960 | Au^{198} | $50\mu\text{c}$ | |
| May, 15, 1960 | | | 2nd sampling |
| May, 21, 1960 | | | 3rd sampling |
| June, 4, 1960 | | | 4th sampling |
| June, 17, 1960 | | | 5th sampling |
| June, 19, 1960 | died | | |

The present material provided a considerable number of tumor cells in mitotic stages available for adequate chromosome analyses. Chromosome counts were made in reliable metaphasic cells, with an immersion $100\times$ objective and $20\times$ ocular.

Normal somatic chromosomes of man were exclusively investigated in tissue culture with the hypotonic pretreatment squash method.

Results of observations

Number of chromosomes: The results of chromosome countings made in five samples are shown in Figure 1. In the 1st sample, most tumor cells counted in the ascites exudate were of uniform size, though there occurred a few giant cells. Chromosome numbers exhibited a major range of variation from 59 to 73, showing two modes, one at 63 (28.0 per cent) and the other at 65 (31.3 per cent). The 2nd sample was obtained 17 days after the administration of Au^{198} . It was found that the tumor cells were variable in size and that a great number of giant cells were present (43.8 per cent), together with damaged cells in large number (Fig. 18). Of five metaphasic plates which allowed exact countings of chromosomes,

three had 65 chromosomes and other two showed 64 and 130 chromosomes, respectively. The 3rd sample showed a sudden decrease of the giant cells as well as of the damaged cells, indicating a regrowth of the tumor with many mitotic cells. The chromosome number in this sample varied widely from 45 to 130. The cells having 65 chromosomes occurred most frequently showing 37.4 per cent, being domi-

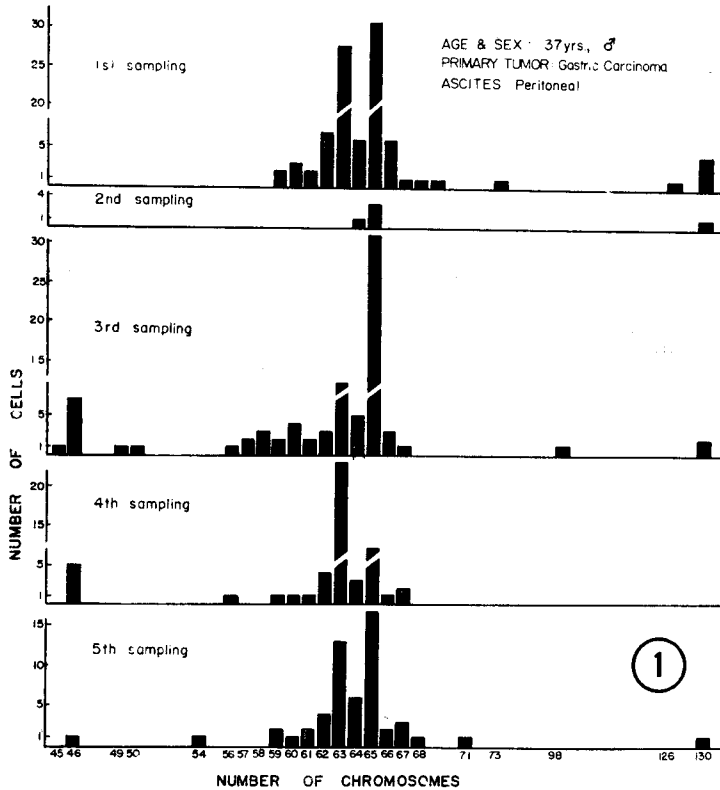


Fig 1. Distribution of chromosome number in five samplings from a human gastric tumor.

nant over the cells having 63 chromosomes at 14.4 per cent. Some cells showed the number of 46 (8.5 per cent). The ascites fluid as the 4th sample was hemorrhagic and showed a further decrease of the giant as well as of damage cells. In this sample, the cells with 63 chromosomes dominated over the 65-cells in occurrence, being 35.5 per cent for the former and 23.6 per cent for the latter, while the cells with 46 chromosomes showed 9.1 per cent in occurrence. The ascites fluid obtained by the 5th sampling was considerably hemorrhagic and contained many tumor cells of uniform size like the 1st sampling. Few damaged cells were observed in

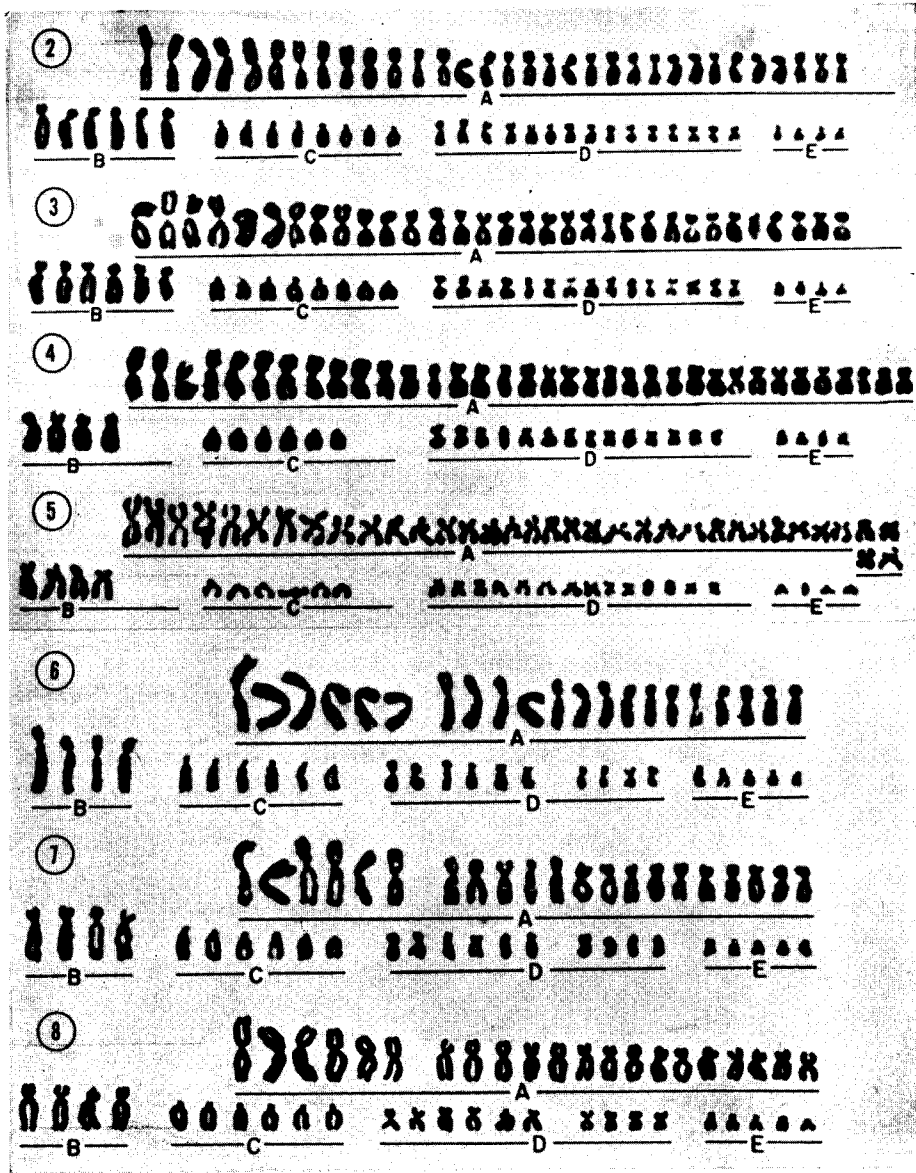
this sample. The cell population was again characterized in this sample by the coexistence of two stem-lines, one represented by 63 cells and the other by 65 cells at 23.2 per cent and 30.3 per cent respectively. A few metaphasic cells having 46 chromosomes were observed in this cell population.

Morphology of the stem-line chromosomes : To facilitate a critical comparison of the idiograms between normal and neoplastic cells, and among different tumor lines, the chromosomes were divided into five morphological groups as follows : group A comprising large and medium sized chromosomes with approximately median centromeres, group B including large chromosomes with subterminal centromeres, group C presenting medium sized acrocentric elements, group D containing relatively small chromosomes with approximately median and submedian centromeres, and group E consisting of very short acrocentric chromosomes (Figs. 2-8). The chromosomes in each group were distinguishable from those of another group by their characteristic size and/or morphology. In reference to the above classification, the number of chromosomes of normal human male complex are 21 for group A, 4 for group B, 6 for group C, 10 for group D and 5 for group E, as shown in Figure 8. The cells having 65 chromosomes showed that groups A, B, C, D, E comprised 32, 6, 8, 15 and 4 chromosomes, respectively (Figs. 2 and 3). The cells with 63 chromosomes, on the other hand, consisted of 35, 4, 6, 14 and 4 chromosomes for groups A, B, C, D and E, respectively (Figs. 4 and 5). It is thus evident that, in addition to the difference of chromosome number, the two stem-line cells differ in their chromosome constitution.

The cells having 46 chromosomes found in the present material were found to have a chromosome constitution similar to normal somatic cells of man, since there were observed 21, 4, 6, 10 and 5 chromosomes in group A, B, C, D and E, respectively, with a morphological similarity of each element, so far as the superficial feature of each element is concerned (Figs. 6 and 7).

Further it was shown that there was no difference of the chromosome constitution in the stem-line cells before and after the administration of Au¹⁹⁸. It is also noteworthy that a sign of endoreduplication was observed rather frequently in tumor cells of this tumor. The frequency of the metaphasic cells with endoreduplicated chromosomes were 9.3, 10.3, 0.59 and 0.89 per cent in the 1st, 3rd, 4th and 5th samples, respectively. Each chromosome count in thirteen endometaphasic plates has shown that eleven endometaphasic cells included 65 diplochromosomes, while the remaining two contained 130. The endometaphasic cells having 65 diplochromosomes showed the chromosome constitution quite similar to the 65-cells in the morphology of the chromosomes. Around the hexaploid range, in which the chromosome counts were made, cells having 130 chromosomes were most frequent. It is highly probable that they have originated from the 65-cells through the endoreduplication mechanism.

Chromosome abnormalities : In addition to the tumor cells with the normal chromosome configuration, there occurred many cells showing chromosome abnor-



Figs. 2-7. Idiogram analyses in the stem-line cells of the gastric tumor. 2-3, 65 chromosomes. 4-5, 63 chromosomes. 6-7, 46 chromosomes.
 Fig. 8. Idiogram analysis of a normal tissue cell cultured (male lung).

Table 2. Summary of data on the frequency of chromosome abnormalities around three ploidy ranges.

| Samplings | Diploid range | | Hypotriploid range | | | | Hexaploid range | | | |
|--------------|-----------------------|--------------------|--|----------------|-------------------|----------------|--------------------|----------------|-------------------|----------------|
| | Without chrom. abnor. | With chrom. abnor. | Without chrom. ab. | | With chrom. ab. | | Without chrom. ab. | | With chrom. ab. | |
| | | | Regular metaphase | Endo-metaphase | Regular metaphase | Endo-metaphase | Regular metaphase | Endo-metaphase | Regular metaphase | Endo-metaphase |
| 1st sampling | — | — | 204 | 21 | — | — | 11 | 1 | — | — |
| | Radioactive | | colloid gold, Au ¹⁹⁸ (50 μ c) | | | | | | | |
| 2nd sampling | 5 | 1 | 3 | — | 22 | — | 1 | — | 14 | — |
| 3rd sampling | 8 | 1 | 77 | 7 | 26 | — | 2 | 1 | 8 | — |
| 4th sampling | 6 | 1 | 78 | 3 | 2 | — | 3 | — | 1 | — |
| 5th sampling | 3 | — | 98 | 8 | 1 | — | 4 | 1 | — | — |

malities in the samples obtained from the peritoneal fluid following the administration of Au¹⁹⁸. Data are given in Table 2. Most metaphasic cells obtained from the 2nd sample showed various types of chromosome abnormalities in very high frequency: they are represented by stickiness of chromosomes, chromatid breaks, somatic translocations and bead-like elongation of chromosomes (Figs. 20–22). They are rather common in highly-ploid cells. In the 3rd sample, the cells with chromosome abnormalities decreased in frequency showing 25.8 per cent. In the 4th sample, chromosome abnormalities showed the frequency of 0.8 per cent. However, no chromosome abnormalities were observed in the endometaphasic cells in any one of the five samples under study. Cells showing very highly-ploid numbers were observed in the samples following the administration of Au¹⁹⁸. Some contained more than 600 chromosomes. The 5th sample exhibited no cells with chromosome abnormalities.

Discussion

Thanks to recent advance of cytological techniques for chromosome analyses in normal and malignant cells both *in vivo* and *in vitro*, accurate and critical knowledge on the human chromosomes has increasingly been accumulated facilitating analysis of the correlation between the genetic constitutions and their functional properties (Hauschka 1953, 1957, Hauschka and Levan 1953, 1958, Sachs and Gallily 1955, Levan 1956, Makino 1956, 1957, Hauschka and Amos 1957, Tonomura and Sasaki 1957, Makino and Sasaki 1958, Sasaki 1958, 1961, Matano and Makino 1961). It has been emphasized that under any certain prevailing condition cells with a certain genetic constitution are most efficient and represent the so-called "stem-line cells" which primarily contribute to the malignant growth. Cells with other genetic constitutions may grow in response to other environmental

conditions. Some experimental procedure could induce shifts of cell populations reflecting the altered environmental conditions (Ising 1955, Levan 1956b, Sasaki 1961, Matano and Makino 1961).

A human gastric tumor in the ascites form on which the present study was based was evidently characterized by two stem-lines. On the basis of the view that a certain genotype has its own environmental optimum, Hansen-Melander *et al.* (1956) and Ford and Mole (1959) emphasized that the tumor cell population with a bimodal or multimodal distribution was in the transitional site. In the gastric tumor here under consideration, however, the bimodal distribution was maintained for 40 days without shift of the stem-lines. Most probably, the two stem-lines of this tumor seem to be very stable, having become adapted to the physiological condition of the host.

It is most likely that the chromosomes of the tumor cells have undergone various structural changes involving the "cryptostructural changes" of Tjio and Levan (1956). The two cell-lines, 63- and 65-cell lines, in the gastric tumor presently under study showed a marked difference in their chromosome constitution beyond the numerical difference. The chromosome analysis revealed that the difference in chromosome constitution in the two cell-lines seems not to be simply accounted for by non-disjunction mechanism. It seems probable that the difference may refer to the difference in functional property of the two stem-lines. The 63- and 65-cell lines showed in the 1st sample different response to the Au¹⁹⁸ while in the 3rd sample the 65-cells were efficient and dominated over the 63-cells. In the 4th sample the 63-cells dominated in turn over the 65-cells. It is then evident that the radio-therapeutic treatment has pointed out a difference in property of the two stem-lines. Further, the difference in functional property between the two cell-lines seems to have connection with the different incidence of the endoreduplication, since most endometaphasic cells were found to contain 65 or 130 diplochromosomes. The above feature seems to supplement the data presented by Levan and Hauschka (1953) showing that c-mitosis and endoreduplication are connected with the functional property of the tumor.

The behaviour of cell population was studied by Koller (1956) in a human malignant effusion after administration of Au¹⁹⁸. He reported an increase of cells with the diploid chromosome number (stem-line number in his material). In the present study, a high frequency of cells with diploid chromosome number was observed to occur in the samples under the influence of Au¹⁹⁸.

On the other hand, the chromosomes of 46-cells showed no visible difference from those of normal male cells and further the incidence of 46-cells decreased under the effect of Au¹⁹⁸. It is highly probable that 46-cells found in the ascites fluid following the administration of Au¹⁹⁸ are normal tissue cells which have recovered their mitotic activity under the effect of Au¹⁹⁸. A similar feature has been observed by the present author in a human tumor following the administration of Mitomycin having a radiomimetic effect on tumor cells.

Summary

The chromosome conditions of a human gastric tumor were studied before and after the administration of radioactive colloid gold, Au¹⁹⁸, with special regard to the genetic constitution and phenotypic property of stem-line cells. This gastric tumor was characterized by two marked stem-lines, one represented by tumor cells with 63 chromosomes and other by those having 65 chromosomes. The chromosome constitutions of these two stem-line cells differ highly from one another. Also the two cell lines differ in response to different amounts of Au¹⁹⁸.

It was found that an alteration occurred in cell population following the Au¹⁹⁸ treatment.

References

- Awano, I. and F. Tuda 1959. The chromosomes of stomach cancer and myelogenous leukemias in comparison with normal complex. *Jap. J. Genet.*, **34** : 220-225.
- Ford, C.E. and R.H. Mole 1959. Chromosomes and carcinogenesis: Observations on radiation induced leukemias. *Progress in Nuclear Energy*, **2** : 11-21.
- Hansen-Melander, E., S. Kullander and Y. Melander 1956. Chromosome analysis of a human ovarian cystocarcinoma in the ascites form. *J. Nat. Cancer Inst.*, **16** : 1067-1081.
- Hauschka, T.S. 1953. Cell population studies on mouse ascites tumors. *Trans. New York Acad. Sci.*, **16** : 64-73.
- 1957. Tissue genetics of neoplastic cell populations. *Canad. Cancer Conf.*, **2** : 305-345.
- , and D.B. Amos 1957. Cytogenetic aspects of compatibility. *Ann. New York Acad. Sci.*, **69** : 561-579.
- , and A. Levan 1953. Inverse relationship between chromosome ploidy and host-specificity of sixteen transplantable tumors. *Exptl. Cell Research*, **4** : 457-467.
- and ——— 1958. Cytologic and functional characterization of single cell clones isolated from the Krebs-2 and Ehrlich ascites tumors. *J. Nat. Cancer Inst.*, **21** : 77-135.
- Hsu, T.C. 1954. Mammalian chromosomes *in vitro*. IV. Some human neoplasms. *J. Nat. Cancer Inst.*, **14** : 905-933.
- , and O. Klatt 1958. Mammalian chromosomes *in vitro*. IX. On genetic polymorphism in cell population. *J. Nat. Cancer Inst.*, **21** : 437-473.
- Ishihara, T. 1959. Cytological studies of tumors. XXXI. A chromosome study in a human gastric carcinoma. *Gann*, **50** : 403-408.
- Ising, U. 1955. Chromosome studies in Ehrlich mouse ascites cancer after heterologous transplantation through hamsters. *Brit. J. Cancer*, **9** : 592-599.
- , and A. Levan 1957. The chromosomes of two highly malignant human tumors. *Acta Pathol. Microbiol. Scandinavica*, **40** : 13-24.
- Koller, P.C. 1956. Cytological variability in human carcinomatosis. *Ann. New York Acad. Sci.*, **63** : 793-816.
- Law, L.W. 1958. Some aspects of drug resistance in neoplasms. *Ann. New York Acad. Sci.*, **71** : 976-992.
- Levan, A. 1956a. The significance of polyploidy for the evolution of mouse tumors. *Exptl.*

- Cell Research, **11** : 613-629.
- 1956b. Self-perpetuating ring chromosomes in two human tumors. *Hereditas*, **42** : 366-372.
- 1958. Relation of chromosome status to the origin and progression of tumors : The evidence of chromosome numbers. *Genetics and Cancer*, 151-182.
- , and T.S. Hauschka 1953. Endomitotic reduplication mechanisms in ascites tumors of mouse. *J. Nat. Cancer Inst.*, **14** : 1-43.
- Lejeune, J., R. Turpin and M. Gautier 1959. Le mongolisme, premier exemple d'aberration autosomique humaine. *Ann. Genetique*, **1** : 41-49.
- Makino, S. 1957. Chromosome cytology of the ascites tumors of rat, with special reference to the concept of the stemline cell. *Internat. Rev. Cytol.*, 6:25-84.
- , T. Ishihara and A. Tonomura 1959. Cytological studies of tumors, XXVII. The chromosomes of thirty human tumors. *Zeitschr. f. Krebsforsch.*, **63** : 184-208.
- , and M. Sasaki 1958. Cytological studies of tumors. XXI. A comparative ideogram study of the Yoshida sarcoma and its subline derivatives. *J. Nat. Cancer Inst.*, **20** : 465-487.
- Matano, Y. and S. Makino 1961. Cytological studies of tumors, XXXVI. Alteration of cell populations in the MTK-sarcoma II after some experimental procedures. *Texas Rep. Biol. Med.*, **19** : 613-624.
- Manna, G.K. 1957. A study on the chromosome number of human neoplastic uterine cervix tissue. *Proc. Zool. Soc., Calcutta*, 95-112.
- Sachs, L. and R. Gallily 1955. The chromosomes and transplantability of tumors. I. Fundamental chromosome numbers and strain specificity in ascites tumor. *J. Nat. Cancer Inst.*, **15** : 1267-1289.
- Sasaki, M. 1958. Cytological studies of tumors, XXIV. An Azo-dye induced ascites hepatoma of the rat, with special remarks on transitional changes of neoplastic characters in the course of serial transfers. *Gann*, **49** : 331-340.
- 1961. Observations on the modification in size and shape of chromosomes due to technical procedure. *Chromosoma*, **11** : 514-522.
- Tabata, T. 1959. A chromosome study in some malignant human tumors. *Cytologia*, **24** : 367-377.
- Tjio, J.H. and A. Levan 1956. Comparative idiogram analysis of the rat and the Yoshida rat sarcoma. *Hereditas*, **42** : 218-234.
- Tonomura, A. 1959a. A chromosome survey in six cases of human uterine cervix carcinoma. *Jap. Jour. Genet.*, **34** : 401-406.
- 1959b. Cytological studies of tumors, XXXII. Chromosome analyses in stomach and uterine carcinoma, *J. Fac. Sci. Hokkaido Univ., Ser. VI, Zool.*, **14** : 149-156.
- 1960. The cytological effect of chemicals on tumors. VIII. Observations on chromosomes in a gastric carcinoma treated with Carzinophilin. *Gann*, **51** : 47-53.
- , and M. Sasaki 1957. Cytological studies of tumors, XIX. A chromosome survey of the MTK-sarcoma II and III after several years of serial transfers. *J. Fac. Sci. Hokkaido Univ., Ser. VI, Zool.*, **13** : 332-337.
- Wakabayashi, M. and T. Ishihara 1958. Chromosome analysis of human mammary carcinoma. *Cytologia*, **23** : 341-348.
-

Explanation of Plate XIII

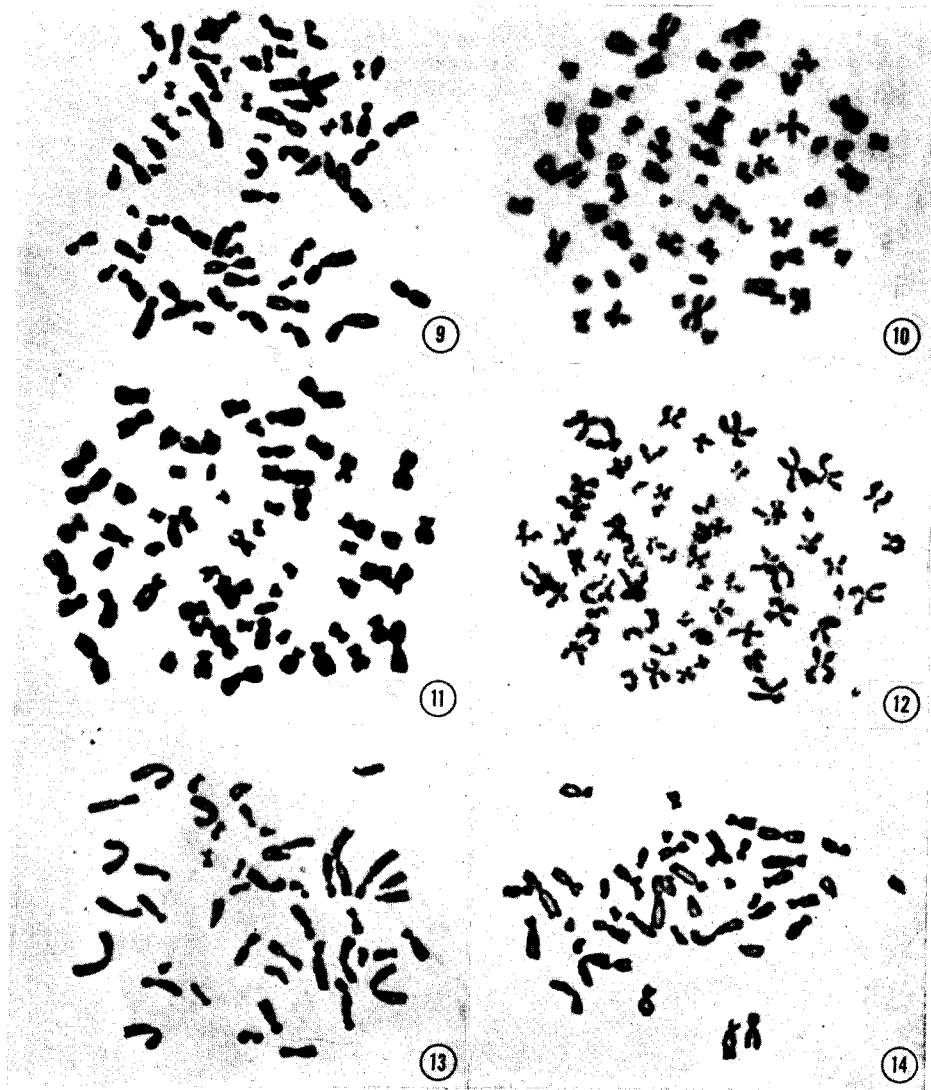
Figs. 9-14. Photomicrographs of chromosomes in the stemline cells of the gastric tumor. 9-10, 65 chromosomes. 11-12, 63 chromosomes. 13-14, 46 chromosomes.

Explanation of Plate XIV

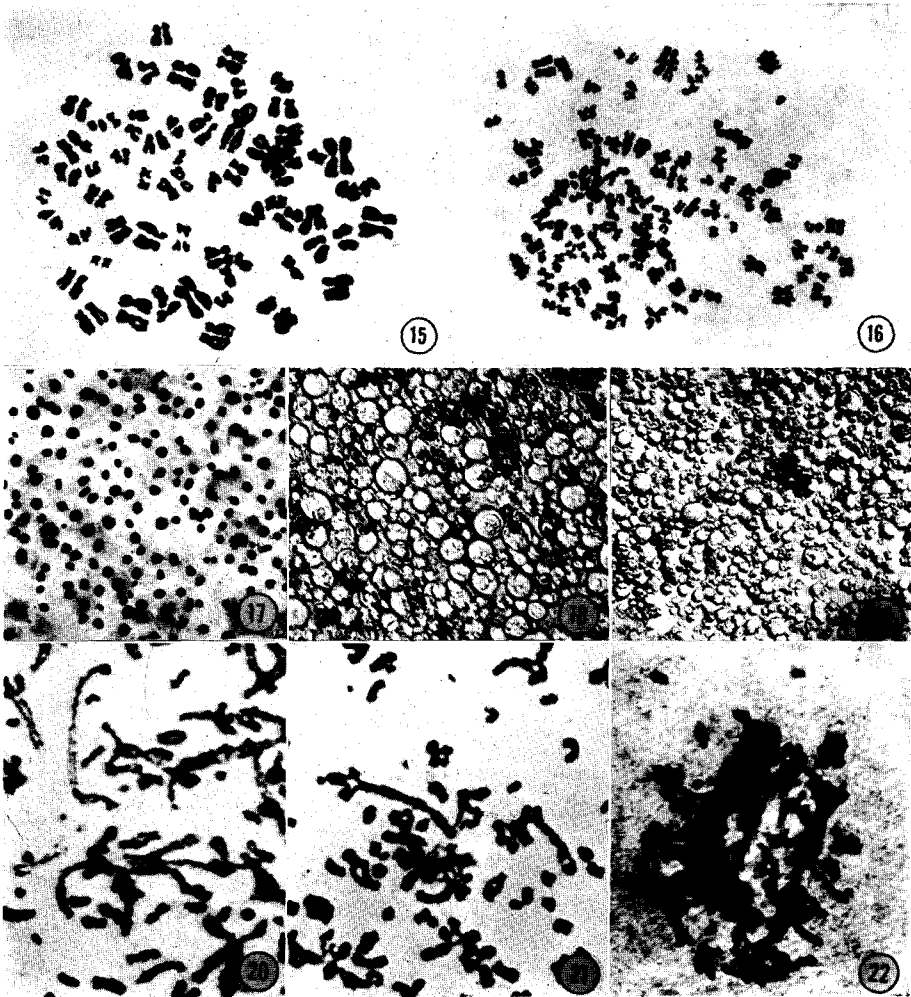
Figs. 15-16. Photomicrographs showing endoreduplication of chromosomes. 15, 65 diplochromosomes. 16, 130 diplochromosomes.

Figs. 17-19. Photomicrographs showing cell populations in the different three samplings from the gastric tumor. 17, 1st sampling. 18, 2nd sampling. 19, 3rd sampling.

Figs. 20-22. Photomicrographs showing chromosome abnormalities caused by Au¹⁹⁸ treatment. 20, fragmentation and bead-like elongation of chromosomes. 21, translocation of chromosomes. 22, stickiness of chromosomes.



M. S. Sasaki: Chromosomes of a Gastric Tumor following Au¹⁹⁸ Treatment



M. S. Sasaki: Chromosomes of a Gastric Tumor following Au¹⁹⁸ Treatment