Cytological Studies of Tumors, XI. Observations of the Multipolar Division in Tumor Cells of Ascites Tumor of Rats by Phase Microscopy[1)]

By

Hiroshi Nakahara

(Zoological Institute, Hokkaido University)

(With 2 Text-figures and 1 Plate)

Many confusing problems remain unanswered in cancer cytology. The analysis of the process of division in cancer cells is of primary importance because of their uncontrollable rapid growth. Previous to a causal analysis on the mechanism of the division a detailed quantitative study of living cells is needed. In one of the former papers (Makino and Nakahara 1953a), a report was presented on the process of the regular mitotic division in living tumor stem-cells[2)] derived from the ascites sarcoma of rats, as observed with the aid of a phase-contrast microscope, beginning with interphase and ending with telophase in the same cell. The multipolar mitosis of tumor cells occurs as a remarkable mitotic abnormality in a large proportion of cases in every kind of tumor. It has been studied by many investigators using both fixed and sectioned material. The present study has been made in an attempt to compare the mitotic events in living tumor cells between the abnormal multipolar division and the regular bipolar division. Since the ascites sarcomas allow microscopical tracing of the behavior and condition of tumor cells in every developmental stage of the tumor, the MTK-sarcoma I (Tanaka and Kanô 1951) provides excellent material for such a study as the present one.

The study was carried out using the new hanging-drop method in combination with the application of liquid paraffin. The technique was described in detail in a former paper (Makino and Nakahara 1953b).

The sample for study was obtained from the tumor-bearing rat 2 to 7 days after the transplantation of the tumor. The tumor ascites at the third day after transplantation proved to be most suitable for observations of the multipolar division because of the prevalence of mitotic figures. The preparations made

---

1) Contribution No. 296 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Aided by the Scientific Research Fund of the Ministry of Education.

2) cf. Makino 1952, and Makino and Kanô 1953.

according to the new hanging-drop method were studied exclusively with the Olympus phase contrast microscope which was set in a chamber equipped with an electric warmer.

Here, the author wishes to express his sincere gratitude to Professor Sajiro Makino for his kind guidance and the revision of the manuscript.

Observations

The successive mitotic stages, followed through the same cells, ranging from interphase to telophase, were recorded in serial photomicrographs taken at adequate intervals with the aid of a Leitz Mikas camera. In addition, a series of motion picutres (16 mm) was taken of the process as a whole, beginning with an interphase cell ending with resting daughter cells, in order to note all visible events, and to time their onset and duration.

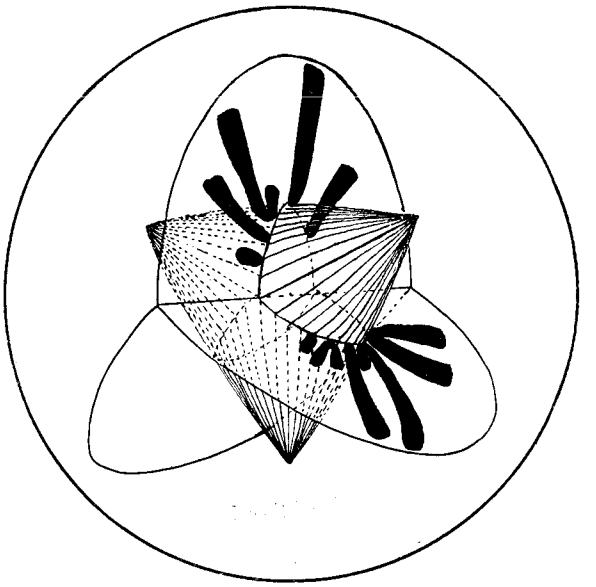
The descriptions of the mitotic events in each mitotic phase of the bipolar division have been presented in the former papers (Makino and Nakahara 1953a, b). In the prophase stage, the distinction of the multipolar cells from the regular bipolar cells was practically difficult. Multipolar division took place not only in mononuclear cells, but also multinuclear cells (Figs. 15–16, 17–20). Further, multipolarity was by no means always restricted to giant cells. Smaller cells showed tripolar spindle, and also there were giant cells of a bipolar nature. In the persent observations, there were cases in which tumor cells having a diameter of 13–16 μ showed multipolar division, while a cell about 17 μ in diameter was bipolar. Rarely, bipolar division was observed in cells of about 20 μ diameter. Generally speaking, it was a common phenomenon that the multipolar division was observed to occur in the cells of larger size, more or less than 20 μ in diameter, having a comparatively larger number of chromosomes. Further, the most usual type of multipolar division was tripolar. The same was stated by Makino and Yosida (1951), reporting that 73 per cent in the total of the multipolar mitoses were tripolar. Quadripolar division was less in number, and the polypolar cell having many poles was very rare in occurrence.

The four different series of the tripolar division are successively shown in Figures 1 to 20 (Plate VIII). Text-figure 2, a to h shows the successive stages of a quadripolar division in a giant tumor cell.

During prophase the nucleus swells up into a well-expanded body. Meanwhile, the chromosomes become partially visible as irregular shrunken bodies inside the nuclear membrane. The nucleoli fade from view. The chromosomes become thicker and shorter. Next the nuclear membrane becomes irregular and wrinkled; then it disappears. The free, now rapidly shortening, chromosomes seem to be entrapped in the spindle. The centrioles, asters and astral rays, were inconspicuous during these stages.

The metaphase plate showing the tripolar spindle with similar numbers of chromosomes in three plates, is formed in three dimensions, as schematically

illustrated in Text-figure 1. The three plates seem to associate with each other at an angle of about 120°. Probably, the poles of this tripolar metaphase lie on the three apexes of a triangle (Fig. 1). In such a cell, the distribution of the chromo-

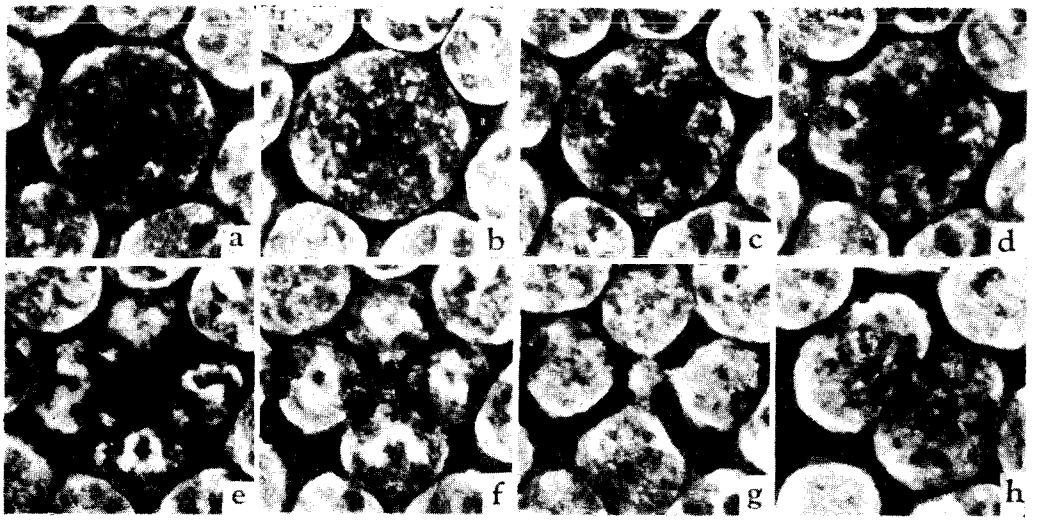


Text-fig. 1. A schematic illustration of the structure of a tripolar spindle.

somes at anaphase is nearly identical at the three poles. The tripolar cell with dissimilar numbers of chromosomes in the three plates usually shows after division unequal distribution of chromosomes at three poles (Fig. 10). In the quadripolar division, the cell usually contains four poles lying on the four apexes of a square, and the metaphase plates are placed in four dimensions (Text-fig. 2, a). Occasionally, the quadripolar cell shows four poles which lie on the apexes of a tetrahedron. In the quadripolar division, unequal migration of the chromosome to four poles takes place very commonly.

The separation of chromosomes at anaphase occurs rather synchronously in multipolar division, though not always (Figs. 2, 11). After the separation of the chromosomes has been completed, the cleavage furrows are formed corresponding to the number of poles (Figs. 5, 13). The formation of the cleavage furrow seems to be nearly identical in process to that which occurred in the bipolar division. After the cleavage furrow has been completely formed, the cleavage stalks as described by Scott (1936) in sea urchin eggs, appear connecting the cell bodies after separation (Text-fig. 2, g). The connection of the separated daughter

cells through the cleavage stalks remains for a considerable length of time. Rather frequently, the two separated daughter cells come together resulting in the formation of giant cells (Text-fig. 2, h). From this observation, the process of the formation of the multinucleated cell can be well understood. The fusion of two cells after separation was rarely observable in the bipolar mitosis, but its frequent occurrence was proved in the multipolar mitosis.



Text-fig. 2. a-h. Photomicrographs of tumor cells from the MTK-sarcoma I, with the phase-contrast microscope. Successive stages of quadripolar mitosis in a single cell, at temperature of 35°C. 1200×. a (taken at 25′ after preparation); late metaphase. b(65′)-c(66′); early anaphase. d(68′); anaphase. e(71′)-f(75′)-g(72′); telophase. h(96′); formation of two giant cells following the fusion of each two separated cells.

Following the completion of cell division, the nuclear membrane appears surrounding the chromosomes inside. By that time, the nucleolar bodies become visible in the nucleus. The process seems to follow a course similar to that taken place in the regular bipolar mitosis.

The duration of each mitotic phase was measured in the successive series of a division process followed through the same cell, beginning with prophase and ending with telophase. The duration of each phase was found to be nearly identical between the regular bipolar division and the multipolar division. The data are presented in Table 1.

Remarks

Multipolar divisions were found to occur sporadically in otherwise normal

tissues. They had already been observed rather frequently in cancer cells as the most remarkable feature by the end of the last century. They have since been repeatedly described by a number of authors. The present study, however, seems to be the first attempt to analyse the multipolar division in living tumor cells through the complete course of mitosis.

Table I. Time relations in the tripolar division of the tumor cell compared with those in the bipolar division.

	Tripolar cell (20 μ) at 35°C	Bipolar cell (20 μ) at 35°C
Prophase	29min.	13min.
Metaphase	45	75
Anaphase	5.5	4
Telophase	30	30
Total	109.5min.	122min.

It has been shown that multipolarity seems to be fairly unconnected with the chromosome number, since highly polyploid bipolar, as well as multipolar configurations which contain a low number of chromosomes, have been found.

Recently, the multipolar divisions together with other mitotic abnormalities have been investigated rather critically in human cancers by Therman and Timonen (1950), and in ascites tumors of rats by Makino and Yosida (1951), both using fixed material. They reported that the most usual type of multipolar division is tripolar. Makino and Yosida (1951), and Makino and Kanô (1951) have shown that for the change of the nuclear contents the multipolar divisions take a significant part as direct or indirect causes, giving rise to cells containing an extremely variable numbes of chromosomes. Obviously, these cells soon become inviable due to their deficient nuclei.

Therman and Timonen (1950) explained the origin of the multipolar division on the assumption that the extrachromosomal changes, *i.e.*, the spindle mechanism, are precocious as compared with the intrachromosomal changes. Though many experimental proofs are needed for this assumption, particularly from the study of living cells, the precocity theory expressed by them is of interest in considering the cytological change in cancer.

The structural configuration of the multipolar spindle was investigated by Wassermann (1929). It is interesting that the structure of the tripolar spindle presented by him shows a close approach in principle to that given by the present author in Text-figure 1.

Of the observed facts in the present study, striking is the fusion of two cells after separation. This phenomenon was found to occur rather frequently in both tripolar and quadripolar divisions in this material. After the separation of cell body took place, the divided two cells come together, resulting in the formation

of a giant cell. The fusion of two cells seems to be caused through the cleavage stalk connection bridging between two separated cells. From this evidence, the process of the formation of the multinucleate cell could be made clear.

It is generally conceivable that the formation of the multipolar spindle is closely related to the occurrence of the extra centrosomes. As shown in the former paper (Makino and Nakahara 1953a), the mitotic organelles, such as asters, astral rays and centrosomes, have not been demonstrated in the living status of the tumor cell. Thus, the existence of the extra centrosomes, together with their origin, has remained unknown until the present time.

Summary

The course of mitosis in the multipolar division was observed in living tumor cells of the MTK-sarcoma I by phase microscopy. Observations were carried out employing the new hanging-drop method devised recently by Makino and Nakahara (1953).

The mitotic events in both tripolar and quadripolar divisions were continuously observed from prophase to telophase in single cells, with particular attention to the change of the spindle body and the behavior of the chromosomes. The duration of each mitotic phase in the multipolar division was measured in a successive series of a division process, under comparison with that in the regular bipolar division.

The process of the formation of the giant cell due to the complete fusion of two cells after separation was traced through successive stages.

Literature

Makino, S. 1952. A cytological study of the Yoshida sarcoma, an ascites tumor of white rats. Chromosoma 4 : 649-674.

———— and Yosida, T. H. 1951. Cytological studies on cancer, I. Morphological and statistical observations on the abnormal mitosis in tumor cells of the Yoshida sarcoma through a transplant generation. Jour. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 10 : 225-242.

———— and Kanô, K. 1951. Cytological studies on cancer, II. Daily observations on the mitotic frequency and the variation of the chromosome number in tumor cells of the Yoshida sarcoma through a transplant generation. Ibid. 10 : 225-242.

———— and ———— 1953. Cytological studies of tumors, IX. Characteristic chromosome individuality in tumor strain-cells in ascites tumors of rats. Jour. Natl. Cancer Inst. 13 : 1213-1235.

———— and Nakahara, H. 1953a. Cytologische Untersuchungen an Tumoren, VIII. Beobachtungen über den Mitoseablauf in lebenden Tumorzellen der Ascitessarkome der Ratten. Zeit. Krebsforsch. 59 : 298-309.

———— and ———— 1953b. Cytological studies of tumors, IX. Further observations on the living tumor cells with an ew hanging-drop method. Cytologia 18 : 128-132.

Scott, A. 1946. The effect of low temperature and of hypotonicity on the morphology of the cleavage furrow in *Arbacia* eggs. Biol. Bull. 91 : 272–287.

Tanaka, T. and Kanô, K. 1951. Cytological studies on cancer, IV. General characteristics of the MTK-sarcomas, new ascites tumors of rats produced by the administration of azo-dye. Jour. Fac. Sci. Hokkaido Univ. Ser. VI. Zool. 10 : 289–301.

Therman, E. and Timonen, S. 1950. Multipolar spindles in human cancer cells. Hereditas 36 : 343–405.

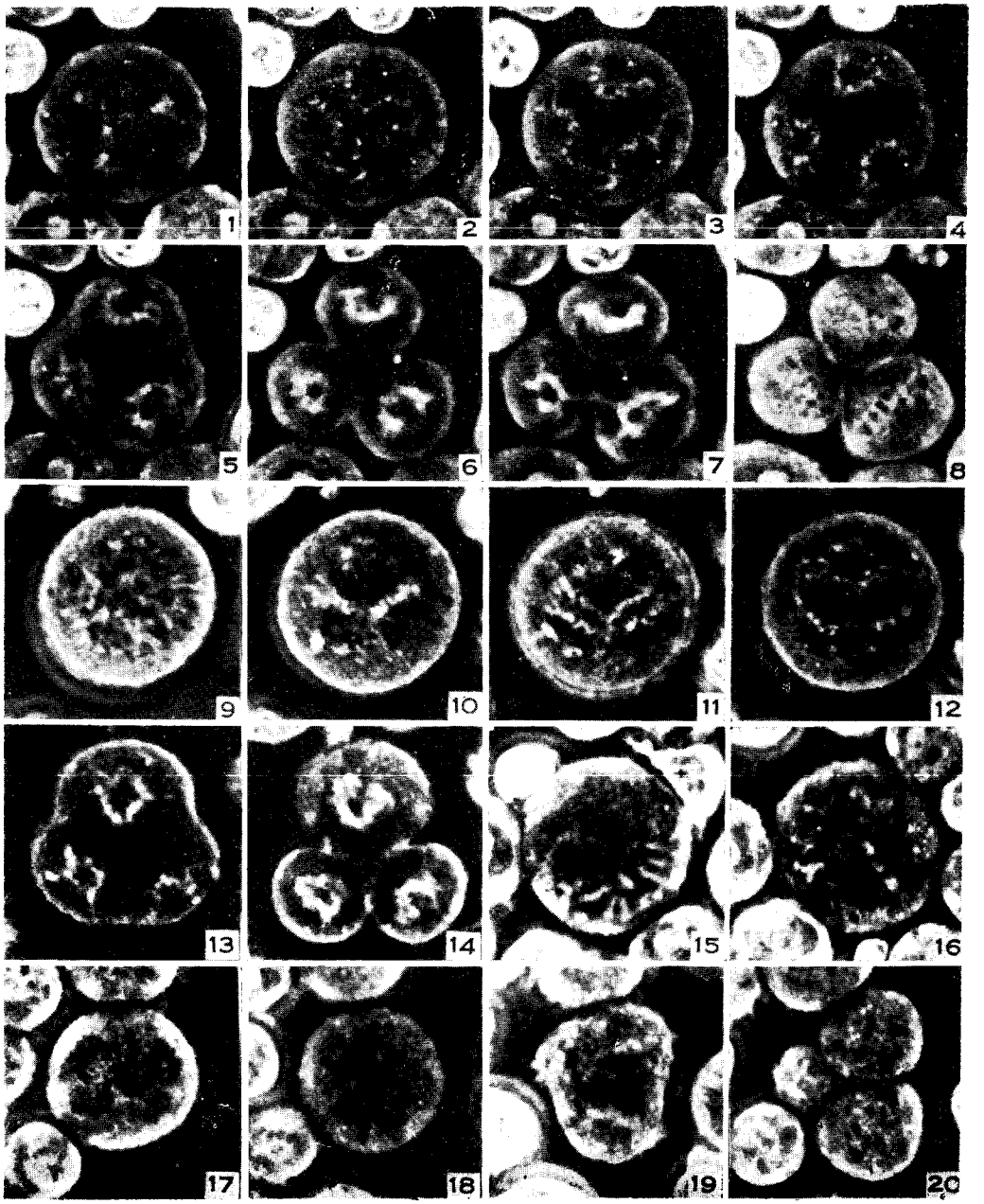
Wassermann, F. 1929. Wachstum und Vermehrung der Lebendigen Masse. Handbuch der Mikroskopischen Anatomie des Menschen. 1/2. Berlin.

Explanation of Plate VIII

Figs. 1–20. All are photomicrographs of tumor cells from the MTK-sarcoma I, taken with the phase-contrast microscope. 1200×. Figs. 1–8. Successive stages of a tripolar division followed in a single cell, at temperature of 38°C. 1 (taken at 7′ after preparation) ; metaphase. 2(10′)–3(10′30″)–4(11′) ; anaphase. 5(12′) 6(14′) ; formation of cleavage furrow dividing the cell into three bodies. 7(17′)–8(24′) ; successive stages showing the process of division of the cell body.

Figs 9–14. Successive stages of an irregular division at temperature of 35°C. 9(15′) ; early metaphase. 10(43′) ; Late metaphase. 11(45′)–12(46′) ; anaphase. 13(50′)–14(55′) ; formation of cleavage furrow dividing the cell into three unequal parts. Figs 15–16. Prophase and metaphase of a tripolar division, at temperature of 35°C. 15(14′) ; prophase containing a nucleus. 16(67′) ; metaphase.

Figs. 17–20. Successive stages of an irreuglar tripolar division from metaphase to telophase followed in a single cell, at temperature of 35°C. 17(20′) ; prophase containing two horse-shoe shaped nuclei. 18(45′) ; metaphase. 19(95′30″) ; anaphase. 20(110′) ; division of the cell body into three unequal parts.



H. Nakahara: Multipolar Division in Tumor Cells