Mitotic Cell Studies Based on *in vivo* Observations

1. Differences of mitotic figures between living cells and fixed cells

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From the recent publication "Analysis of Mitosis" (Wada 1966), cytologists may elicit some data which clarify the dynamic phases of mitotic processes and their continuity in living cells. However, most readers might have felt a tinge of disagreement against the presence of the spindle membrane and denial of continuous fibers (Fig. 1). This disagreement may be attributed mainly to the following preconceived ideas. 1) Since the middle of 1870's all the cytology text-books and their text-figures describe the disappearance of the nuclear membrane and the presence of spindle fibers connecting pole to pole (Figs. 4a, b). 2). Very few cytologists investigate thoroughly the behavior of spindles by *in vivo* observations. 3) Smear methods, squash methods or air dry methods habitually used by most of present cytologists are handle techniques only to preserve the chromosome morphology. These techniques break down the nuclear membrane and show generally no spindle figures.

Under these circumstances cytologists, at present, are liable to disregard the indispensability of the spindle body for cell proliferation. They believe the nuclear membrane breaks down before spindle formation. This is because of what they have learned in cytology text-books and in teaching this to students. The validity of the atractoplasm theory and differences between this theory and others described not only in classical but also in present day cytology books have discussed in detail each mitotic organelle and the mechanism of mitosis in the previous publication (Wada 1966).

This paper will describe the fundamentals of mitosis concisely for each stage of mitosis. We add a new text-figures of mitosis drawn on the basis of the atractoplasm theory (Fig. 2). From the standpoint of the atractoplasm theory, the existing misbelief will be discussed on the subject of mitotic figures and cell proliferation. Also, we should like to evoke reconsideration of the importance of spindle activity for research on cancer cell proliferation.

Comparison of mitotic figures obtained by fixed preparations and *in vivo* observations

Explanations are based mainly on plant cell mitosis. The new text-

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Fig. 1. Electron micrograph of metaphase spindle in meiosis of *Physarum polycephalum*. Preservation of spindle membrane is excellent. Part of dispersed spindle fibrils are visible. Difficulties for preservation of spindle membrane and spindle fibrils depend on kinds of organisms as well as on properties of fixatives used. Pre-treatment with glutaraldehyde and post-fixation with osmic acid. Embedded in styrene-butylmethacrylate. ch: chromosome, chf: chromosomal fiber, sf: swollen spindle fibrils, spm: spindle membrane. $\times 24,000$. (Courtesy of Miss N. Usui)

figures show schematically the fine structure of mitotic organelles drawn not only at microscopic but also submicroscopic levels (Fig. 2).

Mitotic process described in current cytology text-books (Data obtained by fixed preparations)

New blueprint for mitosis based on the attractoplasm theory (Data obtained by in vivo observations and by submicroscopic structures)

1. At the end of the prophase nuclear membrane begins to break down from both

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poles and later disappears entirely.

2. Short spindle fibers appear in cytoplasm at both spindle poles where nuclear membrane already broke down (polar cap stage).

3. Short fibers elongate and fill nuclear cavity after breakdown of nuclear membrane. They constitute spindle body and connect both poles (continuous fiber formation). Nucleoli disappear during this stage.

4. Biochemists assume that proteins in animal cells are polymerized at both centrioles and become fibers.

5. Metaphase plate is formed by bringing kinetochores of each chromosome on a equidistant plane from both poles. This is attained by contraction and expansion of chromosomal fibers.

6. Spindle body is physically a gel body.

7. Continuous fibers those among which happen to contact kinetochores become chromosomal fibers (Figs. 4a, b).

8. Chromosomes can reach poles in anaphase by contraction of chromosomal fibers (Figs. 4a, b).

its shape and organization.

2'. The nuclear membrane in prometaphase protrudes at both polar regions (polar cap formation). Nuclear membrane does not disappear. Fibril precursors accumulate at first in both polar caps and later in the whole nuclear cavity (Fig. 2c). Fibrillation (atractoplasm formation) proceeds from each pole toward the equator of nucleus. Nucleoli disintegrate in this stage.

3'. The protruded nucleus transforms into metaphase spindle. The spindle is filled with atractoplasm and enveloped with spindle membrane derived from nuclear membrane.

4'. Atractoplasm colloidal sol is composed of fibrils as dispersed phase. They are derived from globular dispersed particles of karyolymph colloidal sol. The fibrils are arranged anisotropically due to mitotic cell polarity.

5'. Fibrillation of fibril precursors proceeds from both spindle poles pushing all the chromosomes toward the equator (Fig. 2d). On the other hand, kinetochores of each chromosome show a strong tendency to be arranged in one plane (metaphase plate formation) (Fig. 2e).

6'. Atractoplasm filling spindle cavity is in a sol state the same as karyolymph. Essential differences between both colloidal sols are found in shape and arrangement of dispersed particles. The presence of spindle membrane is indispensable for maintaining the spindle body. It is morphologically independent from the cytoplasm and the centriole-aster system (Figs. 4a', b').

7'. Kinetochores substance secreted from each kinetochores diffuses among already arranged fibrils and reaches both spindle poles (Fig. 2e). In the proceeding of kinetochores substance, fibrils lying in the path of diffusion are bundled to a chromosomal fiber probably by polymerization (Fig. 3a).

8'. Shortening of chromosomal fibers in anaphase occurs by refolding of their fibrils inside of both spindle poles (Fig. 3b).

Figs. 2. Schematic view of plant mitosis drawn based on *in vivo* observations and atractoplasm theory. a, metabolic stage. b, middle prophase: chromonemata individually distinct and show longitudinal doubleness. Increase of nuclear volume during prophase is conspicuous. Text-figures in current cytology books do not indicate this fact. c, polar cap stage: nuclear cavity protrudes at both poles within which fibril precursors begin to accumulate. Most chromosomes lie in the inner periphery of nucleus. In this stage prophase nucleus transforms into karyokinetic spindle. Without analyzing this transformation, no spindle development can be made clear. Unfortunately, most current cytology books omit this stage. No comment is given for the reason. d, prometaphase: fibrillation

9. Spindle becomes phragmoplast in telophase by addition of cytoplasmic elements.

10. Cell plate is formed by accumulation of cytoplasmic granules (phragmosome) or it appears by fusion of swollen continuous fibers at their middle.

11. Daughter chromosome groups at spindle poles are enveloped by nuclear membrane and nucleoli reappear at this stage. The nuclear membrane is formed by arrangement of endoplasmic reticulum-like fragments dispersed around daughter chromosome groups.

Thus the fibers continuously shorten pulling kinetochores toward poles. Consequently, kinetochores of each chromosome are brought to the pole together with chromosome arms (Fig. 2f, Fig. 3b).

9'. In connection with the disintegration of chromosome matrix in reconstruction of the metabolic nucleus in telophase swelling of spindle fibrils is induced. The anaphase spindle becomes phragmoplast by this internal swelling and takes on a barrel-form in telophase (Fig. 2g, Fig. 3c).

10'. Cell plate is formed by accumulation of fibrils lying vertical to phragmoplast fibrils. New fibrils appear at first as two lamellae lying parallel to each other and develop later into a cell wall (Figs. 2g, h).

11'. Daughter nuclear membranes are reproduced from the spindle membrane (or phragmoplast membrane) in both polar regions. Reproduction proceeds enveloping daughter chromosome groups (Figs. 2g, h). Refolded fibrils within the nuclear membrane remain as dispersed particle of karyolymph colloidal sol (Fig. 3c). Nucleoli also reappear in daughter nuclei (Fig. 2i). Phragmoplast substance and its membrane begin to disappear from the periphery of daughter nuclei and young cell wall (Fig. 2h). At this stage mother cell nuclear membranes practically disappears from mitotic cell (Fig. 21).

In regard to the above comparison, some complementary explanation will be added in the next chapter to differences between the common ideas of classical cytology (4-5)¹ and the attractoplasm theory.

proceeds from both poles toward equator. In the drawing fibril precursors remain in the central region. Nucleoli disappear but nuclear membrane remain and transforms to spindle membrane. e, metaphase: all the chromosomal fibers reach both poles. f, late anaphase: details on the chromosome movements are drawn in Figs. 3a and b. g, mid-telophase: anaphase spindle continuously transforms to phragmoplast and young cell plate appears. Daughter nuclear membranes are reproduced from both poles enveloping chromosome groups. Details on these changes are drawn in Fig. 3c. h, late telophase: following reproduction of daughter nuclear membrane, phragmoplast substance and its membrane disappear. Nucleoli reappear. At the peripheral region of the equator, phragmoplast substance and cell plate still remain. i, interphase: nuclei are in a metabolic stage and two daughter cells are separated by completion of cell wall.

¹ Numerals in parentheses here and in the succeeding text indicate the pages of recent publication "Analysis of Mitosis" (Wada 1966). Refer to the pages for detailed explanations, experimental data, evidences or discussion.

Biological significance on the presence of spindle membrane and the denial of continuous fibers

Spindle membrane. As stated on the first page of this paper, the presence of spindle membrane and the fibrillar structure of the spindle ground substance (atractoplasm) seem to have met with strong doubts; these explanations imply a denial of the breakdown of nuclear membrane as well as the presence of continuous fibers. Also, this requires a revision of the

Figs. 3. Schematic drawing of submicroscopic structures of spindle based on *in vivo* observations, birefringence and electron microscope studies. a, end of prometaphase: fibrillation of all the fibril precursors finished. Showing anisotropic arrangement of fibrils and growing of chromosomal fibers from kinetochores toward spindle poles. b, mid-anaphase: showing shortening of chromosomal fibers by refolding of fibrils inside polar region and approaching of kinetochores to pole. Refolded fibrils and dissociated kinetochore substance accumulate inside spindle polar region. c, phragmoplast in mid-telophase: showing reconstruction of daughter nucleus, its karyolymph, nuclear membrane and dissociation of chromosome matrix. Corresponding to these changes, growing of phragmoplast takes place and initial cell plate appears as double lamellae composed of fibrils lying vertically to fibrillar texture of phragmoplast. ~ unfolded fibrils in dispersed phase of atractoplasm colloidal sol. — strained fibrils composing of chromosomal fibers. ▧ folded or refolded fibrils as dispersed phase of karyolymph. ∴ kinetochore substance (98-106) (Wada 1966).

description as orthodox explanations in current cytology text-books. In the previous publication (Wada 1966), we have reached the conclusion that the disappearance of nuclear membrane and the presence of continuous fibers are mainly attributed to inadequacies of fixatives.

Concerning the first problem we have succeeded in demonstrating the spindle membrane by improved fixatives (16-30). The organisms which we tested are most familiar and standard materials for cytological work: root

tip cells of *Vicia* and *Allium* (Wada, Nagao and Awasawa 1963), PCM's of *Tradescantia* (Wada and Kusunoki 1964a), sea urchin eggs (Wada, Hanaoka and Niitsu 1964). Ehrlich ascites tumor cells (Wada and Kusunoki 1964b) and spermatocytes of grasshopper (Wada and Fukunaga 1957, Nakao, Nakanishi and Wada 1968). If we would spend enough time for research and prepare adequate treatment and fixatives for organisms other than those mentioned respectively above, the presence of spindle membrane will be demonstrated in all of their mitoses. More delicately devised techniques will be required to demonstrate fine structure of the spindle membrane at the electron microscopic level. Excellent electron micrographs of spindle membrane have been presented in the mitosis of amebas by Roth (1964). Very recently Miss Usui has demonstrated the presence of spindle membrane in the meiosis of *Physarum polycephalum* (Fig. 1). The presence of spindle membrane in these organisms and the spindles within the nuclear membrane in Algae and Fungi are generally dealt with as exceptional cases in classical cytology. No interpretations have been undertaken to generalize the presence of spindle membrane in the mitosis of higher plants and animals. If the spindles of amebas, slime molds, Algae and Fungi would be exceptional, this exception means that the spindle membranes of these organisms are preserved with routine fixatives for chromosome studies. The fixatives are generally effective for preservation of chromosomes but destructive to spindle membranes.

In modern cell biology, it has been revealed that the interior of most cells is elaborately compartmentalized by intracellular membranes or many membranous structures. Most of them have been observed by electron microscope studies. They couple morphological frameworks with physiological functions at molecular levels. Among these membranes, the plasma membrane and the nuclear membrane are most conspicuous and were studied in classical cytology.

The *raison d'être* of the plasma membrane has been profoundly studied as physiological problems for a long time. However the visualization of its morphological features has just begun by electron microscope studies. On the contrary, the presence of the nuclear membrane was morphologically confirmed since the middle of 1870's. Inside the nuclear membrane, chromonemata develop into chromosomes in meristematic cells passing through G₁-, S-, G₂- and M-phases of DNA-cycle. This process is biochemically and genetically confirmed to occur in the nuclear cavity and independently from cytoplasm in higher organisms. The genetic material is maintained undefiled and transported as chromosomes to the daughter nuclei in M-phase.

On the basis of this viewpoint, the *raison d'être* for the nuclear membrane as well as the spindle membrane becomes very clear. In spite of this biological necessity for genetic material, current cytology text-books do not doubt the breakdown of nuclear membrane at the end of prophase in higher plants and animals. This is because no cytologists consider the breakdown

of nuclear membrane as an artifactual feature induced by inadequate fixatives. It also may be attributed to results of specialization in modern cell biology. Cytomorphologists take little interest in biochemical genetics and biochemical events and the biochemist is apt to disregard the biological significance of intracellular membranous structures because of technical difficulties.

With regard to the presence of spindle membrane, conditions are different, at present, from the days of foundation of cytology. The spindle membrane gets to be demonstrable in fixed preparations by improving treatment. It has been clarified that the breakdown or the maintenance of the spindle membrane are attributed to cytological techniques but not to the intrinsic nature of organisms as well as to resolving power of microscopes. No spindle membrane will be observed even under electron microscope, when the membrane is inadequately treated and fixed (10-30).

The demonstration of spindle membranes has just begun by improving fixatives. Consequently, functions and biochemical and biophysical properties of the spindle membrane remain for future research. At present, however, the *raison d'être* of the spindle membrane is assumed as follows. The spindle membrane, especially at both polar regions, play an important role during mitosis. Regulation of the shortening of chromosomal fibers, refolding of spindle fibrils, energy transport for chromosome movements and many chemotaxis-like functions are considered to be performed through the intervention of the spindle membrane (98-104).

Spindle fibrils. In classical cytology the continuous fibers are considered as one of the essential constituents of the spindle (Fig. 4a, b). Recently by using the isolated mitotic apparatus, biochemists have regarded continuous fibers equivalent to chromosomal fibers and astral rays. From the standpoint of morphology and *in vivo* observations, the origin of astral rays is cytoplasmic and that of the so-called continuous fibers is nuclear. The former fibers are fragile and in an unstable state, while the latter fibers are stable and consistently appear in the mitosis of both plants and animals (Wada, Hanaoka and Niitsu 1964). Matuszewski (1966) demonstrated the nuclear origin of the spindle in oogenesis of *Oligotrophus schmidtii* and described the independency of the spindle from the cytoplasm.

In electron microscope studies, the continuous fibers are reported: 1) as space appeared homogeneously by treatment with neutral osmic acid, 2) as fibers or tubules mixed with fine granules and vesicles by fixation of glutaraldehyde, and 3) anisotropically arranged fibrils by treatment with CdCl₂ solution and ethanol. No conclusive structure has been obtained, at present, by electron microscope studies on the spindle fibers (37-52). On the other hand, the birefringence of spindles demonstrated by Inoué (1953) in the mitosis of living cells has been taken as a decisive evidence for the presence of fibrous structures. Most cytologists have accepted the birefringent patterns as an optical image produced by the presence of continuous fibers.

However, an alternative interpretation is possible for the same birefringence.

We have reached a conclusion which denies the presence of continuous fibers (35-38). No strong birefringence is visible at both spindle poles where the continuous fibers, if really existing, would converge densely. The birefringence of the spindle ground substance implies the absence of continuous fibers which connect pole to pole. The homogeneous brightness of the spindle body is interpreted as follows (Figs. 5a-f). Atractoplasm is composed of innumerable uniformly dispersed fibrils. The fibrils arrange in parallel, showing positive birefringence to the longitudinal axis of the spindle. In other words, the birefringence is induced by the anisotropically arranged dispersed phase of the attractoplasm colloidal sol (Fig. 4a', b'). Consequently, the so-called continuous fibers are nothing but artifact features produced by the aggregation of dispersed fibrils of the attractoplasm, when the spindle is fixed and dehydrated (Figs. 4a, b). If the continuous fibers really exist, they would obstruct the chromosomes from their poleward movements. Cytologists who believe in the presence of continuous fibers have stated nothing about the *raison d'être* of the continuous fibers.

Fig. 4. Comparative diagrams of mitosis based on classical cytology (a-b) and on the attractoplasm theory (a'-b'). a and a' showing anaphase spindles in animal cell and b and b' those in plant cell. Spindle fibers in (a) end surrounding centrospheres and those in (b) end near poles. Both diagrams (a and b) are familiar in cytology books but meaningless to learn physicochemical basis for chromosome movement and spindle activity. Anaphase spindle (a' and b') represent presence of spindle membrane, anisotropic arrangement of fibrils and independency of spindle from cytoplasm. In animal mitosis (a') it is most important to show the fact that centriole-aster system lies outside of spindle-membrane having no participation in spindle fiber formation (cf. Wada 1966 Fig. 10, Nakao *et al.* 1968 Figs. 14 and 15).

Another interpretation that some of the continuous fibers which happen to contact the kinetochore become chromosomal fibers, is too ideological and has no experimental corroboration. On the contrary, the hydrostatic pressure experiments by Pease (1946) are very important. According to his data,

Figs. 5. Birefringence of karyokinetic spindle, phragmoplast and cell plate in the same endosperm cell in *Zephyrantes candida*. Mitosis occurs in cells cultured in endosperm fluid. Cells in upper line (a-c) in early anaphase and those in lower line (d-f) in mid-telophase. Cells in first column photographed by ordinary light microscope, those in second and third by polarization microscope. Positive birefringence of spindle to its axis (b and e) changes to a negative one (c and f) by rotation of 45° to the axis. Uniform brightness of spindle ground substance implies presence of evenly dispersed anisotropically arranged fibrils and absence of continuous fibers converging to spindle poles. Negative (e) and positive (f) birefringence of cell plate indicate that constituent fibrils of cell plate lie vertically to the dispersed fibrils of phragmoplast ground substance (33-37). (Courtesy of Dr. Niitsu and Hanaoka)

chromosomal fibers start from the kinetochores of each chromosome and grow toward both spindle poles. In the growing of fibers, the arranged fibrils play a role of a pathway. Along the path, kinetochore substance can diffuse toward both spindle poles by capillary phenomenon. By flowing out of the

kinetochore substance the fibrils on the path are bundled to a chromosomal fiber probably polymerized in a reversible state. By this process each chromosomal fiber can constantly connect the kinetochore with the spindle pole (43-49). The *raison d'être* of the anisotropically dispersed fibrils in the spindle becomes very clear according to the above explanation. Any other reasons than this cannot be imagined for the birefringence of the spindle in living cells (Fig. 3a).

Recently biochemists analyzed the isolated mitotic apparatus of sea urchin eggs. They discussed the constituents of the apparatus and explained the polymerization process of the fibers. I have strong doubts about the biological meaning of such biochemical treatment and results. In order to isolate the so-called mitotic apparatus, sea urchin eggs are hardened by oxidizing agents. As a result of such treatment, innumerable spindle fibrils aggregate and form continuous fibers of the mitotic apparatus. Therefore, the biochemical explanation of the spindle fiber formation in the isolated mitotic apparatus implies that artificially induced spindle fibers are analyzed biochemically. The fibrils of the spindle in living cells will be found biochemically as well as biophysically in another state different from the fibers explained in current biochemical cytology books.

The biochemical analysis of mitotic apparatus should be undertaken by separating the karyokinetic spindle from the centriole-aster system because the former belongs to nuclear substances and the latter to cytoplasmic ones. If such separation be impossible by present biochemical techniques, the publication of the results must be conscientiously prudent. The centriole-aster system has no participation in the formation of karyokinetic spindle fibrils (Nakao, Nakanishi and Wada 1968). Dietz (1959) already pointed out that the bipolar spindle in *Pales crocata* is formed without the participation of centrioles. The spindle fibril formation in animal cells is intrinsically identical with that in plant cell mitoses (Nakao *et al.* 1968). The latter have no centriole-aster system (98-106).

In the interior of cells, various kinds of metabolic, physiological or morphological changes can take place simultaneously for the cell is composed of many differentiated compartments as mentioned above. There is no objection to consider the karyokinetic spindle as one of intracellular compartments. Chromosome movements in the spindle proceed being separated from the cytoplasm. It is very difficult to obtain biologically significant results of spindle constituents only by biochemical procedures. Also any behavior of mitotic organelles cannot be explained by morphological investigations alone. It is very important that the results of both kinds of research are always carefully and unifiedly interpreted in comparison with the dynamic state of living cells.

Some remarks on cancer cell proliferation

Some remarks on cancer cell proliferation supplemental to the previous publication (Wada 1966) will be added in this chapter from the pure cell biological point of view. As mentioned in foregoing chapters, the independency of the spindle is disregarded in current cytology books. Most animal cytologists misunderstand the origin, development, and fibrous structures of the karyokinetic spindle together with those of the centriole-aster system. The

Fig. 6. On the basis of *in vivo* observations and the attractoplasm theory, relationships among mitotic organelles are indicated diagrammatically from prophase to end of mitosis. Mitotic organelles and their constituents connected with lines indicate continuous changes in their forms and colloidal states (Wada 1966). This diagram is a tentative plan for mitosis. We desire earnestly that this blueprint will be brought to completion in the future by cooperative research in various fields of cell biology.

majority of cancer cell research workers also have similar misconceptions about the mitotic process.

Indispensability of the karyokinetic spindle for cancer cell proliferation.

The most important biological significance of the spindle lies in the fact that it is an indispensable mitotic organelle for cell proliferation. The spindle is the only space in which chromosomes separate longitudinally and migrate poleward in a regular manner. In other words, no nuclear division would occur without reproducing karyokinetic spindle in meristematic cells (123-127).

Cancer cells continuously repeat mitosis and produce a large cell aggregation. Their mitotic process is normal in young cell aggregation, while it becomes irregular in old one. The influence of an old and large cell aggregation on the host is pathological and virulent as a whole. This property of cancer cells is generally considered as a result of unbalance between cell proliferation and cell differentiation.

By the rapid development of autoradiographic experiments there are hundreds of researches on DNA-cycle in meristematic cells of plants and animals including cancer cells. Some of these researches seem to be undertaken to shed some light on the solution of fundamentals of cancer cell proliferation. It should be seriously reconsidered that the reproduction of chromosomes in meristematic cells is one of the necessary conditions but by no means the absolute condition for bringing the cell into proliferation. The indispensable condition for cell proliferation is the reproduction of the karyokinetic spindle and its activity in meristematic cells (123-127). This is not an exception to cancer cell proliferation as well. I believe that research on the spindle, especially on its reproductive cycle of the atractoplasm in meristematic cells may give some clues to distinguish the behavior of ordinary somatic cells from cancer cells. The development of atractoplasm is concerned with the alteration of colloidal state of karyolymph and chemical and physical properties of nucleoli including their disintegrated state (66-74, 114-116). The relative quantity of nucleoli to the nuclear contents will present an important problem for cancer cell research.

Biological significance of chromosome aberrations in cancer cell. As fundamental research on cancer cells, it seems to be fashion to study chromosome aberrations. They are certainly significant markers of cancerous tissue cells. However it may not be too strong to say that the analysis of chromosome aberrations in cancer cells will contribute little to the solution of carcinogenetic processes. This is because chromosome aberrations are detected always as secondary phenomena. After repetition of mitosis several times, ordinary meristematic cells discontinue cell proliferation and enter into cell differentiation. In the case of cancer cells, they continue mitosis but never cease in order to commence cell differentiation as a tissue to which the cells belong. Under these circumstances, it is assumed that the metabolites produced for cell differentiation would be accumulated within the cell. I imagine that some of these metabolites or excess nutrients may induce chromosome aberrations, polyploid or heteroploid nuclei.

This idea is not mere imagination. It is comparable with the occurrence of chromosome aberrations in cell cultures. The ordinary purpose, techniques and nutrient media for cell cultures are centered on inducing active cell proliferation. However no invention has been made to analyze the cause for the preservation of cell differentiation. Similarly little consideration has been given to analyze the cause for occurrence of chromosome aberrations

in culture cells. The nutrient state of cells in cell cultures shows a close resemblance to that of cancer cells. Both cell groups are supplied with excess nutrients and repeat mitosis without entering cell differentiation.

From the above comparison, we can reach the conclusion that chromosome aberrations may be induced by excess nutrients accumulated in meristematic cells. In the case of cancer cell aggregations, excess nutrients may be accumulated as a result of genetically induced repression of cell differentiation (128-139). In the case of culture cell aggregations, excess nutrients may be supplied artificially and the cells are cultured under inadequate conditions for cell differentiation. In other words, excess nutrients or metabolites accumulated among meristematic cells may induce various kinds of chromosome aberrations when the cells are genetically or artificially repressed from entering into cell differentiation. Therefore, the chromosome aberrations of cancer cells are mere secondary phenomena produced in grown cancer cell aggregations. The mitosis of incipient cancer cells is normal. This fact has been demonstrated in the mitosis of stemline-cells by Makino (1957).

Importance of analysis for behavior of individual cancer cells. Many theories or hypotheses have been presented for the solution of carcinogenetic processes by medical doctors, biologists or biochemists. They have induced conclusions from the results of research in physiology, enzymology, immunology or virology of cancer cells. Strictly speaking, their conclusions are based on the analysis of phenomena and properties manifested by the aggregation of cancer cells but not on the behavior of individual ones. No solution for birth of a cancer may be attained from the results of research on cancer cell aggregations, although this research is very important and necessary for cancer therapeutics. The properties of incipient cancer cells are greatly altered secondly, thirdly, or pathologically by the metabolic conditions in aggregated cell states. Aberrations found in chromosomes are only one instance among many.

First of all the biological significance of anarchic growth or unlimited repetition of cell proliferation in cancer cells should be reinvestigated at the individual cell level but not in cell aggregate states (123-128). In biological research it is a matter of common sense that clarification of morphological changes takes precedence over commencement of physiological or biochemical research. This is not an exception to cancer cell research.

Interpretations on the mechanism of mitosis are constantly progressing by *in vivo* observations together with advances in other fields of science. Notwithstanding much progress in morphological studies on mitotic organelles, the descriptions of mitosis in current cytology text-books stoutly stick to the interpretations of classical cytology since the middle of 1870's. We expect that biologically significant research on carcinogenesis will find clues by practical application of this new blueprint for mitosis (128-141).

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