Mitotic Cell Studies Based on *in vivo* Observations
IV. The *raison d'être* for the spindle membrane and
the atractoplasm being composed of fibrils

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Introduction

Recently three papers which are worthy of reconsideration have been published. Bajer (1968) and Dietz (1969) discussed the behavior and fine structure of spindle fibers, and Aldrich (1969) reported both the presence and absence of a nuclear envelope during the entire mitosis in slim mold and amoeba. The authors of the first two papers have already worked on living mitotic cells and stated their original opinions concerning the problems of mitosis. However, they are not perfectly free from the ideas of classical cytology. Both authors believe in the breakdown of the nuclear membrane and the presence of continuous fibers even in living cells.

Bajer (1968) stated: "the spindle contains numerous microtubules (MTs)". In his working hypothesis he assumed that the chromosomes are transported toward the poles by a shortening of the kinetochore fibers accompanying the simultaneous elongation of continuous fibers. His interpretation is based on the idea that the kinetochore fibers and the continuous fibers are composed of microtubules. By means of cine-micrography, Dietz (1969) studied in detail the behavior of chromosomes during meiosis I and II of spermatocytes in *Tipula*. He revealed the to and fro wandering of chromosomes in the formation of metaphase plate and that of sex-chromosomes in anaphase I. Based on this chromosome wandering, he assumed that the chromosome movements in the meiosis are attributed to partial shortening or elongation of each chromosomal fiber. This change of chromosomal fiber length is interpreted as caused by the association or dissociation of molecular constituents of these microtubules. Both authors give importance to the microtubules in reference to the chromosome movements.

All the mitotic organelles are functional only in living cells having approximately 80% of water content.

I cannot follow the interpretations mentioned above which introduce the microtubules as effective factors in the chromosome movements in nuclear divisions. The movement of chromosomes is an apparent phenomenon taking

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place in living cells having approximately 80% of water content. The microtubules under electron microscopes can be observed only in the fixed and dehydrated state of mitotic cells. At present, the electron microscope possesses no power to demonstrate microtubules in the living state of mitotic cells.

We wonder why cytologists do not question the inconsistency mentioned above. In classical cytology and even in present day advances, little objection is presented against the data observed in fixed preparations, as being proven facts; it were as if the images under the microscope would identically exist in living cells. Such organelles which do are practically confined to those intrinsically in a gel state such as chromosomes, plastids, mitochondria or cytoplasmic granules. They can, when fixed and dehydrated, keep their features without drastic changes. On the contrary, nuclear sap, spindle ground substance, i.e. the atractoplasm, spindle fibrils, spindle membrane, plasma membrane, and other ultrathin membranes or membraneous structures, are in a sol state, or are composed of molecular films and suffer from drastic deformations by fixation and dehydration. Some of them are observed as deformed artifacts by coagulation and some others disappear through destruction.

Based on the above viewpoint, the author published a monograph "Analysis of Mitosis" (Wada 1966). This is the author's earnest desire to bring about a revision of those static features seen in mitosis of material killed by fixation, and to supplant them with the dynamic state in living cells. This may be the first blueprint of mitosis to interpret the morphology and physiology of living mitotic cells with approximately 80% water content. Unfortunately, Bajer (1968) and Dietz (1969) did not cite the "Analysis of Mitosis" (Wada 1966). They seem to experience no doubts about introducing the idea of a breakdown of the nuclear membrane and the presence of microtubules, when they interpret mitotic phenomena in living cells. Nearly one hundred years have past, since cytology was established on the basis of fixed materials. Cytologists are accustomed to study fixed materials instead of living ones. They can have become indifferent to the very fundament that the mitosis takes place only under the conditions in which cells can live.¹

Morphological aspect of the mitotic organelles in living cells with approximately 80% water content

In vivo observations of the mitosis are simultaneous studies on the changes of the biocolloidal states in living cells.

¹ In 1954, the results of *in vivo* observations appeared for the first time in a foot-note in a cytology book ("General Cytology", 2nd ed. p. 248): "Wada (1950) maintains that the nuclear membrane merely changes its structure, and persists from prophase to telophase. He attributes its absence in cytological preparations to artifacts produced by the fixatives." In the 3rd edition of this book (1960) which carries increased content the concept of the spindle membrane is stricken out. The descriptions are restored to the static morphology of fixed mitotic cells including electron microscope studies: the breakdown of the nuclear membrane, the presence of continuous fibers and so on. Again mitosis problems are lacking a certain vitality.

Most cytologists are taught and believe in a breakdown of the nuclear membrane before the appearance of the nuclear spindle in the cytoplasm. They do so because this is an orthodox explanation of mitosis described in current cytology books. At present, scores of works report the dynamic states of living mitotic cells by cine-micrography and time lapse photomicrography. All possible efforts were exercised by pursuing the chromosome movements in meta- and anaphase, but neither the breakdown process of the nuclear membrane nor the behavior of cytoplasmic granules or of vacuoles, which come in contact with the nuclear membrane, were clarified.

Fig. 1. Time lapse photomicrographs of an endosperm cell mitosis in *Zephyranthes candida* cultured in a medium of 1% sucrose and 0.5% agar. Photographed with ordinary light microscope without compressing the cell to the disc. Focused on spindle membrane and granules (g_1 , g_2) lying at outer surface of membrane. Arrows indicate positions of membrane. a' - c' are drawing of above photographs. a and a' , late prophase. Chromosome arms stretch out inside of nuclear membrane (photographed 17:10). b , and b' , atractoplasm (clear zone) appears surrounding chromosomes (so-called somatic contraction) in prometaphase. No breakdown of nuclear membrane occurs: granules stand still on nuclear membrane (17:15). c and c' , early anaphase. Nuclear membrane transforms into spindle membrane and granules (g_1 , g_2) are still lying on the membrane (17:29). $\times 625$. (Wada and Kusunoki 1964a).

Since the proposal of the atractoplasm theory by Fujii (1931), we have investigated thousands of time lapse photomicrographs of living cells of *Tradescantia* staminal hairs. The photographs were focused mainly on the nuclear membrane, and also on the tonoplast membrane, or on the cytoplasmic

granules which come in contact, or are adsorbed by the nuclear membrane before and after the appearance of metaphase spindles (Fig. 1). The data of *in vivo* observations have been summarized and discussed in detail in the author's recent monograph (Wada 1966) and more concisely in the first report of this series (Wada 1969a).

The entrance of the prophase nucleus into metaphase is represented by the transformation of dispersed protein particles of a nuclear sap colloidal sol into fibrils.

In living mitotic cells, the prophase nucleus is composed of chromosomes, nucleoli, nuclear sap and nuclear membrane. The first two nuclear components keep their independency in the nuclear sap, and the nuclear sap maintains its independency, enveloped in the nuclear membrane, from the cytoplasm. When prophase nuclei enter into prometaphase, the nuclear sap changes into the atractoplasm and the nuclear membrane transforms into the spindle membrane. This membrane transformation and its existence are supported in living cells by the fact that the entrance of the cytoplasmic granules, plastids, and mitochondria is arrested at the circumference of the metaphase spindle, just as they are at the surface of the prophase nuclear membrane (Fig. 1).

From the results of polarization microscope studies on the spindle, it is already confirmed that the basic structure of the nuclear spindle shows birefringence and the spindle constituents are optically anisotropic and parallel to the spindle axis. The possible relationships between the dispersed particles of nuclear sap colloidal sol and the spindle fibrils have been demonstrated by the electron microscope studies on the development of meiotic spindles of PMC in *Lilium* treated with CdCl_2 -containing fixatives (Satô 1961).

The parallel arrangement of the spindle fibrils presumably by molecular forces among fibrils forms, as a whole, a tactoid. Therefore, in spite of growth, elongation or other deformation of spindles, the deformed spindles can always recover their spindle form by forming a tactoid (Fig. 2). There is no room for maintaining a breakdown of the nuclear membrane, the appearance of any kind of microtubules, or of continuous fibers observed in the fixed preparations. The transformation of the nuclear membrane into the spindle membrane is demonstrated in the plasmodium metaphase spindle which is enveloped in a double membrane known as a metabolic nuclear membrane (Aldrich 1969).

There are no definite biochemical conditions which make possible the polymerization of spindle fibrils into continuous fibers in the spindle, although many animal cytologists and some biochemists still follow the misinterpretation of classical cytology that the centrioles may be the kinetic centers of the spindle fiber formation.

With regard to the chromosomal fiber development, we can infer the activity of the kinetochore substance in each chromosome. This substance

may become diffusible in prometaphase. At this time, the diffused kinetochore substance may be led, probably by capillary phenomenon, toward both the spindle poles organizing chromosomal fibers. Consequently, each chromosomal

Fig. 2. Deformation of karyokinetic spindle by centrifugal force (2800 rpm for 30 min) and recovery. Time lapse photomicrographs of a cell of *Tradescantia* staminal hair cultured in 2% sucrose solution. a'-e' are drawings of above photographs. a and a', showing deformed metaphase spindle pushed to centrifugal side (downward) of cell. Large vacuoles appear in centripetal side (upper part) of cell (photographed immediately after the stop of centrifugation; 12:46). b and b', showing recovery of early anaphase spindle. Spindle fibrils form a tactoid by rearranging themselves in parallel. No chromosomes reach elongating spindle pole in vacuole (12:54). c and c', upper spindle pole continues growing (12:56). d and d', late anaphase. By growing of spindle or that of tactoid body, position of cell plate is led at middle of cell (12:58). e and e' late telophase. Mitosis is finished normally (13:15). $\times 1,000$. (Wada unpublished, photographed July 13, 1933).

fiber is made up of a bundle of arranged fibrils and the kinetochore substance. This process of chromosomal fiber development is supported by the well known birefringent images of the fibers in living mitotic cells. Each chromosomal fiber appears compact near the kinetochore of each chromosome and

becomes loose and slightly spread near the polar regions of the spindle. This image of each fiber corresponds to the outflowing locus of the fluid substance from kinetochores among parallel arranged fibrils (Wada 1966, 1969a).

Evolutional aspect of the spindle membrane in lower and higher organisms¹

The nuclear membranes are preserved during all the mitotic stages in organisms whose nuclei become morphologically and functionally independent of the cytoplasm.

Concerning the spindle membrane, Aldrich (1969) demonstrated with excellent electron micrographs the presence of a double membrane which persists during the entire division of the intranuclear spindle mitosis in the diploid plasmodium of *Physarum flavicomum*. With the same treatment, however, he observed the breakdown of the nuclear membrane during prophase in haploid myxamoeba, where centrioles are present.

In Aldrich's work mentioned above, it happened that two types of mitosis were found as a consequence of the same treatment. He stated: "There appear, then, to be few parallels between mitotic spindles of higher plants and the myxomycetes, although similar mitotic apparatus can be found among the protozoa, the algae, animals, and possibly the higher fungi." So long as the breakdown of the nuclear membrane is believed to be fact, it would be difficult to grasp the intrinsic significance of the spindle membrane in the mitoses of higher and lower organisms in both plant and animal kingdoms. According to the attractoplasm theory (Fujii 1931), the presence of the nuclear membrane, or that of the spindle membrane throughout the nuclear division, is essential to keep the independency of the nuclear content from that of the cytoplasm. By improved fixation techniques, we have demonstrated the presence of the spindle membrane in some higher plants and animals (Fig. 3). Heretofore, the nuclear membranes of these organisms were thought to breakdown at the end of prophase. Consequently, the independency of the metaphase spindle from the cytoplasm, at least, ideologically was disregarded.

The spindle membrane is an essential organization for the organisms which proliferate by mitosis.

In primitive organisms such as bacteria, no nuclei differentiate morphologically independent of the cytoplasm, although DNA, as genetic material, is present in the cytoplasm. In the Cyanophyceae, the nuclear substance is recognizable in the cytoplasm. However, the problem still remains that the nuclear substance is morphologically independent of the cytoplasm.

During the course of evolution, unicellular organisms appear in water.

¹ In the second report of this series, it had been stated that the evolutional aspect of the centriole will appear in the fourth report; it will, however, be postponed until the fifth report.

In each individuum, a nucleus differentiates morphologically and functionally from the cytoplasm and makes an independent system enveloped in the nuclear membrane. These organisms swim about in water to obtain food and to meet

Fig. 3. Spindle membranes in higher plants and animals. Arrows indicate positions of spindle membranes. a-c, root tip cells of *Vicia faba* fixed with CdCl_2 -containing fixative and stained with iron alum hematoxylin. a, rising of nuclear membrane at beginning of polar cap formation. b, spindle membrane at late prometaphase. c, spindle membrane (=phragmoplast membrane) in which daughter chromosome groups, phragmoplast substance and cell plate are enveloped. (Wada, Nagao and Awasawa 1963). d, a side view of spindle at metaphase II of grasshopper spermatocyte meiosis. Centrioles lying close to out of upper spindle pole. Treated with MnSO_4 solution and fixed with Bouin's fluid. (Nakao, Nakanishi and Wada 1968). e, spindle membrane in sea urchin egg mitosis. Spindle axis is slightly declined, so that spindle membrane appears as a part of circular line at optical section. Pretreated with $1/10 \text{ M MnSO}_4$ solution and fixed with Bouin's fluid. (Wada, Hanaoka and Niitsu 1964). f, metaphase spindle of PMC in *Tradescantia* treated with CdCl_2 solution and ethanol without staining. (Wada and Kusunoki 1964a). Magnification: a-c and f, ca. $\times 2,000$; d-e, ca. $\times 1,000$.

partners for reproduction. For the swimming function, centrioles, cilia, or flagella differentiate from the cytoplasm. The centrioles are primordia of locomotive organelles for cell movement.

It is natural that the differentiation of the nuclear membrane and of the

centrioles is inherited by each cell in multicellular organisms in the course of evolution. Obeying the general principles of the evolution of organisms and of the differentiation of organs, it is utterly unthinkable that the independency of the nucleus would be lost by the breakdown of the nuclear membrane, whenever cells of multicellular organisms enter into the nuclear division. It is also unnatural to think that the centrioles, i.e. primordia of locomotive organs, would participate in the spindle fiber formation. From this evolutionary viewpoint, it becomes clear that the centrioles in each cell of the higher animals are preserved in an inactive state except the centrioles in male gametic cells (Nakao, Nakanishi and Wada 1968, Wada 1969b).

The explanations of mitosis described in current cytology books disregard these fundamental principles in the evolution of organisms and of cell differentiation. Most cytologists have overlooked the importance of the intranuclear spindle mitosis as a primitive but a basic feature of mitosis. Unfortunately, classical cytology has dealt with the intranuclear spindle mitosis as an exceptional case. By this misinterpretation, cytologists have completely lost the clue to grasp the mechanism of mitosis in higher plants and animals according to one consolidated principle. According to the atractoplasm theory and based on *in vivo* observations, we have already explained that karyokinesis is carried out on one and the same principle in both plant and animal kingdoms. Simultaneously, many inconsistencies were pointed out with regard to the mitotic organelles described in current cytology books (Wada 1966, 1969a).

The greatest misinterpretation which is currently hindering advancing knowledge about the mechanism of mitosis and the opening up of a correct approach in modern cell physiology is the preconceived idea about the breakdown of nuclear membrane and the presence of continuous fibers. The continuous fibers appearing in the so-called mitotic apparatus are nothing but the coagulated state of the spindle fibrils composing a tactoid.

A solidly made spindle membrane in lower organisms and a fragile but efficiently made spindle membrane in higher organisms

As repeatedly stated, the appearance or disappearance of the spindle membrane is a problem due to fixation techniques but not to an intrinsic difference in the nature of the organisms in higher and lower plants and animals.

In the various kinds of unicellular organisms and in algae or fungi, when each individuum or each nucleus in the thalli or hyphae enter into nuclear divisions, it would not be necessary for the entire nuclear sap to change into the atractoplasm to transport the minute chromosomes to the spindle poles. Therefore, the newly organized spindle and the surplus nuclear sap maintain their independence from the cytoplasm by the presence of the prophase nuclear membrane. This is a feature of the intranuclear spindle mitosis. In this case, the constitutional changes in the nuclear membrane may be very

little or nil, when the nuclear membrane is transforming into a spindle membrane.

In general, the spindle membrane of lower organisms are solidly made and have enough resistance to the routine fixatives and dehydration, whether or not the metaphase spindles accompany the nuclear sap.

When higher plant and animal cells enter into mitosis, all of the nuclear sap changes into the atractoplasm in prometaphase. As already stated in the foregoing chapters, the dispersed fibrils of the atractoplasm form a tactoid body made by a parallel arrangement of these very fibrils. Corresponding to the tactoid formation, the nuclear membrane passively transforms its shape from sphere to spindle form. There was no biological reason requiring the nuclear membrane to be broken down in living mitotic cells having approximately 80% water content. In spite of many common fundamental characters in the spindles among both lower and higher organisms, there still may be a conspicuous difference in the biophysical property of the spindle membrane. The spindle membranes of higher plants and animals are so fragile that the membranes are easily destroyed by treatment with routine fixation techniques.

The characteristic of the spindle membrane in higher organisms is interpreted as follows. When a nuclear membrane transforms into a spindle membrane, it is inferred that the former alters its own organization at the molecular level to raise the physiological activities of the latter. Presumably, the spindle membrane may be obtaining the same biophysical properties as the plasma membrane has. No physical strength is demanded to keep the independence between the atractoplasm and the cytoplasm.

To conserve the spindle membrane of higher organisms in fixed preparations, it is necessary to harden the membrane structure by pre-treating it with various kinds of metal salt solutions. Their kind and concentration should be determined according to the kind of organisms and tissues (Fig. 3). And it is also important to reduce the concentration of acetic acid as much as possible from the post-fixation fluid (Wada, Nagao and Awasawa 1963, Wada and Kusunoki 1964a, b).

Physiological cell aspects and relation to the spindle membrane

Tactoid formation by parallel arranged spindle fibrils is an indispensable condition for spindle activity.

According to the general principle of cell physiology, the nuclear membrane is an effective barrier at the nuclear surface. Loewenstein and Kanno (1963) measured the resting potential of the nucleus in a gland cell of *Drosophila*, and it was 15 mv negative with respect to the cytoplasm. This makes a diffusion bottleneck restraining the mixing of otherwise freely mobile intracellular electrolytes. The spindle membrane in metaphase may behave as a diffusion barrier for electrolytes and many other metabolites as that the

nuclear membrane does. Therefore, there seems to be an unequal distribution of some electrolytes across the spindle membrane. Consequently, there must be a potential difference across the membrane. Yasui and Fujii (1951) by a special staining technique have demonstrated that almost no transfer occurs of electrolytes across the spindle body.

The electrostatic phenomena in the mitotic cells were once considered by Lillie (1905, 1911) to be an available mechanism underlying chromosome movement in anaphase. His hypothesis, however, remains unestablished due to the lack of electrophysiological considerations and also to a misunderstanding of the spindle organization (Fig. 4).

Fig. 4. Schematic diagrams showing hypotheses on mechanism of mitosis by R. S. Lillie's electrostatic theory (a-c) and Wada's hypothesis based on attractoplasm theory (a'-c'). a, cell before metaphase. b, establishment of two poles. c, conditions at anaphase, when negative chromosomes move toward negative poles (a-c are drawn after Fig. 15 in "Mitosis" by Schrader 1953). a', cell in prophase showing evenly distributed ions, electrolytes and metabolites in cytoplasm. Distributions other than ions across nuclear membrane and ATP in cytoplasm are omitted. b', establishment of metaphase spindle by fibrillation and growth of tactoid enveloped in spindle membrane. Ions, electrolytes and metabolites converge around both spindle poles. c', showing poleward movement of chromosomes by chromosomal fiber shortening and refolding of fibrils constituting dispersed phase of daughter nuclear sap colloidal sol at both polar regions. No centrioles participate in spindle activity.

A remarkable development of electrophysiological techniques in the last two decades made it possible for us to consider regional differences in permselectivity of the spindle membrane in a mitotic cell. Recently, Naitoh and Eckert (1969) found that an electrical response (therefore a behavioral

response) in a ciliate protozoan, *Paramecium caudatum*, could be induced by a mechanical stimulus (such as a light tap of the specimen by a fine glass rod) but only when it was applied to the terminal region (anterior or posterior end) of the specimen. The middle region was essentially insensitive to a mechanical stimulation. Furthermore, the electrical response to an anterior stimulation was found to be in the opposite direction to that of a posterior stimulation. This is, the anterior response was depolarizing and the posterior response was hyperpolarizing. They also found that the depolarization was due to an increase in permeability of the anterior membrane to calcium ions, whereas the hyperpolarization was dependent on potassium ions.

These findings together with a resemblance of the body shape of *Paramecium* to that of a spindle body, suggested to the present author to attempt to evaluate from up-to-date electrophysiological considerations and observations the old idea, namely that the electrical phenomena across the spindle membrane might be a possible mechanism underlying the movement of chromosomes within a spindle body.

It may be natural to consider that the potential differences across the nuclear membrane are maintained also across the spindle membrane. In prometaphase, the nuclear membrane transforms from sphere to spindle-form by the development of a tactoid within the membrane. Corresponding to this change of the membrane form, the electrolytes which are evenly distributed across the nuclear membrane may change into a bipolar distribution across the spindle membrane (Fig. 4). It may then be electrophysiologically possible to elicit the changes of permeability confined to the polar regions of the spindle. It may be concluded consequently that the changes of the electrochemical gradient and the transfer of metabolites take place at both polar regions across the spindle membrane. The accumulation of adenosine triphosphates and their energy transfer may then concentrated around both spindle poles, and the necessary energy for the spindle activities in anaphase is furnished at the both spindle poles.

On the other hand, the dynamic states of mitotic organelles confirmed by *in vivo* observations are ingenious responses to the cell physiological requirement for accomplishing the chromosome movement in anaphase by the following processes: 1) tactoid formation by the fibrillation of the dispersed particles of nuclear sap colloidal sol; 2) increase of permeability at the polar regions of the spindle membrane; 3) migration of electrolytes and metabolites around the spindle poles; 4) increase of electrochemical activities and energy transfer at the both spindle poles; and 5) finally disintegration of chromosomal fibers and poleward movement of chromosomes by the shortening of chromosomal fibers.

Kinetic centers of the spindle activity lie at the polar regions of the spindle membrane.

Various physical and chemical changes may take place at the both polar

regions of the spindle as mentioned above: some of them require energy and the others release it. 1) The disintegration of chromosomal fibers into individual fibrils, with their consequent shortening and pulling of chromosomes toward the poles; 2) the transformation of fibrils into globular particles; 3) an increasing of their entropy in the dispersed medium of daughter nuclear sap; 4) reorganization of nucleoli; 5) the dissociation of the chromosome matrix;

Fig. 5. Partial suppression of spindle development and chromosome behavior in *Tradescantia* hair cell mitosis by irradiation of UV microbeams¹. a'-d' are drawings of above photographs. a and a', showing a very young polar cap which appears by rising of nuclear membrane. Cross-lines indicate position of irradiation (photographed 15:25). b and b', deformed polar cap and blocked metaphase chromosomes by sticking each other at irradiated pole side (15:58). c and c', chromosome behavior at non-irradiated pole side in anaphase. Chromosomes are prevented from reaching the pole by sticking with chromosomes at irradiated pole side. However changing of atractoplasm into phragmoplast occurred normally (16:07). d and d', by swelling of phragmoplast, sister chromosome groups at non-irradiated pole side was pushed and fused with other chromosome group. These phenomena revealed normal development of atractoplasm, phragmoplast and cell plate, but not that of chromosomal fibers (photographed in the next day of irradiation). ca. $\times 800$. (Wada and Izutsu 1961).

6) the appearance of chromonemata in daughter nuclei; and 7) the replication and growth of the daughter nuclear membrane,—all these changes start, at first, from the both polar regions of the spindle and some of them continuously spread through the whole spindle cavity. The relationships and the continuous morphological changes among the mitotic organelles, which appear from prophase to the end of mitosis, were illustrated diagrammatically (Wada

¹ a and b were photographed with camera equipped with and mounted on the UV microbeam irradiation instrument; c and d with Leitz "Makam" after irradiated cells were replaced in a moist chamber for culture.

1969a, Figs. 2 and 6). The developmental as well as shortening processes of chromosomal fibers at the submicroscopic level were also schematically shown in the previous papers of this series (Wada 1969a, Fig. 3).

The fact that the spindle poles are the kinetic centers for the migration of chromosomes was experimentally demonstrated by giving a fatal wound to one of the polar regions of the spindle by irradiation with ultraviolet microbeams (Wada and Izutsu 1961). Mitotic spindles of *Tradescantia* staminal hair cells were irradiated with an UV microbeam about 2μ in diameter and the induced aberrations of chromosome behavior were traced by *in vivo* observations.

A complete suppression of the spindle development occurred, when a polar cap was irradiated at the very beginning of its appearance, and a partial suppression occurred, when it was irradiated at a slightly later stage after its appearance (Fig. 5). We infer that the first irradiation induced a hinderance to the fibrillation and the second irradiation induced the suppression of the chromosomal fiber development and the chromosome stickiness at the irradiated spindle pole side. No abnormal spindle activities were found, when the UV microbeam irradiations were made on the outside of the spindle poles or on the equatorial region of the spindle.

Needless to say, there is no biological indication to assume the breakdown of the nuclear membrane at the end of prophase, nor the presence of continuous fibers, nor the presence of microtubules in the living mitotic cells. Again, the author feels compelled to repeat that cytologists should keep in mind the fact that mitosis is carried out only under the conditions in which the cells can live and that all the mitotic organelles are functional only in the state having approximately 80% of water content.

Concluding remarks

1. Although the explanation about the mechanism of mitosis based on the atractoplasm theory may not be perfect, we believe that this is more logical and valid than any other hypotheses described in current cytology books. The reliability of this blueprint of mitosis may be backed up with the fact that all the dynamic states of mitotic organelles confirmed in living cells essentially respond to the cell physiological requirements for the movement of chromosomes in anaphase.

Before the authors had reached the present blueprint of mitosis the original hypothesis was published in 1950. After that, the blueprint of mitosis was partially revised several times introducing new findings which contributed advances to the mitotic problems in living cells. The author had commenced studies on mitosis by micrurgical experiments on the living mitotic cells of *Tradescantia* staminal hairs over 40 years previously. Since then, the effects of physical or chemical treatment of various kinds, and the influence of radiation or UV microbeams on the mitotic cells *in vivo* were causal-

analytically studied. More than 50 papers have been published each time a project was finished.

However, our contributions to the analysis of mitosis have been generally disregarded as was mentioned in the foregoing sections (See the foot-note on page 484), because the results confirmed by *in vivo* observations do not always coincide with the commonly accepted ideas about the mitotic figures described in current cytology books. The disagreements are especially conspicuous with regard to the origin, structure, and function of the karyokinetic spindles.

2. Further studies aimed to arrive at the perfecting of the blueprint of mitosis should primarily depend on biophysical research. The first objective should be to fill in the insufficient knowledge of the colloidal properties of the nuclear sap and its transformation process into atractoplasm. With regard to the appearance of the atractoplasm in prometaphase and the reappearance of daughter nuclear sap in telophase, the possibility of reversible unfolding and refolding of dispersed protein particles in the nuclear sap colloidal sol should be ascertained by biophysical or colloid chemical research. We infer that the dissociation of nucleoli before spindle formation may play a role as a trigger for the fibrillation of dispersed particles of nuclear sap colloidal sol.

Different from the qualitative analysis of mitotic organelles, the quantitative analysis of the intranuclear constituents is practically impossible with present techniques of biophysics or colloid chemistry to reach a clarification of the physical and/or colloid chemical properties of intracellular organization such as the nuclear sap, nuclear membrane, the spindle membrane, the spindle fibrils, and the formation of chromosomal fibers.

3. To elucidate the mechanism of mitosis, biophysical or colloid chemical research take precedence over biochemical research. The present biochemical treatments are conducted completely with disregard for the intracellular organization. Such treatments are simple, easy and apt to be in fashion in comparison with *in vivo* observations, micrurgical operations, and polarization microscope studies on living mitotic cells.

Any protein fibers biochemically ascertained by a mass analysis of the so-called isolated mitotic apparatus from sea urchin eggs¹ are biologically of very little significance. This is because the result can identify no characteristics of specific fibers organizing the mitotic apparatus, such as 1) continuous fibers: a coagulated feature of tactoid fibrils; 2) chromosomal fibers: coagulate tactoid fibrils incorporated with kinetochore substance; 3) coagulated interfibrillar substance; and 4) coagulated astral rays and centriolar substance.

¹ Concerning the isolated mitotic apparatus, we cannot overlook Prof. Schrader's respectful attention stated in his famous monograph "Mitosis" (1953): "Their method (Mazia and Dan 1952) makes possible the gathering of spindles for mass analysis but of course opens the possibility that such spindles do not correspond to normal, untreated spindles in all their properties." At present, little consideration is paid to this attention, when cytologists and biochemists work on isolated mitotic apparatus but biologically interpret their results.

The author does not except much from further biochemical research, at least at present, for any elucidation of the problems in mitosis. The most important effort in this problem is to reveal the molecular architecture of each spindle component and not necessarily to know the kind, size, weight, or structure of the molecules themselves. Specifically studies must clarify the arrangement of the biological macromolecules of each mitotic organelle which may bring its functional specificity in approximately 80% water content. The brilliant contributions of the DNA molecule itself to the gene activity in genetics is a specific case of an exception among many exceptional examples.

4. Closing these concluding remarks, the author should like to add a few words to the cancer cell problem. It is the author's long-cherished opinion that any fundamental solution to cancer cells is impossible without an elucidation of the mechanism of mitosis (Wada 1966).

Hundreds of research projects on the fundamentals of cancer cells are reported yearly in all the fields of modern biological science: chromosome aberration, DNA replication patterns, regulation of mitosis, virus problem, and so on. All these results, however, contributed little to exclude the deadblock "anarchic growth" or "autonomic growth" of cancer cells. These words are generally used as a definition for cancerous cells. And yet the meanings of the word are dependent on a non-scientific utterance, although the researchers may ideologically understand the content of these definitions as the repetition of cell proliferation without cell differentiation. However, they concretely know nothing about what kind of mitotic process has a direct relation to the origin of cancer cell appearance among ordinary tissue cells. And also, current cytology books give no answers or even clues to elucidate this problem.

Based on the comparison of mitotic cell behavior among ordinary tissue cells, damaged tissue cells, and tumor cells, we have reached the conclusion that a somatic cell mutation may induce an incipient tumor cell. The findings on the tumor formation in the cut epicotyles of *Stizolobium hassjoo* and the various evidence to support the somatic cell mutation hypothesis for *Stizolobium* tumor are described in detail in the monograph (Wada 1966). The possibility of extending this hypothesis to the origin of cancer cells stands of course on the fact that nuclear divisions and somatic gene mutations are carried out under common principles among the cells of plants, animals, and human bodies.

In stead of the definition describing the "anarchic or autonomic" growth for cancer cells, the author should like to propose a concrete scientific expression standing on a purely cell biological viewpoint, namely, cancer cells may be caused by a somatic gene mutation affecting the atractoplasm formation. In other words, the deletion or deficiency of the suppressor gene or genes for the atractoplasm formation result in the suppression of cell differentiation. Simultaneously, the one-side activation of the inducer gene or genes for

atractoplasm formation results in the repetition of cell proliferation. The possibility of gene mutations mentioned above in pretumor cells are explained in the monograph (Wada 1966).

In future, if the substance suppressing atractoplasm formation should be successfully extracted from differentiating but ordinary tissue cells, then the substance would be theoretically the only effective substance having total-organ specificity to suppress the repetition of cancer cell proliferation.

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CORRIGENDUM

CYTOLOGIA Vol. 34, No. 3: 400 and 406

S. C. Saxena and V. Aditya: "Histopathology and Histochemistry of the Insects Treated with Chemosterilants" I and II

Figs. 1 to 6 on page 400 are to be replaced by

Figs. 1 to 6 on page 406 and *vice versa*;

the legend of the figures, however, are correct in both the papers.