CHAPTER 7 Colloidal State of Spindle Body

1. General remarks

With regard to the colloidal state of the karyokinetic spindle, the viscosity change in protoplasm during a mitotic cycle has been measured by means of centrifugation, micrurgy, Brownian movement and other methods. Together with the viscosity of protoplasm, that of the nuclear spindle has been investigated by direct or indirect methods. Concerning this problem, the references in the work by Milovidov (1949) should be consulted. It is generally accepted that the nuclear spindle or the karyokinetic spindle may be in a gel state; this idea seems to be especially so with respect to the so-called half spindle.

Recent advances in research on mitotic spindles have clarified the morphology of each constitutional element in the spindle, such as fine structures as well as physical and chemical properties of chromosomal fibers, unit fibrils of karyokinetic spindles, those of phragmoplast and their membranous structures as mentioned in the previous chapters. Considering all these findings, it proves to be of little significance to discuss the colloidal state of the spindle as a whole by techniques hitherto used. For this purpose, it will be essential to clarify by experiment the physical properties of each constitutional element of the spindle in living cells.

In classical cytology, 1) by the preconceived ideas on disappearance of the nuclear membrane before the formation of karyokinetic spindle and 2) by its plastic appearance including no granular elements except chromosomes, it is ideologically concluded that the spindle must be in a gel state. However, the first premise has been proved to be not correct as explained in Chapter 2. In this chapter, it will be proved that the second premise is also erroneous. According to our integrated results from analysis of each mitotic organelle, the atractoplasm is not a gel body but is in a sol state in living cells.

Using the centrifugation method on dividing eggs of Chaetopterus, Heilbrunn and Wilson (1948) reported that the spindle is considered to be in a gel state. Their experiment certainly indicates the change in rigidity of mitotic cells as a whole, but may not prove directly the viscosity change in the spindle itself. Mazia and Dan (1952) succeeded in isolating the mitotic apparatus from sea urchin eggs. They obtained isolated mitotic apparatus by hardening with S-S bridge formation. This treatment is an inevitable precondition for isolating the spindle from the cytoplasm. In other words, this procedure implies that the spindle in the living cell may not be too rigid to isolate the mitotic apparatus without hardening. Recently, Kane (1965) in isolating the mitotic apparatus in sea urchin eggs elucidated the
physical-chemical factors which bring about isolation and stabilization of the mitotic apparatus. Viewed from the biological standpoint of the cell, the importance is not only concerned with stabilization of the mitotic apparatus by killing but also with its colloidal state in living cells as to whether it is in a gel or in a sol state.

In animal cells, the so-called mitotic apparatus is described as an unbounded region within the cell (Mazia 1961). However, our improved cytological techniques have revealed that in this unbounded region, there exists a karyokinetically spindle which is nuclear in origin and by its own membrane is independent from other parts of the mitotic apparatus (Figs. 9, 10, 18). Moreover, the common idea that the so-called spindle fibers are organized from centrioles with cellular proteins has been denied and proved to be an *ad hoc* interpretation from animal mitoses, because this phenomenon cannot be realized in plant mitoses where there is no centrosome system.

The colloidal state of the spindle taken up for discussion here is defined as that of the atractoplasm, which plays a role as a field for chromosome movement in the meta- and anaphases. Therefore, the so-called spindles in *Barbulanympha* or in grasshopper neuroblast mitosis are disregarded in the discussion, because they play no role as a field in which chromosomes move poleward in the anaphase. Although the central spindle of grasshopper neuroblasts has been proved experimentally to be in a gel state (Carlson 1946, 1952), such demonstrations may not provide evidence to maintain that the atractoplasm must be in a gel state. Vivid movement of chromosomes on the screen demonstrated by excellent cine-micrographs of cell divisions in grasshopper, *Haemantheus* endosperm cells or ascites tumor cells may make the observers believe that the chromosomes are migrating in a fluid medium toward the spindle poles, discounting the fact that the apparent speed of chromosomes in the screen is strikingly accelerated by time lapse photography. In practice, our microdissection experiments on the spindle of *Tradescantia* hair cells *in vivo* (Wada 1932, 1933, 1935) revealed a sol state of the atractoplasm which maintains its shape by its own membrane (Fig. 1), and its position by the polar cytoplasmic strands in the cell (Fig. 5a).

2. Microdissection experiments

By means of microdissection techniques, Chambers and Sands (1923) found that the spindle of *Tradescantia* pollen mother cells embedded in anther slime is in a gel state. However, in regard to spindle rigidity, Chambers himself (1924) obtained different results in animal cells from those of *Tradescantia* cells. He reported: "In the insect spermatocyte, the cytoplasm and spindle substance are both fluid. A needle may be inserted through the cytoplasm into the spindle, and can be moved from side to side without dislocating the chromosomes unless they are touched with the needle." This inconsistent result described by Chambers caused us to repeat the
experiment with the same material, the pollen mother cells of Tradescantia, to reinvestigate by micrurgical operations the colloidal state of the spindle.

By applying a dilute solution of neutral salts of various kinds as observation medium, Yamaha and Ishii (1932) succeeded in keeping meiotic figures of pollen mother cells, including chromosomes, in a visible state although the cells are in a slightly coagulated state. Even in this state of the meiotic cells of Tradescantia embedded in 0.2 M KCl solution, Wada (1933) found that the spindle substance flows out easily, together with the chromosomes, through a torn place in the cell membrane made by microneedles. This finding reveals that the atractoplasm of Tradescantia cells is fluid similar to that of insects as reported by Chambers as mentioned above.

Figs. 25a–d. Drawing of disturbance of chromosome movement in spindles induced by micrurgical operation in staminal hair cells of Tradescantia reflexa. a, from a hole punched with microneedle at a corner of metaphase cell wall, atractoplasm together with chromosomes flow out instantly. Chromosomes belonging to opposite pole are retained within swollen atractoplasm and faint fibrous structure appears when the cell coagulated. b, through flowing out of polar cytoplasmic strands from punched hole, chromosomes belonging to this pole drawn to hole but others remain in original position. c, on breaking down of polar cytoplasmic strands, a spindle pole at punched cell wall side becomes flat showing clearly the sol state of atractoplasm. d, in telophase, atractoplasm transforms into phragmoplast by swelling. Spaces occupied by disintegrated chromosomal fibers in polar regions of daughter nuclei (black) known as “Polfeld”. (Wada 1935)

In comparison with these experiments, the inconsistent results between the fluid spindle of insects and the gel spindle of plant cells demonstrated by Chambers are considered to be due to the following fact: the spindle of insect spermatocytes may be easier to keep in a living state than that of pollen mother cells. Aside from technical difficulties, it can be concluded that the atractoplasm in both plants and animals is intrinsically in a sol state, although it becomes a gel state to various degrees according to necrotic changes or by death of cells. Shimakura (1934) pointed out that the keeping alive of meiotic cells of Tradescantia outside the anther can be attained only when the pollen mother cells are in a strictly isotonic medium of chemically pure sucrose solution.
In his microdissection operations on the dividing neuroblast cells of the grasshopper, Carlson (1946, 1952) reached the conclusion that the spindle at metaphase is a semi-solid body which undergoes liquefaction during anaphase and shows an abrupt rise in viscosity in the telophase. The result of his experiments clearly demonstrated that the central spindle is in a gel state in metaphase, but a sharp line must be drawn between this neuroblast spindle and the ordinary one in which chromosomes can migrate. The migration of anaphase chromosomes in the neuroblast cell takes place in the space outside the so-called central spindle.

We may call to mind another piece of evidence about the sol state of the atractoplasm as confirmed by micrurgical operations on the staminal hair cells of *Tradescantia* (Wada 1935). The atractoplasm, together with the chromosomes, can flow out instantly through a fine hole punched on the cell wall by insertion of a microneedle after the needle has been withdrawn (Fig. 25). This phenomenon reveals that the atractoplasm must be intrinsically in a sol state. The intensity of physical attack which would affect the spindle's colloidal state by the insertion of microneedle is so extremely small and localized that any liquefaction or thixotropy of the whole spindle cannot be imagined, although Milovidov (1949) pointed out the flowing out of the whole spindle may be the result of induced thixotropy.

The most important point of our microdissection experiments mentioned above should emphasize the fact that the atractoplasm is so fluid that it can instantly flow out through the fine hole in the cell wall, but not the fact that the streamed out atractoplasm assumes a semi-solid state outside the cell (Mazia 1961). The punched hole usually has a diameter which can allow the chromosomes to pass, but when a chromosome lies crossway against the hole it can block up the hole. In this case, the remaining atractoplasm in the cell changes not only into a gel state but also temporarily shows fibers among the scattered chromosomes in the coagulating cell (Fig. 25a).

Very recently, Bajer (1965b) reported that in the mitosis of *Haemanthus katherinae* endosperm cells the chromosomal fibers are visible *in vivo* under the phase contrast microscope. Reversible appearance of spindle fibers were already demonstrated experimentally by Lewis (1923) in the cell of living chick embryos; he demonstrated that gelation of spindle fibers occurs in a slightly acidified state of the cell.

3. **New interpretations on the data obtained by hydrostatic pressure experiments**

*Chromosomes are the only materials which present a gel state in the spindle of living cells:* Concerning the colloidal state of the nuclear spindle, Pease (1946) has given us very important data. He intended to investigate the colloidal state of the spindle by applying hydrostatic pressure
on meiosis in pollen mother cells of *Tradescantia paludosa*. According to his explanations, each mitotic element shows different resistance to the pressure: the fibrous structure of the spindle is completely obliterated under 6,000 lbs. pressure per square inch, the chromosomal fibers under 8,000 lbs. and all of the kinds of oriented fiber structures under 10,000-15,000 lbs., while the deformation of chromosomes occurs at 4,000 lbs., i.e. undue shortening and rounding of chromosomes occur. Total fusion of chromosomes and rounding appear at 15,000 lbs.

From the data mentioned above, Pease concluded that the spindle is in a gel state, and that this gel structure is essential for anaphase movement. Furthermore, he assumed: 1) by means of gel-sol-gel transformation, motion and force responsible for anaphase chromosome movement is imparted, and 2) the deformation of spindle constituents is induced by thixotropic phenomena of the spindle colloidal gel.

Unfortunately, Pease's interpretation seems to rely on the basis of the preconceived idea that the spindle is in a gel state. If we reconsider the data presented by Pease's hydrostatic pressure experiments according to physical laws without giving consideration to the common ideas of cytology, we should reach a different conclusion concerning the colloidal state of the spindle. First of all, most importance rests on the fact that the rounding of chromosomes takes place at 4,000 lbs., while the obliteration of spindle fibrous structures occurs for the first time under 6,000 lbs. This phenomenon shows that when unduly shortening and rounding of chromosomes occur, the spindle still maintains its normal state. Therefore, the deformation of chromosomes implies that the reduction of their free surface by rounding is a possible occurrence in the normal colloidal state of the spindle substance. If the spindle would be in any kind of gel state and composed of so-called continuous fibers as described in classical cytology, the deformed chromosomes would not be able to show rounding by reducing their free surface. Needless to say, as a physical phenomenon, the rounding up of the substance can occur only in a liquid medium, when both substances are in a liquid state and cannot mix with each other. Therefore, if we take all these data into consideration, the interpretation by Pease, as well as the explanation in classical cytology, on the gel state of the spindle body are physically and, of course, biologically not correct; the intrinsic state of the karyokinetic spindle is a colloidal sol maintaining its shape by its own membrane.

The colloidal state of the spindle cannot be considered to be as simple as that in non-living substances. The thixotropic phenomena of biocolloidal gel may occur probably in special portions of protoplasm but rarely in a highly organized organelle such as the spindle body. Another important finding in Pease's experiment is concerned with the recovery process of chromosomal fibers; his data revealed conclusively that the chromosomal fiber develops from the kinetochore of each chromosome toward the spindle
pole. The details on this problem are described already in Chapter 4.

*Interpretations of spindle obliteration under hydrostatic pressure at the molecular level:* With regard to Pease's hydrostatic pressure experiments, cytologists should keenly reconsider the fact that among other mitotic organelles the solution of chromosome gel occurs most easily by hydrostatic pressure (under 4,000 lbs.). The deformation of the spindle body itself and the obliteration of the fiber structures require strikingly high hydrostatic pressures (under 6,000-15,000 lbs.). Concerning the change of colloidal states in cell organelles, Lehmann (1943) stated that the more the gelation of the endoplasm advances, the stronger is the centrifugal force required to obtain a solated mass by the disjunction of molecular networks. As this principle is available for hydrostatic pressure experiments, the force required to solate a gel structure in the case of Pease's experiment may increase in proportion to the rigidity of organelles. If the spindle were really in a gel state, the data obtained by Pease's hydrostatic pressure experiments would lead to the contradictory result that the spindle body might have been in a more rigid gel state than the chromosomes themselves. This is an altogether unimaginable phenomenon.

Considering various aspects of the data presented by the hydrostatic pressure experiments, it is reasonable to conclude that the force induced by hydrostatic pressure may have been used for phenomena other than the gel-sol transformation of the spindle body itself. Here we have to present a new interpretation. As already explained, the nuclear spindle in living cells is intrinsically in a sol state as it is in the karyolymph. There is no reason to support the explanation that the spindle is physical body in a gel state as described in classical cytology.

According to the atractoplasm theory, the obliteration of fibrous structures under hydrostatic pressure should be interpreted as follows: Under high hydrostatic pressures of more than 6,000 lbs. per square inch, it is assumed that the unit fibrils or the dispersed phase of the atractoplasm may be forced to change their fibrillar forms into globular ones. Concerning the obliteration of chromosomal fibers, it is assumed that the relaxation of the constitutional unit fibrils occur at first and then each unfolded fibril may change to a globular particle as the dispersed phase of colloidal sol. Thus, as all the fibrillar elements transform to globular ones, the spindle can no more maintain its tactoid-like structure or its spindle form as a whole. The difference of the resistibility between the spindle unit fibrils and the chromosomal fibers have been demonstrated by various experimental treatment: i.e. by temperature experiments (Inoué 1952a, b), UV microbeam irradiation (Wada and Izutsu 1960) and by various kinds of chemicals (Wada 1952a, 1953, Niitsu 1958, Sawamura 1964, 1965). This resistibility difference may be attributed to the constitutional difference of both fibrils: the spindle unit fibrils are naked in atractoplasm, while the same fibrils may be protected with kinetochore substance in the
chromosomal fibers. Corresponding to this difference, Pease's experiment indicated that the obliteration of the former fibrils occurs under 6000 lbs., while that of the latter ones under 8,000 lbs. per square inch.

When the cell is released from the pressure and all the other mitotic organelles also resume their activities, the dispersed particles may at first recover their fibrillar form. Then the fibrils distribute anisotropically, converging toward both spindle poles and taking a tactoid-like form. On the basis of microscopic observations of spindle in fixed preparations, Ehrenberg and Östergren (1942) suggested that the spindle is tactoid-like in nature. This interpretation has been supported also at the electron microscopic level by Sató (1959, 1960). The secretion of kinetochore substance and the recovery of the fibrillar texture of the atractoplasm may be called for as a premise to the reappearance of chromosomal fibers. Under this condition, some of the unit fibrils lying between the spindle poles and the kinetochores of each chromosome organize chromosomal fibers together with the flowing out kinetochore substance. Consequently, each chromosomal fiber grows out from the kinetochores toward the spindle poles. In this way, the spindle as a whole recovers its fibrillar structures and functional conditions.

At the molecular level, the obliteration of the karyokinetic spindle and its recovery may be interpreted as a reversible change of folding and unfolding of the spindle unit fibrils. As to the mechanism of reversible folding and unfolding of spindle unit fibrils we know nothing at present. However, it is not entirely impossible to conjecture that the force caused by hydrostatic pressure must be strong enough to overcome the interfibrillar force which may cause fibrils to be arranged in a tactoid form under the bipolar force of mitotic cells. Also, the force may be powerful enough to induce relaxation of the molecular force which keeps the dispersed protein molecules in a strained fibrillar state.

The change of spindle fibrillar structures can be induced by treatment with chemicals of various kinds, or by physical treatment of various sources. The results of these treatments may generally induce irreversibility but sometimes reversibly the disintegration of unit fibrils, of chromosomal fibers, or both of them in various degrees. Thus, chromosome doubling, binucleate cells, multinucleate cells with multisepta, and others appear as the results of aberrations against spindle activities. In Tradescantia tests in vivo, the cause-and-effect relations on these mitotic aberrations are analyzable step by step continuous observations, but in the generally used Allium test, interpretations may depend on researchers' judgement and conjecture because the observations are carried out on killed static figures of mitotic cells in fixed preparations.

**Conclusion**: Concerning the plasticity of the atractoplasm, it can be concluded that an increase in spindle contents by change of colloidal states may give rise an apparent increase of spindle rigidity and that of its mem-
brane tension. Consequently, there is a possibility of misjudging the spindle as a gel body, in spite of the liquid contents, just as in the case of a thin rubber balloon filled up with air. In classical cytology, the presence of the spindle membrane has been disregarded for a long time as a result of inadequate fixatives. Therefore, the rigidity of the spindle was attributed to the hardness of spindle content itself and this misunderstanding induced the conclusion that the spindle is a physical body in a gel state (Mazia 1961).

Apparent rigidity of the karyokinetic spindle is maintained by its own membrane according to its tension but not by rigidity of its contents.

From the results of various experiments on mitotic organelles, it is concluded that the chromosomal fibers are real threads composed of a bundle of innumerable unit fibrils growing from kinetochores of each chromosome toward the spindle poles. Other fibrous structures, such as continuous fibers or interzonal connections, are nothing but optical images of anisotropic distribution of unit fibrils as the dispersed phase of atractoplasm colloidal sol. Consequently, the karyokinetic spindle is in a sol state as long as the cell is alive. The formation of the spindle shape may be attributed on one hand to the bipolar force in the mitotic cell and, on the other hand, to the molecular interactions of the fibrillar dispersed phase of atractoplasm colloidal sol, probably by similar intermolecular forces in the case of the tactoid formation with inorganic or organic fibrillar macromolecules.

4. Interfibrillar liquid

Dispersion phase of atractoplasm colloidal sol: The interfibrillar liquid cannot be ignored in the living state of the mitotic spindle, but it seems to be practically disregarded in the study of fixed cells. Brownian movement of fine granules in the spindle has been reported by in vivo observations on plant cells (Bělař 1929, Wada 1936a, b, Bajer 1956). The granules can move in a narrowly limited space and oscillate for longer distances parallel to the spindle axis. This result of in vivo observations indicates decisively that it is incorrect to assume the spindle as a whole to be in a gel state. Based on the fact of unique Brownian movement of granules in the spindle and the anisotropic distribution of fine fibrils demonstrated by electron micrographs, it is most reasonable to conclude that the interfibrillar liquid is nothing but the dispersion phase of the atractoplasm colloidal sol. In this case, the unit fibrils, of course, distribute evenly in the dispersion phase. Each unit fibril in living cells may be a single or a small aggregation of molecular chains, so that their presence together with water molecules may be a cause to excite Brownian movement, but not an obstacle to suppress the oscillation of the granules whose sizes are in microscopical dimensions. Therefore, the narrow spaces in which granules oscillate longitudinally are interspaces among the chromosomal fibers, but not those among the so-called continuous fibers.

Concerning the origin of such granules in the spindle, most cytologists
can scarcely doubt that granules may have migrated from the cytoplasm, so long as they believe in the old concept that no interfacial border is present between the spindle substance and the cytoplasm. However, as repeatedly explained, no cytoplasmic granules can enter into the spindle body; the origin of these granules may originate in the spindle. Fortunately, we had several opportunities to confirm the appearance of granules in the spindle by in vivo observations of Tradescantia hair cells.

In plasmolysis experiments treated with sucrose solution or in dehydration experiments by dried air, it was found that fine granules appeared in the spindle body of the Tradescantia hair cells whenever the treated cells were brought back to a normal state of cell hydration (Wada 1936a, b). These granules are small enough to perform Brownian movement and are inferred to be produced by aggregation of submicroscopic particles. Concerning the appearance of the granules, Bélař explained nothing in his plasmolysis experiments but it may originate by the same reasons as mentioned above. In the case of dehydration of the cell, it may be possible to assume that the particles as reported by Roth (1964) and Satô (1958, 1959), or other particles scattered in the atractoplasm, aggregate by their dispersity change during dehydration and become visible under the microscope. The granules which once appeared in the spindle during meta- and anaphase remain in the phragmoplast in telophase and most of them are discharged into the cytoplasm when the phragmoplast disappeared.

On the basis of the fine structure of the spindle and phragmoplast, the interfibrillar liquid implies the dispersion phase of the colloidal sol composing of the atractoplasm and the phragmoplast substance.

Conclusion: Summarizing the data mentioned above, it is most reasonable to conclude that the unit fibrils in the spindle are nothing but the dispersed phase of the atractoplasm colloidal sol. The dispersed phase of karyolymph colloidal sol can transform the individual shape from globular form to fibrillar when the karyolymph changes into atractoplasm in the prometaphase. Under the influence of bipolar force presiding over the mitotic cell, the fibrils disperse not only anisotropically but also organize, as a whole, a tactoid-like structure and there is no reason to consider the spindle as a gel state. The so-called interfibrillar liquid is the dispersion phase of the atractoplasm colloidal sol in which no fibers are present except chromosomal fibers.