Dynamics of Spindle Formation and Its Inhibition by Chemicals

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PLATES 111 AND 112

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ABSTRACT

The formation of the mitotic spindle of the newt cell in tissue culture has been studied, using polarized light. The rate of formation was measured and it was shown that the spindle increased in length at a constant rate until the maximum was attained. During metaphase the spindle shortened to about 50 to 60 per cent of its original length, reaching a minimum just before anaphase. No birefringence was detected in late anaphase in the spindle region after the chromosome masses had separated.

The effects of certain compounds which are believed to inhibit protein synthesis were investigated. Chloramphenicol added in early prophase prevented the formation of a spindle of normal length. The possible relation of chloramphenicol to the synthesis of spindle proteins is discussed.

Polarized light has been used to study the mitotic spindle in a variety of living cells (1–4). The most detailed studies, however, have been concerned with the changes in birefringence which accompany the anaphase movement of the chromosomes (1, 5), while the formation of the spindle has been neglected. Since most of the work on spindle formation comes from fixed preparations, the present study was undertaken to follow the process in the living cell using polarized light. The newt (*Triturus viridescens*) was selected because the cells are large and changes in the mitotic figure are easy to measure.

Classical cytologists have defined two types of spindles, the anastral and the amphiastral (6). The amphiastral type is divided into two classes. In the majority class the asters separate and migrate to opposite poles of the nucleus and then the spindle fibers radiate out, penetrating the nuclear region. In the minority class, which includes the newt, the spindle forms between the asters while they are separating.

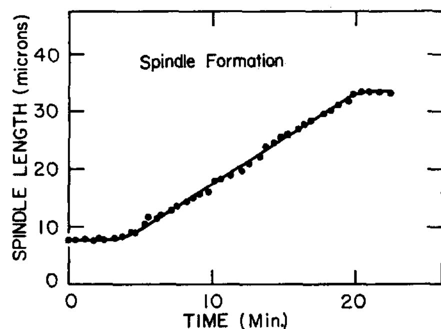
The effects on cell division of a great variety of compounds have been studied (for a review see Hughes (7)). In the cells used in the present study, at least half the protein of the spindle must be synthesized for each division. Therefore, some inhibitors of protein synthesis were tested in an attempt to obtain information regarding synthesis of the spindle protein.

Materials and Methods

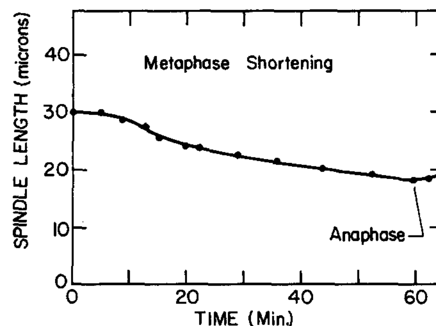
The experiments were performed on thin slide cultures obtained from fragments of the heart of adult newt (*Triturus viridescens*) by a technique outlined previously (8). For the inhibitor experiments the chamber was opened, and the top coverslip, to which the cells adhere, was rinsed quickly and transferred to a new culture chamber containing the inhibitor dissolved in amphibian Tyrode solution or Eagle's basal medium diluted to amphibian isotonicity.

The observations were made with a Leitz Wetzlar petrographic microscope with a polaroid plate and a Leitz 10x objective substituted for the Nicol polarizer and condenser. Light from a 1000-W high pressure mercury arc (General Electric AH-6) was imaged on a pinhole aperture which was in turn imaged in the object plane by the substage objective. By this method a region no larger than the cell under study was illuminated; this reduced stray light from other objects in the field. The contrast of the birefringent material was increased by the use of a Leitz $\lambda/10$ elliptical compensator, as described by Swann and Mitchison (9). The observations and photographs were made with a 45x Leitz objective and an 8x eye piece.[1] All measurements were carried out at room temperature (23°C.).

[1] The author is indebted to Dr. R. B. Uretz who developed the optical system used in this study.



TEXT-FIG. 1. Rate of spindle formation.



TEXT-FIG. 2. Rate of spindle shortening during metaphase.

TABLE I

Formation of Individual Mitotic Spindles

Rate of formation	Maximum length
microns/min.	*microns*
1.15	32.0
1.35	42.0
1.33	32.0
0.85	34.0
1.15	31.3
1.39	33.3
1.15	31.3
1.50	26.3
1.10	50.0
2.43	36.3
2.08	39.3
1.43	30.3
1.53	31.0
1.50	33.5
1.43	34.0
Standard deviation, 0.39	Standard deviation, 5.7

OBSERVATIONS

Formation of the Spindle.—In a few favorable cases the centrosome can be detected in early prophase in the cytoplasm (Fig. 1, *a*). It consists of a pair of birefringent bodies, (the asters) united in a single structure. This structure migrates into the nuclear region and then elongates to form the spindle (Fig. 1 *b* to *f*). Usually the asters are first observed in the nuclear region after the breakdown of the nuclear membrane. At completion of its formation, the spindle is a prolate spheroid which may reach a length of up to 50 microns. A typical curve for spindle formation is shown in Text-fig. 1. It should be noted that throughout most of the process the length increases linearly with time. The results of a series of determinations of the rate of formation (*i.e.*, the slope of the linear portion of the curve) and the maximum length attained are shown in Table I.[2] The rate of formation and the maximum length vary by as much as a factor of two for this group of cells.

During the period of spindle elongation, the chromosomes shorten and thicken and begin to migrate onto the metaphase plate. About 1 hour usually elapses between the end of spindle growth and the beginning of anaphase. However, soon (*i.e.*, 2 to 4 minutes) after the spindle has formed to maximum length it begins to shorten and thicken. This shortening phase is illustrated by the curve of Text-fig. 2 and the photographs of Fig. 2. The spindle reaches minimum length just before anaphase, having shortened to about 50 to 60 per cent of its maximum length.

During anaphase the chromosomes move to the poles and the spindle elongates again. The newt chromosomes are large and tend to obscure the spindle birefringence during anaphase, but when the chromosome groups have separated there is no detectable birefringence remaining between them in the spindle region (Fig. 2 *i*).

Inhibition of Spindle Formation.—Several compounds are known which inhibit protein synthesis in microorganisms (10, 11). If these inhibitors also block protein synthesis in metazoan cells, they might be expected to have a specific effect on cell division by preventing the synthesis of the spindle protein. Chloramphenicol and two amino acid

[2] A single phase contrast film (1-47) from the collection of Dr. Zirkle and Dr. Bloom clearly shows spindle formation. On this film the spindle happened to be outlined by a large number of tiny fat droplets. The rate of formation and the maximal length of this spindle fall within the limits obtained above.

TABLE II

Effect of the Time of Addition of Chloramphenicol

Time of addition	Maximal length of spindle	Effect on division
min.	*microns*	
+5	34	Normal anaphase
−4	28	" "
−4	23	" "
−12	31	" "
−12	23	" "
−13	21	" "
−14	13	" "
−17	15	" "
−19	20	" "
−22	20	Rosette
−23	18	Normal anaphase
−28	11	" "
−29	18	" "
−32	8	Rosette
−34	8	"
−43	5	"
−48	8	" with division*
−55	5	"
−60	5	" with division*
−60	5	"
−68	5	"
−75	8	"
−80	8	" with division*

* Apparently false anaphase.

analogues, *p*-fluorophenyl alanine and β-2-thienyl alanine, were tested. The culture was transferred to a medium consisting of amphibian Tyrode solution plus 1 mg./ml. of the inhibitor. Cell divisions was not inhibited by the amino acid analogues; spindles of normal length were formed at a normal rate for at least 2 to 3 hours after the inhibitor was added. Chloramphenicol, however, produced a striking inhibition of cell division. Cells which had already begun to form a spindle proceeded through division at a normal rate, while cells which had not yet begun spindle formation produced short spindles or no spindles at all. An intermediate concentration of inhibitor (750 μg./ml.) was selected, and a series of experiments was carried out to determine whether there was a correlation between the maximum length of spindle formed and the time at which the inhibitor was added. The results are shown in Table II.

The zero of time was chosen as the time at which asters were clearly visible in polarized light; thus the entry "−19 minutes" means that the drug was added in prophase and the asters were detected 19 minutes later. The time scale is somewhat subjective since the earliest time at which the asters are clearly seen depends on their position in the cell. Nevertheless, a general trend is evident. Spindles formed within about 10 or 15 minutes after addition of the drug (times between −10 and −15 in Table II) were normal. For addition times of from −15 to −30 minutes, the spindles were shorter than normal, although anaphase was still possible. For addition times earlier than −30 minutes, the spindle was very short, a metaphase plate was not formed, and the chromosomes grouped themselves around a small birefringent body which may be a pair of asters or a very short spindle. Since the kinetochores point to the inside, this configuration has been termed a rosette (8). Some cells in this configuration divide by what appears to be the "false anaphase" process described by Bloom, Zirkle, and Uretz (8), in which whole chromosomes separate into two groups, each group being in a rosette configuration. This step is followed by normal constriction.

DISCUSSION

The results on the formation of the spindle in the newt confirm the work of classical cytologists. The rate of elongation and the maximum length of the spindle vary from cell to cell by as much as a factor of two, but the average rate of formation (1.43 μ/min.) is comparable to the average rate of spindle elongation in anaphase (unpublished observations).

During metaphase the spindle becomes shorter and thicker. This effect has not been stressed by previous authors. It cannot be attributed to a rounding up of the cell, since it is found in cells which remain elongated during metaphase. It may be caused by a crowding of the chromosomes on the metaphase plate, which forces the chromosomal fibers apart and thus increases the diameter of the spindle. On the other hand, it may be an integral part of the anaphase motion.

Individual fibers can be resolved in the spindle. By rotating the culture, up to nine fibers of varying cross-section have been distinguished. Usually they appear to connect with the chromosomes, although occasionally a fiber can be seen stretching from pole to pole. The majority of the birefringence in the newt cell appears to be due to chromosomal fibers. When the chromosomes separate in anaphase, no birefringence can be detected in the region between the chromosome groups. Chick cells (12) and human liver cells in

tissue culture (unpublished observations) show considerable birefringence after the chromosome groups have separated. Therefore, we cannot generalize about spindle structure until several different organisms have been studied.

The configuration obtained in the inhibition experiments, which we have called a rosette, has also been described by Barber and Callan (13) in newt larval tail-tip cells treated with colchicine or subjected to low temperatures. These authors refer to this configuration as a "star metaphase," and they also show a "bifocal star," which resembles our false anaphase.

The effect of chloramphenicol on the formation of the spindle can be explained if the spindle protein is being synthesized during the period of about an hour before the formation of the spindle. Chloramphenicol may block protein synthesis, and the cell constructs a spindle (sometimes undersized) from the protein already present before the drug was added. Evidently protein synthesis is complete before visible spindle formation begins.

Thienyl alanine has been reported to be ineffective on protein synthesis in chick heart cells grown in a medium containing both protein and amino acids, since the cells preferentially utilized the protein (14). In our experiments both an explanted tissue piece and a plasma clot are present and these may contribute material which reverses any inhibition. Unlike the amino acid analogues, chloramphenicol is a non-competitive inhibitor, and similar effects are obtained whether it is added in Eagle's basal medium or in Tyrode solution. The concentration used in these experiments is many times greater than that which is needed to block protein synthesis in microorganisms. Recent experiments (15) on isolated thymus nuclei show an inhibition of protein synthesis in the range 200 to 2000 μg./ml. which is comparable to the inhibition range found in our experiments. However, ribosenucleic acid synthesis was also blocked, so the effect is not so simple as was originally believed. The interpretation that we have proposed must be regarded as speculative until further evidence is obtained from metabolic studies. The newt is unsuitable for this purpose since the cells grow too slowly to provide sufficient material for chemical analysis. Further experiments are planned with mammalian cultures.

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Explanation of Plates

Plate 111

Fig. 1. Representative stages in spindle formation in newt cells in tissue culture as seen by polarized light. Photographs *b* to *f* are from the same cell. In each case the arrow points to a centriole; *a*, cell in very early prophase. In this material *a* represents the earliest stage observed in the formation of the spindle. The two centrioles (the diplosome) are still very close together. As the centrioles move apart the spindle develops between them, as seen in Figs. *b* to *f*. *b*, early stage of spindle formation showing separate asters. *c*, 6 minutes after *b*. *d*, 9 minutes after *b*. *e*, 12 minutes after *b*. Chromosomes are beginning to migrate onto metaphase plate. *f*, 18 minutes after *b*. Spindle elongation complete. Metaphase plate has formed.

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PLATE 111
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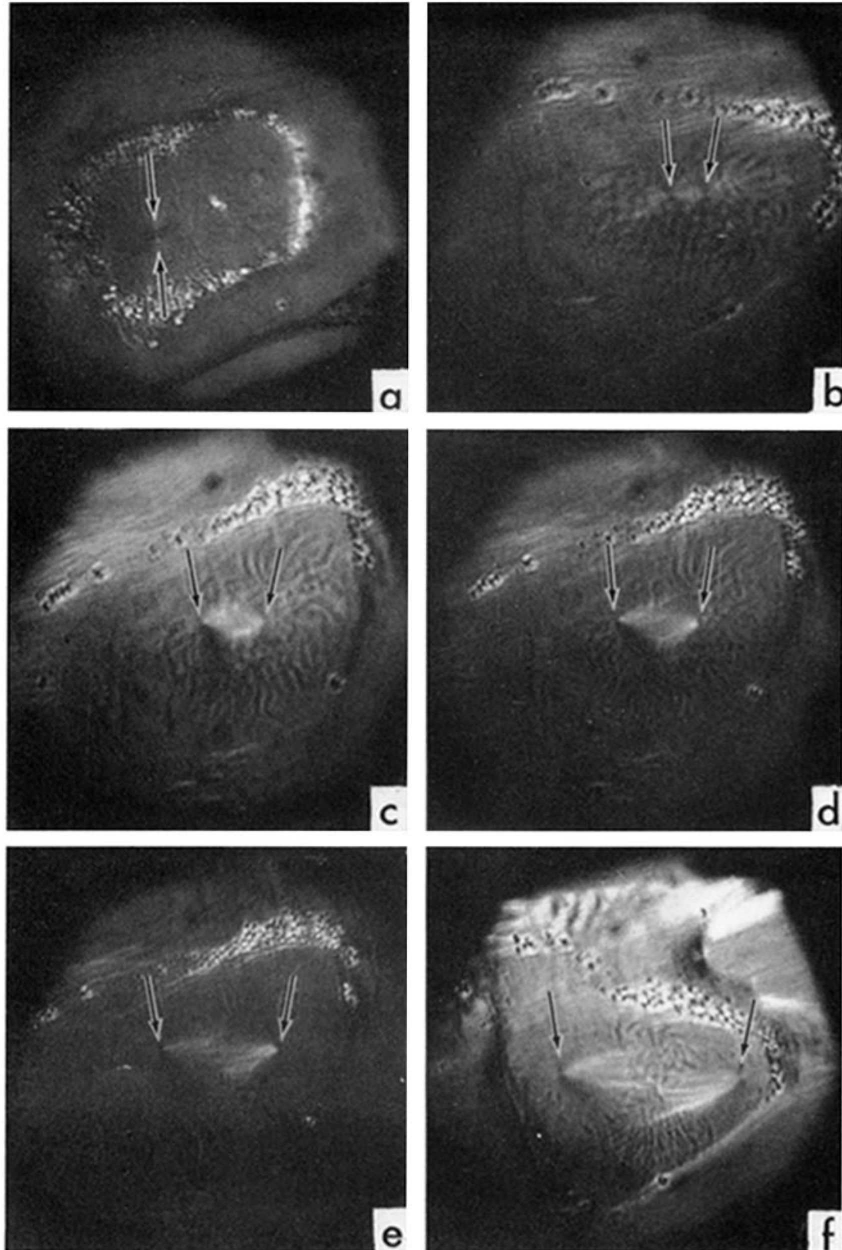

(Taylor: Dynamics of spindle formation)

Plate 112

Fig. 2. Shortening of the spindle in a newt cell during metaphase. *a*, spindle elongation stage has just been completed. Note the long narrow spindle. *b*, 8 minutes after *a*. *c*, 19 minutes after *a*. *d*, 35 minutes after *a*. *e*, 45 minutes after *a*. *f*, 62 minutes after *a*. Spindle at minimum length. *g*, 64 minutes after *a*. Anaphase begins. *h*, 66 minutes after *a*. *i*, 69 minutes after *a*. Note there is little or no birefringence between chromosome groups.

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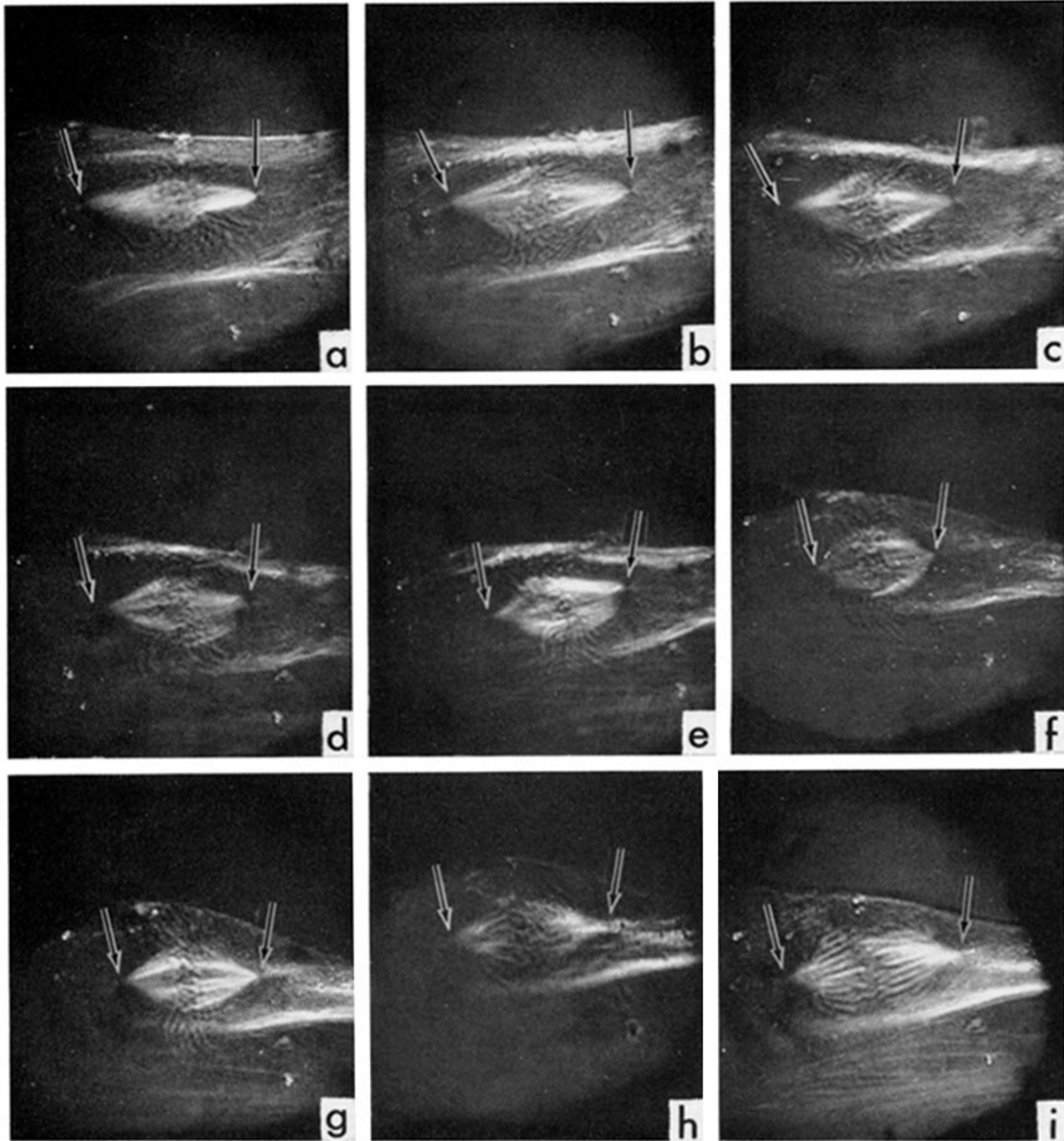

(Taylor: Dynamics of spindle formation)