CHAPTER 3

Spindle and Cytoplasm

3.1: Colchicine and Spindle Fibers

More metaphases than anaphases or telophases collect in tissues treated with colchicine, creating an impression that chromosomes appear stranded between the two poles. Obviously colchicine blocks the mechanism that regularly moves them to the respective poles (Fig. 3.1*A,B*). Interference seems to be localized at the spindle fiber; consequently, arrested metaphases pile up in greater numbers per given area than do the other mitotic stages.[28, 58, 1]

A disproportion of metaphases was pictured by Pernice in 1889. His illustrations[35, 34] show many arrested metaphases with very few anaphases; the contact between the drug and intestinal cells of the dog blocked mitosis (Fig. 1.4).

If the spindle fiber is the substrate where colchicine acts — and there are many data to support this assumption — then cytological and biochemical methods should show us more clearly what reactions occur. The basic cause for a mitotic arrest undoubtedly is to be found in the chemistry and physiology of the spindle fiber and attending mechanisms.[31]

Provisionally, let us say that colchicine alters rather than totally destroys the spindle substance. Such assumptions are consistent with cytological tests. It is known that arrested metaphases fail to show the usual spindle fibers as linear structures; therefore, conversion of a fibriform element into a corpuscular one becomes a tempting suggestion, with attractive possibilities for explaining, at one level, how the spindle fiber and colchicine interact.[73, 38, 47, 95, 72, 37]

Molecules of colchicine reacting with a molecular system of spindle substrate have been considered as one of the basic relationships between the two substances[55, 38, 39, 57, 73] Such an explanation can be given on a quantitative basis. The destruction or inhibition of the fiber then appears to be a quantitative reaction, because the concentration of colchicine is a critical factor.

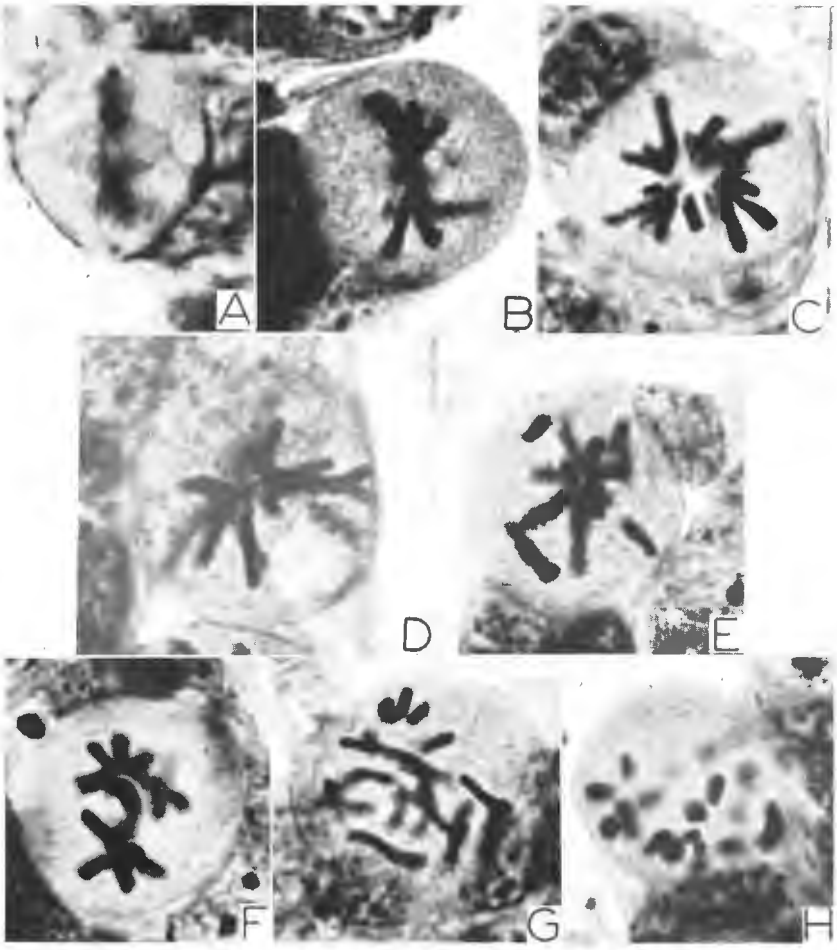


Fig. 3.1—Photomicrographs from embryo of grasshopper, sectioned 13 microns, stained with iron hematoxylin. **A.** Untreated cell at metaphase, spindle fibers differentiated. **B.** Cell treated, 25×10^{-6} M, 30-minute exposure; spindle fibers reduced by treatment but chromosomes not dispersed. **C.** Concentration of, 2.5×10^{-6} M, 90 minutes; star metaphase with some spindle activity. **D.** Clear spherical area, which is not stained, is the hyaline globule, that increases when spindle substance disappears as a result of treatment with colchicine. **E.** Chromosomes outside the star, 120 minutes, with 2.5×10^{-6} M concentration. **F.** Multiple stars, three in one cell, 2.5×10^{-6} M, 180 minutes. **G.** Exploded c-metaphase derived from prometaphase treatment, 2.5×10^{-6} M, 15 minutes. **H.** Chromosomes shortened after 180 minutes, 2.5×10^{-6} M, settle to bottom of cell. (Photographs provided through courtesy of Drs. M. Gaulden and J. Carlson. Adopted from **Experimental Cell Research** 2:416–33, 1951.)

Wide ranges of concentration induce a wide variety of reactions. These range from extremely minute changes involving the spindle orientation, the *tropokinesis,*[38] to the full c-mitosis, *stathmokinesis,* obtained by strong doses.[28, 38, 73, 25] These two reactions represent the extremes, between which there can occur many intermediate changes.

Before proceeding further, we should recall the old argument about spindle fiber reality as opposed to "artefact." If we are dealing with a specific molecular problem, the possibility that spindle fibers are artefacts would seriously influence our proposition. Perhaps the whole concept would be annulled. But excellent results, obtained from treated and untreated cells and from living and fixed materials, have opened up new approaches. Hence, the argument that spindle fibers are not real is almost extinct. An entirely new series of studies with phase contrast microscopes, polarization microscopes, cinematography, and other techniques has shown that fixed and stained fibers are similar to the living functional linear structures.[47] Colchicine has been employed most effectively in these studies.

A high specificity can be demonstrated between colchicine and spindle fibers.[15, 59, 9, 88, 54] Moreover, this specificity can be quickly destroyed if the chemical structure of the drug is changed only slightly. Pharmacobiologists have known for a long time that certain derivatives such as colchiceine are less active pharmacologically than colchicine. Numerous chemical derivatives of colchicine are accurately known by chemists and these have become available to biologists.[80] For example, isocolchicine is a transformed molecule of colchicine, that involves a shift in the position of keto and methoxyl groups on ring C. By this change the specificity between spindle fiber and colchicine is reduced.[88] Isocolchicine is one hundred times less active in producing a c-mitosis than colchicine.

The specificity between colchicine and spindle appears to be on the order of the enzyme and substrate specificity.

Admittedly, the spindle fiber mechanism is complex, highly organized, and delicately coordinated. But much is understood of this mechanism in animals and plants. Cytologists agree that two sets of fibers are formed at each regular mitosis: the continuous and the chromosomal.

The reaction between colchicine and the several components of the spindle appears, then, to have a quantitative basis. Some portions of the spindle can be inactivated leaving other portions activated. Such fractionating possibilities have been demonstrated,[55] and this fact merits attention.

3.2: Spindle Inhibition

Every mitotic cycle builds anew the spindle fibers. Cytoplasmic separation, a function of cytokinesis, is closely coordinated with the fiber and spindle functions.[94] Colchicine prevents the formation of a spindle at prophase, precludes a nuclear mitosis, delays chromosomal separation, inhibits daughter nuclei, and effectively blocks cleavage processes.

Among plants, the inhibition starts at the polar cap stage when polarity makes an appearance.[95] The first sign that colchicine acts upon a spindle is noticed at the polar cap stage.[95] Among animals, the preliminary spindle inhibition is an interference with the development of the astral rays, and functioning of the centriole outside the nucleus.[5] The initial inhibiting influence is seen at the time nuclear membranes are about to disappear and the centrioles begin their movement.

The prophase orientation of chromosomes in animal cells may or may not be destroyed by colchicine. Likewise, plant cells, e.g. in *Dipcadi,* have a prophase orientation that is determined from the previous telophase. These arrangements are not disturbed by colchicine. Thus, colchicine may inhibit the spindle without changing a basic chromosomal arrangement at prophase,[55] although strong solutions may interfere with the orientation before membranes disappear.

The bipolar mitosis is effectively prevented by colchicine acting at late prophase, and progressive changes from interphase into prophase are not inhibited by colchicine.

Undoubtedly there is an action upon resting cells if strong concentrations are used.[67, 68] Nuclear poisoning,[12] intranuclear precipitates,[44] chromatin condensation,[13] pycnotic destruction,[28, 31, 24] and nuclear degeneration[60] before mitotic arrest, are possible actions of colchicine. Deeply stained inclusions in cells of Amphibia were observed after strong treatments.[66] In most cases concentrations above the threshold for c-mitosis induce the changes. Neuroblastic cells of grasshopper, usually very responsive at prophase, metaphase, and anaphase, require a tremendous concentration ($1000 \times 10^{-6}\ M$) at interphase or late telophase.[37]

The mitotic stage at which colchicine is most effective in lowest concentration, is late prophase. There is no doubt that colchicine interferes with transformations of karyolymph, because the regular linear arrangements of fibers do not develop. These structures normally are formed 20 minutes after disappearance of the nuclear membrane; but in the presence of colchicine, fibers do not form. Instead, there is formed a hyaline globule in grasshopper neuroblastic cells, which is nonfibrous.

Similarly for *Tradescantia*, fibers do not develop at prophase with concentrations of 0.05 per cent or 0.1 per cent colchicine.[95] There are other cases, but these two are enough to prove that the first stage of spindle inhibition sets in at prophase.

Full strength solutions applied at prophase cause total inhibition; no vestige of the mitotic spindle can be observed. Partial inactivations are only found at the threshold levels.[73] The continuous fibers and astral rays rather than chromosomal fibers are then the ones inhibited during a partial inactivation. That is, enough colchicine is present to inhibit the exterior spindle, but the interior spindle develops. Such partial inactivation leads to a star metaphase.

Spindle material may be converted into such bodies as hyaline globules,[37] (Fig. 3.1*D*), the lakelike substance in *Arbacia*[5] (Fig. 3.5), achromatic sphere of *Allium*[29, 7] (Fig. 3.6), or the deformed atractoplasm among *Tradescantia*.[95] All these structures are closely associated to karyolymph; consequently, the inhibiting process of a normal spindle fiber is in reality transformation to another form of substrate.

Electron microscopic analysis of colchicine-treated polar cap stages in *Allium* indicated a "solubilization" and "fragmentation" of fibrous strands. These changes are interpreted as spindle fiber transformations. Submicroscopic interpretations are difficult, but the evidence is consistent with other microscopic data.[82]

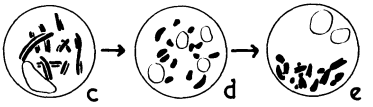
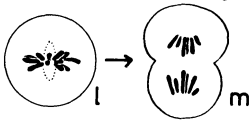
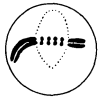
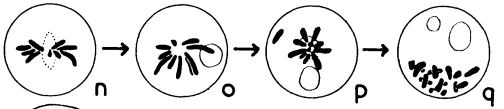
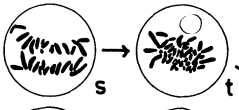
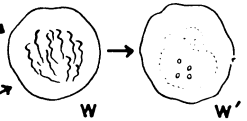
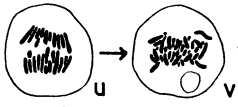
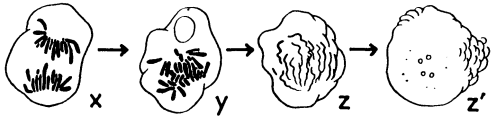
A primary effect of colchicine is the inhibiton of a mitotic spindle.[7] Secondary effects stemming from this action are colchicine pairs, chromosomal changes, desynchronization of mitotic processes, delayed separation of chromosomes, and restitution nuclei instead of daughter nuclei.[7]

Originally the term *colchicine-mitosis* designated an "effect of colchicine on the course of mitosis" that is entirely specific.[55] Additionally, in a colchicine-mitosis the spindle apparatus is totally inactivated, and this causes completion of a "chromosome mitosis without nuclear or cellular mitosis." [55]

3.3: Destruction of the Spindle Fibers

That colchicine inhibits the spindle at late prophase is well established. Less familiar are the facts about colchicine when applied to a mitotic spindle that has developed as far as anaphase (Fig. 3.2*s–v*).

To establish these facts, special techniques had to be developed. Individual cells must be observed at the critical stage, anaphase, and the chemical must be applied at a precise moment when the mitosis has reached a certain stage. Fortunately, several excellent methods for plants and animals[47, 5, 95, 64, 37] have been developed, and we may now learn what happens when the drug is added to a cell after a spindle has formed.

Mitotic stage treated	Colchicine x10-6 molar	SUCCESSIVE CHANGES
late prophase 	50.25	→  a → b
	2,5	→  c → d → e
	1,9	→  f → g → h
prometaph. 	25_2,5	→  i → j → k
	0,2	→  l → m
metaphase 	25	→  n → o → p → q
	2,5	→  r
anaphase   	50.25	→  s → t →  w → w'
	50_25	→  u → v → w → w'
	50_25	→  x → y → z → z'

The spindle fibers at anaphase can be destroyed by the proper concentration of colchicine. Thus, in addition to an inhibitive action upon a spindle at the start of the mitotic cycle, the spindle fibers can be reduced after they have been formed (Fig. 3.1*A–G*). The destructive action at anaphase follows a regular order, and there is a quantitative as well as a qualitative basis for the change.

3.3–1: *Neuroblast cells of grasshopper.* The technique developed by Professor J. Carlson, University of Tennessee, and used effectively in cooperative research with Dr. M. E. Gaulden, Oak Ridge Laboratories, Tennessee, has given a new insight to the relationship between colchicine and spindle fibers. Continuous observations upon living cells, together with the application of the chemical at a specific stage and in variable concentrations, have been a valuable addition. In fact, the answer to our question about anaphase and colchicine demands this kind of special method for watching an action upon the fiber (Figs. 3.1 and 3.2).

Cells at early, middle, and late anaphase were chosen. Strong concentrations (50 and 25 $\times$ 10^{-6} M) were used, and in each instance the spindle was "impaired almost immediately"[37] (Fig. 3.2*t*). The chromosomes stopped in their movement to the poles; the two groups intermingled, fused, and formed into a single telophasic nucleus (Fig. 3.2*s–w'*). This restitution nucleus was tetraploid, since the anaphasic separation of centromeres had taken place before the drug was applied. Four nucleoli appeared instead of two, and the "uncoiling" processes were only slightly delayed by colchicine (Fig. 3.2*w'*). Spindle fibers were destroyed at anaphase.

When the concentration was reduced to 2.5 $\times$ 10^{-6} M for the same stage, an anaphase, no detectable results were observed. The chromosomes continued to move to the respective poles. Yet this same concentration invoked a definite reaction at an earlier mitotic stage, i.e., late prophase or pro-metaphase (Fig. 3.2*c*).[37]

Fig 3.2—Mitotic stage when treatment began, shown in right column. Concentrations are expressed in molarity. Successive stages are lettered **a** to **z'**. **a** and **b**: prophase reversions occurring 10 to 20 minutes after treatment with this strong concentration. Chromatin resembles early prophase. **c** to **e**: chromosomes lie at random, no spindle formed, exploded c-metaphases, chromosomes continue to shorten, then clump together in groups at bottom of cell, hyaline globules formed in **d** rise to top of cell. **f** to **h**: the evolution of a star metaphase. **i** to **k**: star metaphase that becomes increased to multiple star and lost chromosomes. **l** to **m**: weak solutions do not fully inhibit spindle but reduce the size. **n** to **q**: the metaphasic spindle is reduced, hyaline globules form in **o**, chromosomes settle to bottom and globules rise in cell. **r** cell divides when concentration is too weak to destroy spindle completely. Compare figure **r** and **c**, that received same concentration, but applied at different stages. Anaphase spindles are reduced if concentration is 25 x 10^{-6} **M** or more. Chromosomes fuse and intermingle in **t** and **v**, hyaline globule forms in stages **t**, **v**, and **y**. Four nucleoli in **w'** and **z'** indicate a tetraploid restitution nucleus. These stages show the interaction of concentration, stage of mitosis, and length of exposure. (Diagrams adapted from M. Gaulden and J. Carlson, **Experimental Cell Research** 2:416–33, 1951)

A fully formed metaphasic spindle was reduced by weaker concentrations than those necessary for anaphase. Specific concentrations applied to the fully formed metaphasic spindle led directly to a star metaphase (cf. Chapter 2). These stars formed by treated metaphases persisted for five or six hours. During this time the Brownian movement shown by the mitochondria was actively increasing. While the activity of the protoplasmic material was increasing, the metaphasic spindle fibers were being reduced.

With further reduction of concentrations and with application to metaphase, no obvious reduction of the spindle was obtained. This concentration (2.5×10^{-6} *M*) had no effect on anaphase, but produced a slight retardation of the spindle at metaphase. Yet this same concentration applied to earlier stages, the prophase, induced visible and truly inhibitive effects. No visible changes were observed at full metaphase by the concentration 1.9×10^{-6} *M*.

Pro-metaphase, an earlier stage than metaphase, responded (Fig. 3.2*i–k*) immediately to a strength (2.5×10^{-6} *M*) that was without detectable action at anaphase. The spindle formed at late prophase was immediately reduced, and the chromosomes scattered in the cytoplasm: a typical exploded metaphase. Doses without influence at anaphase and with only slight effectiveness at metaphase were totally effective at pro-metaphase, or late prophase (Fig. 3.2*c–e*).

Reduction to a concentration of 1.9×10^{-6} *M*, effective at metaphase and now applied at prophase, created the star metaphase. Under these conditions, several focal points for the star remained after treatment (Fig. 3.2*f,g*). Hence, this concentration usually led to the multiple star metaphase (Fig 3.2*j*). The particular concentration inducing stars was effective only at prophase. Now, compare the difference between an effective concentration at prophase, $.2 \times 10^{-6}$ *M*, with the concentration required to reduce the anaphasic spindle,[37] 25×10^{-6} *M*. The difference is significant.

Since, as one approaches interphase from anaphase, correspondingly weaker concentrations are required, it becomes a point of interest to note requirements for detectable results at interphase, or resting stage, or even late telophase. The concentration was raised to 1000×10^{-6} *M* before any changes were noticed, and then the toxic action as well as pycnotic changes were the only results obtained. From all these tests there appears to be a critical point in the mitotic cycle when spindle fibers can be reduced with a minimum concentration.[37] That stage is late prophase and pro-metaphase.

Three important conclusions were reached:[37] (1) Effectiveness in destroying the spindle or interference with its further development depends upon concentration; the greater the concentration, the greater the effectiveness upon the spindle, within certain limits. (2)

A greater concentration is necessary to destroy the more advanced spindle, i.e., at anaphase, than a spindle at an early stage, pro-metaphase. (3) The form of a particular spindle is directly related to the characteristic type of metaphasic pattern that will develop after treatment such as the star, multiple star, ball, exploded, or other arrested metaphase.[37] Configurations depend upon stage at time of treatment, concentration, and duration of treatment or recovery.

After sober reflection upon these conclusions no one can disregard the importance of a specific concentration, the type of cell, and, most interesting of all, the particular mitotic stage at the time the drug enters the cell. Specificity between chemical and spindle fiber is supported by these investigations.

3.3—2: Staminal hair cells of Tradescantia. Techniques with the *Tradescantia* material were used quite as effectively as with the neuroblastic cells just reviewed. The central feature and main advantage lie in the possibility of applying colchicine at a particular stage and following the progressive development of mitosis thereafter. *Tradescantia* staminal hair cells have been a favorite material for mitotic studies *in vivo* for a long time. The first studies to be conducted with colchicine and plant cells were accomplished with the staminal hair cells.[72]

Colchicine applied to a cell when the spindle was well developed stopped further development and reduced the spindle within a short time. A deformed atractoplasm appeared in the cell after destruction of fibers by the chemical. Stronger concentrations were necessary to induce changes if the spindle was very far along in development. As the drug began its action, Brownian movement on the spindle was increased, indicating that the colchicine was acting upon the fibers. This action took place suddenly, as the chemical reached the cell.

Phragmoplasts, which are spindle materials of cytokinesis, were stopped in their further development and also reduced by colchicine. A cell wall partly developed from each side of the cell can be stopped by the drug.

At metaphase, activity upon the spindle is immediate. The c-pairs are formed as the spindle fibers are destroyed. Within 13 minutes, granular changes upon the spindle showed that action had set in. Within 1 hour and 36 minutes, the entire group of chromosomes returned by a precocious reversion to an interphase. Such quick results required strong solutions (2 per cent). Generally, lesser concentrations (0.05 per cent and 0.1 per cent) were used to effect spindle fibers.

Regardless of the stage from prophase to anaphase, even as late as the phragmoplast, an application of colchicine stopped movement, destroyed the spindle, and returned the chromosomes to interphase

by regular uncoiling processes, similar to the regular telophasic transformations. During later stages a "cytoplasmatization" of spindle or "fluidity" was created.[72] By this process the spindle was transformed.

Metaphasic spindles were destroyed in pollen cells of *Ephedra*. The concentration was a strong one (2 per cent), and reversion to interphase was rapid. The total time for a cell to proceed through a regular mitosis was no different from the time taken for a reversion. A full c-mitosis would have taken a longer time. This rapid conversion back to interphase led to the conclusion that colchicine did not delay the mitotic cycle. Preliminary results unpublished by the authors show that concentration is a most important consideration for *Ephedra* as well as other cells. Reversions can proceed very rapidly under the action of colchicine.[47]

The data from *Tradescantia* and neuroblasts confirm an opinion stated earlier that the destructive action is quite as notable for colchicine as its inhibitive activity. The main difference lies with the concentration. Stronger solutions are required to destroy a fiber at anaphase than to inhibit its formation during prophase. That is why a broad range of concentrations is imperative to obtain a full picture of c-mitosis.

3.3–3: Arbacia punctulata. Colchicine applied to eggs of *Arbacia* at a specific time after fertilization, showed a disintegrating action upon the astral ray.[5] They faded out shortly after the drug entered the cell, and a "lakelike" body appeared at one end of the mitotic figure (Fig. 3.3). The chromosomes were massed in the center of the cell. If the drug entered the cell when two polar regions had already developed, then two lakelike bodies were seen, one at each end. Finally, a still later stage showed the chromosomes in two anaphasic clumps and a lake area encircled the entire figure.

There is a critical time beyond which the colchicine does not stop cleavage, but then a fluidity may be developed around each set of chromosomes even though separate cells were formed.

The disintegration of amphiasters was rapid, and restitution nuclei were formed after a scattering of chromosomal portions was obtained. The destruction of the mitotic spindle at metaphase blocked cleavage effectively. Thus, the spindle components are vitally important to cleavage. The independence of the spindle action and a rhythm of viscosity changes of the cortical layers, independent of mitosis, have been demonstrated. The two processes may go on simultaneously. These have been shown by methods for observing the changes at the outer layer of the cytoplasm.[20, 72]

There can be no doubt that spindle fibers already formed can be destroyed. The specificity between drug and fiber is necessary for such action. A confirmation from materials representing diverse biological

sources has been effectively concluded. Therefore, colchicine acts either by an inhibition before mitosis or by destruction after spindles have been formed.

3.3–4: The polarization microscope. Submicroscopic structures were followed with an improved polarization microscope adapted for specific biological purposes. The birefringence pattern is clear because

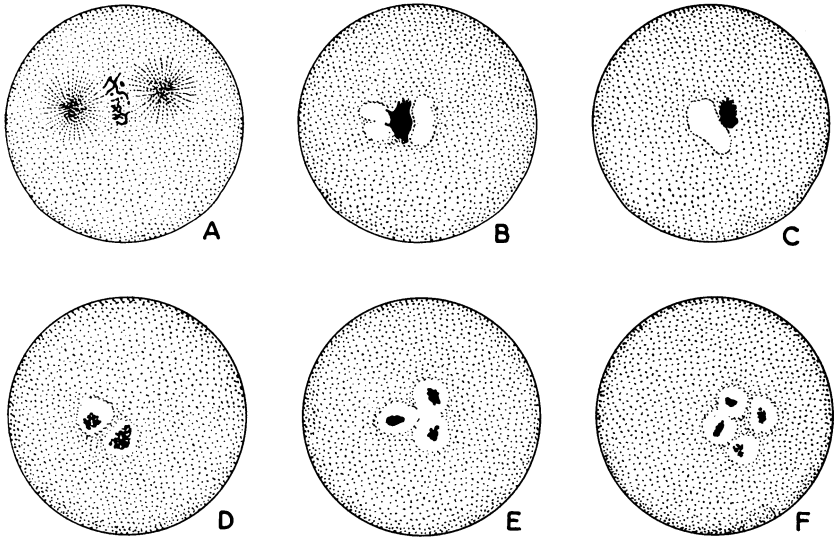


Fig. 3.3—Effects of colchicine upon first cleavage in **Arbacia punctulata**. The area where colchicine causes spindle destruction is a "lakelike" body. Compare **A**, the control, with **B**, a treated metaphase. **A.** Spindle fibers of untreated egg at metaphase. **B.** Colchicine applied when egg was at metaphase, both polar areas laked and chromosomes are clumped. 0.0002 molar concentration of colchicine in sea water applied 10 minutes after fertilization, temperature 22° to 24.4°C. **C.** Prophase when treated causing liquefaction of spindle and asters at one side. **D.** Spindle destroyed, chromosomes separated, but no cleavage furrows. **E.** Three groups of chromosomes. **F.** Four groups of chromosomes with laked areas around each group. (Drawings adapted from photomicrographs by Beams and Evans, 1940)

spindle fibers are optically anisotropic. The fibers, therefore, shine brightly, as compared with a dark grey for the chromosomes.

The disappearance of the spindle was correlated with the disappearance of the birefringence pattern. Therefore, as colchicine acted upon the spindle, a reduction was noticed by a definite fading out of the light pattern. Obviously the fibers changed their form under an attack by the chemical. This general procedure made it possible to perform some critical experiments.[47]

The first maturation division of the egg, the metaphasic spindle of a marine annelid worm, *Chaetopterus pergamentaceus*, was chosen

for these experiments.[47] Normal metaphasic patterns are well known for this species at 25°C. Thus it was possible to judge the exact time when a fully formed metaphasic spindle could be expected. Accordingly, at this stage, the spindle fibers shone brightly and chromosomes were less brilliant against the light background of spindle fibers when viewed through this polarization microscope.

An egg cell in metaphase immersed in colchicine-sea water, showed

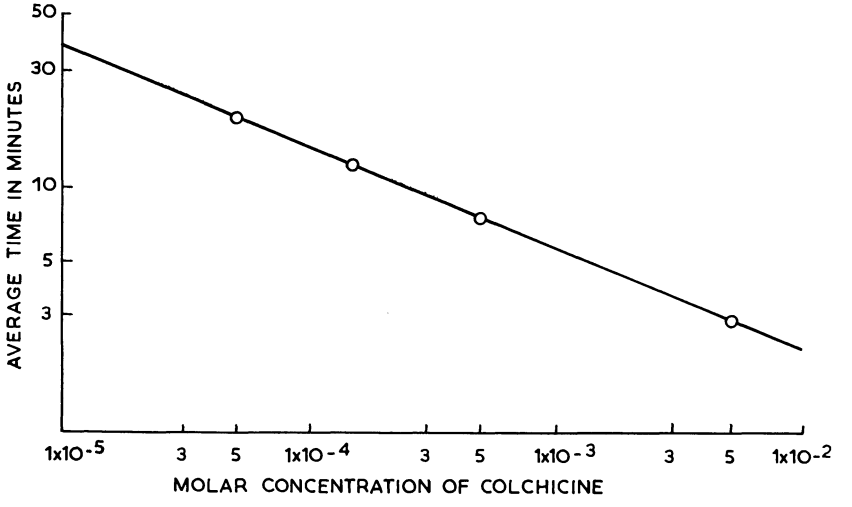


Fig. 3.4—The average time for disappearance of metaphasic spindle of **Chaetopterus** egg, disappearance measured by polarized light pattern. The stronger the concentration, the shorter the time for complete disappearance of spindle. Temperature of sea water 25°C. (Adapted from Inoue, **Experimental Cell Research** Suppl. 2:305–18. 1952)

a steady disappearance of the spindle. This meant that colchicine was destroying an already formed metaphasic spindle. The rate for a disappearance was directly correlated with concentration. In line with previous data, then, the greater the concentration, the more rapid the destruction of the spindle. Figure 3.4 shows these relationships clearly. For example, in one test, the disappearance of spindle occurred in 30 minutes with the concentration 5×10^{-4} *M*. But an increasing concentration (5×10^{-3} *M*) reduced the same stage of a spindle within 3 minutes. Moreover, these observations were made by continuous records from living cells and not fixed structures.[47]

By an entirely new technique the destructive action of colchicine was traced from a fully formed metaphase spindle to the complete disappearance. Finally, the quantitative relation between concentration and disappearance supports the proposition that specificity has a quantitative basis.

Several other similar observations were made at the same time spindle disappearance was studied. The continuous fibers are the first to disappear along with the astral rays. These observations confirmed previous work. Accordingly, the last fibers to lose their birefringence were the chromosomal fibers. These data also fit other results. The order in which the component spindles disappear is important to an explanation for the star metaphase. Active chromosomal fibers and suppressed continuous fibers create the star figure.

While stronger solutions cause the most rapid disappearance of the spindle, the shortening of the spindle during its disappearance is not the same for each strength. Rapid destruction showed very little shortening, whereas weak solutions, which require a long time, showed much shortening during destruction. The shortening process carried the chromosomes up to the periphery of a cell. While this reduction in length of spindle occurred, the chromosomes were always maintained at a midway point between two poles. At the same time chromosomes retained their metaphase position on the equator.

Another important detail was noticed just before the final disappearance of the metaphasic spindle. The chromosomal fibers were the last to disappear, and as soon as the last vestige of spindle faded out, the chromosomes scattered. Their position in the equatorial plate evidently was maintained by chromosomal fibers. Thus chromosomal fibers are responsible for equatorial orientation. Chromosomal fibers once destroyed caused a scattering of the chromosomes and a typical exploded metaphase.

Spindle retardation, measured in millimicrons, showed that changes in spindle measured against time, and plotted accordingly, showed a rapid decrease at first then a slowing down of this process (Fig. 3.5). An exponential decay curve was obtained for this activity.

Confirmation of an action of colchicine along similar lines was obtained by a phase contrast microscope in which no spindle fibers were detected 24 hours after treating testis cells of *Melanoplus differentialis* with colchicine.[90] By other methods and with different chemicals, the spindle fibers were studied as bodies that operated during a mitosis. These could be destroyed, or transformed into other structures. The net result was c-mitosis.[46]

Fibers that appeared anisotropically active, linearly differentiated with micellar particles arranged end to end, changed in their structural pattern. Birefringence showed that colchicine destroyed the fibrous arrangement progressively, step by step. First the continuous fibers and asters disappeared, then the chromosomal fibers. These critical tests with a polarization microscope deal a solid blow to the argument that spindle fibers are cytological artefacts. Not only can the spindle fibers be demonstrated by a light pattern but their changes

under an influence of colchicine are traceable. Finally a quantitative relation between concentration and rate of spindle reduction has been established (Figs. 3.4 and 3.5).

3.4: Changes in Spindle Form

The *Allium* root tip cells treated by the research group at Brussels showed that a differentially stainable body was formed in the col-

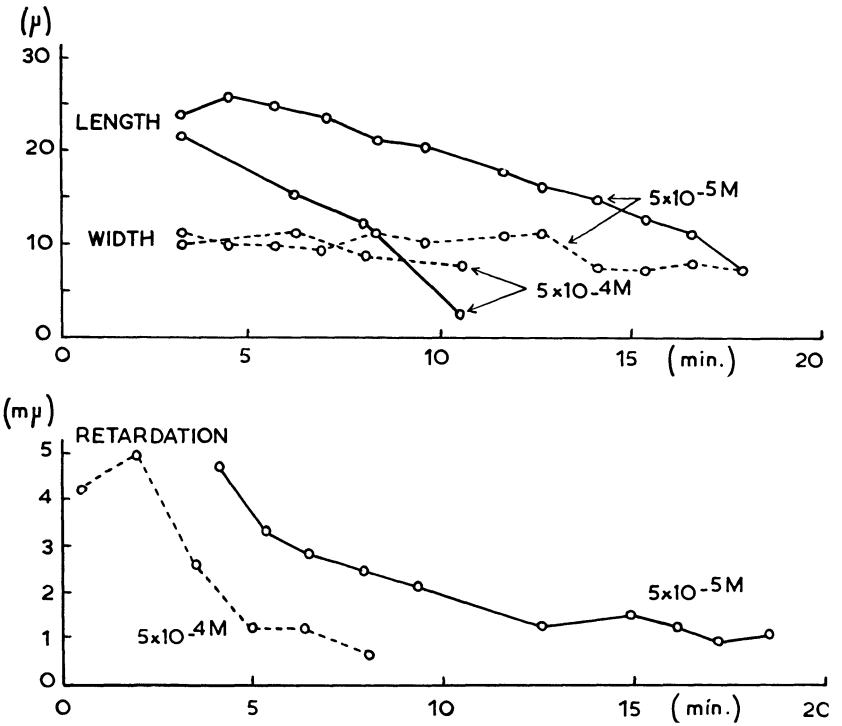


Fig. 3.5 — The shortening of spindle as it disappears differs according to the concentration. The strong solutions cause rapid disappearance and not much shortening of spindle. The width does not change as much as length of spindle. Measurements of retardation in millicrons show rapid retardation at first, then gradual slowing toward the end. Top group shows decrease in length compared to width for two concentrations. Bottom group indicates the sharp drop at the beginning and slower rates of retardation until final disappearance. (After Inoue)

chicinized cells.[29] The chromosomes were clustered about this body (Fig. 3.6). Such structures persist through the interphase and become prominent in the large amoeboid restitution nuclei (Fig. 3.6).

Although the relation to spindle was not suggested until later,[63, 85] the role of the deformed spindle has been mentioned for a number of

cases. Specifically, this was called the achromatic sphere and the pseudospindle. Related to this same structure from observations with neuroblasts is the hyaline globule.[37]

These bodies do not show polarity, their staining properties are distinct from cytoplasm, and their relationship to spindle material or karyolymph is a good one. It was believed that the c-pairs regularly

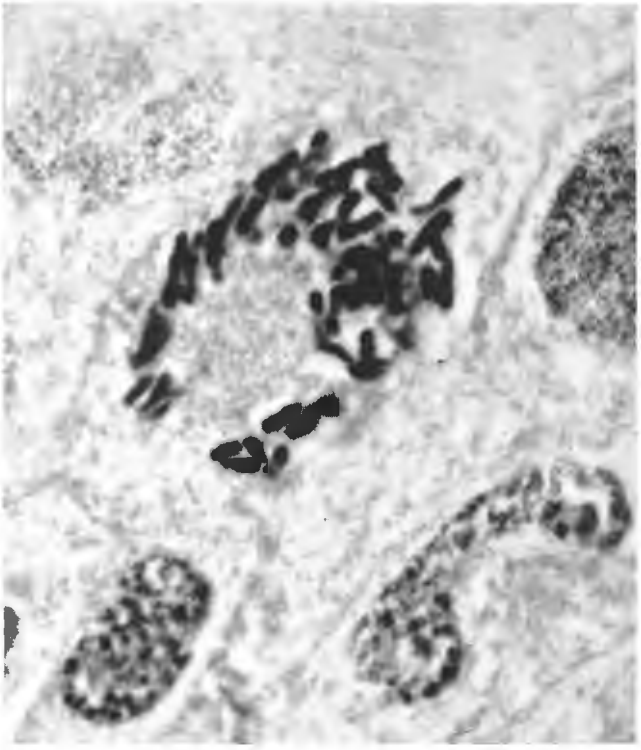


Fig. 3.6—Cell of **Allium** root treated with colchicine showing the spindle substance around which chromosomes are grouped. Another amoeboid nucleus shows the influence of this substance. (Photomicrograph made from slide of the A. P. Dustin Collection, University of Brussels. An unpublished photo similar to diagrams by Havas, Dustin, and Lits, 1937)

associated around the pseudospindle, and that this structure accounted for the exploded metaphase. Indeed the chromosomes were distributed by this body, and the specific distributed c-mitosis was seemingly related to the pseudospindle, but no further direct associations can be made.[37, 95, 61] Different subjects tend to show different kinds of material. The clear area around chromosomes[17] and the lakelike bodies of *Arbacia* may all be related to these deformed spindle materials.

Some materials, such as *Spinacia*[7] and *Lepidium*,[77] do not show the body. Not all cells of *Allium* develop the achromatic sphere. There may be some progressive relational development, or a specific concentration may be required for producing the achromatic sphere and other similar bodies. That a definite progressive stage is followed was carefully shown by the work with neuroblasts.

Until the final answer is obtained, our present observations have led to the idea that fibriform materials, that is, substrate making the spindle fibers, are converted into a corpuscular form instead of the usual fibrillar arrangements. Colchicine plays a role in directing the spindle fiber substance into these modifications noticed for many cells. The course of development of the spindle to its disappearance in neuroblasts and the progressive enlargement of the hyaline globule as the spindle fibers disappear, point to the fact that a spindle material is converted into another form and this form is shown by the hyaline globule. Such a body has definite optical characters, size relationships, and is, in fact, a structure that must be given serious consideration as a changed form of spindle substrate.

If the globules form at prophase, then karyolymph is suspected to be the original material. When metaphasic and anaphasic stages are studied, the spindles have been developed and quite another view comes into focus. In such cases, colchicine progressively reduces or destroys the spindle, and globules form as spindles disappear. Such globule formation requires a longer time at metaphase or anaphase than at prophase. Again, both concentration and stage of spindle are important factors in converting the spindle into globules[37] (cf. Subsection 2.4–3).

When 25 and 50 $\times$ 10^{-6} *M* colchicine solutions are applied during anaphase, the spindle disappears and a hyaline globule forms[37] (Fig. 3.1*D*). The globule occupies a position near one of the poles. The formation of a globule, as the drug acts, leads to a correlation between spindle and globule. Since concentrations determine spindle destruction, the globular formations are likewise dependent upon concentration. These facts are clear.

In agreement with reports on the hyaline globule specifically noted in treated neuroblasts, a similar structure, the achromatic sphere, has characteristics in common with the hyaline globules. Very likely these are similar, just as the spindle fibers of mitoses in cells of plants and animals have certain similar properties. Characteristics of the hyaline globule are: (1) it is spherical; (2) diameters vary from 3 to 15 microns; (3) rate of formation is related to speed of spindle destruction; (4) it is opaque, homogeneous, of high viscosity, not surrounded by membrane, and is optically indistinguishable from karyolymph or spindle; (5) it tends to lodge at top of cell while chromo-

somes settle to bottom.[37] Finally after all these characteristics are cited, the fact remains that in colchicine-treated neuroblasts, the hyaline globule increases when disorientation of chromosomes and spindle destruction take place. Observations such as these support the idea that, as colchicine acts, spindle structure becomes altered rather than annihilated.

The spindle fiber analyzed by electronic microscopy can be described as compound, measuring from 600 to 800 Å at the polar cap stage.[82] When colchicine is applied to *Allium* root tip cells for 30 minutes, the fibers lose their compactness. After one-hour exposures the fibers are disoriented and fragmented. After 2 hours the fibers appear swollen as well as increasingly fragmented. In the untreated cell, fibers remain as such regardless of the type, whether they be chromosomal fibers, continuous fibers, or fibers of the polar cap stage. With long exposure to dilute solutions or short exposure to stronger concentrations, a decided swelling and a tendency toward "solubilization" of their substance were apparent.[82]

3.5: The Arrested Metaphase and Spindle Mechanisms

Interaction between colchicine and spindle fibers ultimately determines the arrested metaphase. The two types, oriented and unoriented,[2] both depend upon several variables existing during a treatment or during a recovery from the drug. As mentioned before, concentration of colchicine, mitotic stage at time of action, length of exposure, recovery processes, type of cell, and conditions favorable to mitosis, all play an important role in the production of the particular arrested metaphase, whether oriented or unoriented.[37]

A pattern such as the star metaphase (Fig. 3.1*C*) is far too regular to be regarded wholly as a random occurrence. During a recovery, the star is characteristic, as is also the multiple star (Fig. 3.1*F*). These types do not reach a peak in a recovery until some time has elapsed between application and the dissipation of drug. A majority of the bipolar mitoses follow the star metaphases, thereby indicating that recovery was nearing completion. The star metaphases are the last colchicine effects to appear during recovery. The *Triton* material that was fixed[2] directly out of colchicine and stained at three hours and at succeeding intervals, shows that stars appear at once and build up much faster than in *Triturus*.[74] When the stars reach a peak in *Triton*, unoriented types, rather than bipolar mitoses, become the most prominent mitotic figures.

Any pattern, whether star or exploded metaphase, should be regarded as a response to colchicine, operating primarily through the spindle fibers. Two basic components are accepted as established for plants and animals; these are (1) continuous fibers and (2) chromo-

somal fibers (Fig. 3.1). Sometimes these two are called the exterior and interior spindles,[5] or the centrosomic and centromeric spindles.[74]

The birefringence pattern for a metaphasic spindle[47] in *Chaetopterus* egg, disappearance due to the action of colchicine, registers the fading of continuous fibers and astral rays first, while the chromosomal fibers are the last to disappear. Action upon astral rays before the interior portions has been demonstrated with other material.[98] Hence, data on the living cell and on fixed tissue are in accord as to the action upon the several parts of the total spindle.

Acenaphthene is 1000 times slower in action upon a spindle than colchicine.[55] This slower activity permits a better analysis, because the exterior spindle is destroyed before the interior. Colchicine acts so totally and abruptly that this delicate difference is frequently overlooked. Until the threshold concentrations are employed, a partial action showed that colchicine in dilute solution, like acenaphthene, destroyed the exterior spindle before the interior. That is, continuous fibers are first to be affected. This experience is like dissecting an organism into its essential parts.[55]

Certain concentrations of colchicine applied to the metaphasic spindle in neuroblasts cause star formations (Fig. 3.1). The continuous fibers are inactivated, but chromosomal fibers remain intact. The centromeric portions of chromosomes are drawn to one focal point (Fig. 3.1). There, however, is another way to produce a star metaphase in neuroblastic cells. To obtain the correct concentration for prophasic treatment, enough colchicine is used to inhibit the continuous fiber in its development, but such a concentration does not act in the same manner on the chromosomal fiber. These interactions lead to a star metaphase.

Now a final explanation for *Triton*[2] and *Triturus*[74] appears to be at hand. *Triton* cells removed from colchicine show star metaphases at 3 hours, build up to a peak within 12 hours, and are succeeded by unoriented metaphases. Colchicine acts progressively more strongly as the peak is being built. During the action, continuous fibers were destroyed before chromosomal fibers, giving cause for stars in *Triton* cells. Finally, the whole spindle was inactivated when colchicine reached full effect and unoriented types took precedence (cf. Chapter 2). Inspection of data from *Triturus*[74] leads to another observation. The stars appear later, and after the peak is reached, the bipolar mitoses occupy the prominent position among dividing cells. As recovery was taking place, the colchicine was becoming more dilute. At a certain point the continuous fibers were inhibited but not the chromosomal fibers. Then at last, both continuous and chomosomal fibers developed, and bipolar mitosis predominated among the dividing cells. Among cells of *Triton* the stars appear as the effect of col-

chicine begins. The stars were the "arrivals" in this case. While *Triturus* cells developed, the star showed that the effect of colchicine was "departing."

We may conclude that the star forms when centriole, centromere, and chromosomal fibers interact while continuous fibers are suppressed. A mitotic polar metaphase appears much the same as the star, but the latter has very small, if any, stainable achromatic core. The size differences have been demonstrated in several instances.[74, 8, 37]

Chromosomes occasionally fall outside the star cluster. Lagging chromosomes may be observed in untreated cells. Neuroblasts, treated with very weak solutions of colchicine, consistently show lagging chromosomes. The lost chromosome is confirmation that a partial spindle inactivation takes place when these particular types form.[69]

Multiple stars (Fig. 3.2*j*) are basically the same as the single star, except for several focal centers instead of one. If two or more chromosomes fell outside the first star, a second could form. This type is most common when cells are recovering in *Allium* root tips. Increasing the number of chromosomes shows a corresponding increase in the number of multiple stars. Multiplex stars have been demonstrated in both plants and animals, during recovery as well as during active treatment. *Triturus* showed the bimetaphase and trimetaphase, equivalent to multipolars, five to six days after recovery.[74]

Distorted stars[2] are not proved as easily as the star formation. Two explanations have been given. One, the action is a response of centromeres and a centrosomic center, but the staining procedures did not bear out these assumptions. Two, the hyaline globule which forms when spindle fibers disappear, becomes wedged between the chromosomes, distorting the star.[37] Either explanation may be considered valid until more information is at hand.

Unoriented metaphases, such as ball, clumped, prophase-metaphase, or exploded types, do not show activity on the chromosomes or any part thereof. The term *unoriented* is entirely appropriate[2] for such figures (Fig. 3.1*G*, 3.2*d*).

An exploded or scattered arrangement has been observed in many plants and animals (cf. Chapter 2). If the disappearance of a metaphasic spindle is followed by the birefringence pattern,[47] one may assume some mechanical explanation for the exploded type, for as soon as the spindle disappears completely, the chromosomes seem to scatter as if they were held on the equatorial plate to the very last moment. Disappearance of the continuous fibers did not permit the scattering. Not until chromosomal fibers disappeared did the chromosomes disperse. This confirms that the exploded metaphase originates when both chromosomal and continuous fibers are destroyed. Such observations support the concepts that a full c-mitosis may involve an

exploded metaphase and that complete spindle inactivation is fundamental to the unoriented type or full c-mitosis.

Presence of the pseudospindle[61] or the achromatic sphere[85, 7] (Fig. 3.9) has helped to explain the scattered arrangement in some cases, notably in *Allium* root tips (Fig. 3.7). C-pairs are closely appressed around an achromatic sphere. But comparable cells in regenerating liver exhibit excellent exploded metaphases without a stainable sphere. Other scattered types are not comparable to the special case of *Allium*.

The assumption[2] that a single centrosomic spindle operates in pushing the chromosomes to the periphery of the cell is hardly tenable, for staining has not proved the case, nor have the other techniques subtantiated such mechanisms. It would hardly be consistent to classify as an unoriented type, one that had such a mechanism as a central spindle pushing the chromosomes to the edge.

Whatever the final answer will be as to their disposition, they seem profusely scattered, and seem to lie in the cytoplasm as if each repulsed the other.

The exploded metaphases are a striking type.[14, 16] They would seem to result from the total inactivation of both the continuous and the chromosomal fibers.

The ball metaphase is more common than the exploded metaphase; it increases in frequency as the concentration increases. A toxic or poisoning action is logically the basis of a ball metaphase. The chromosomes are definitely unoriented and are often massed in a clump. For that reason the c-mitosis has been called *clumped*, a type related to the ball metaphase.[33, 77]

Prophase-metaphase formations (Fig. 3.2) are more nearly described by the term *arrested prophase* (cf. Chapter 2), for they represent leftover prophasic arrangements. With no spindle action, chromosomes remain stranded in a pre-prophasic arrangement.[33] In fact there is complete inactivation. Prophase orientations are not necessarily disturbed by colchicine, as noted for *Dipcadi*.[55] Here the chromosomes are disposed in a pattern determined by the previous telophase. If the concentration is partially inactivating, a star metaphase results; total inactivation leads to the prophase-metaphase type.[24, 78] The prophase-metaphase merges into the ball metaphase and clumped metaphase depending on the concentration. There may be return by recovery to a multinucleate cell. The prophase-metaphase and clumped c-mitosis seem to be more characteristic of meristematic cells of stems than of roots.[96]

Distributed c-mitoses have attracted much attention because they were described as a "somatic meiosis" (cf. Chapter 2). These are a subtype of the exploded metaphase. The main difference between exploded and distributed metaphase is seen in the disposition of the

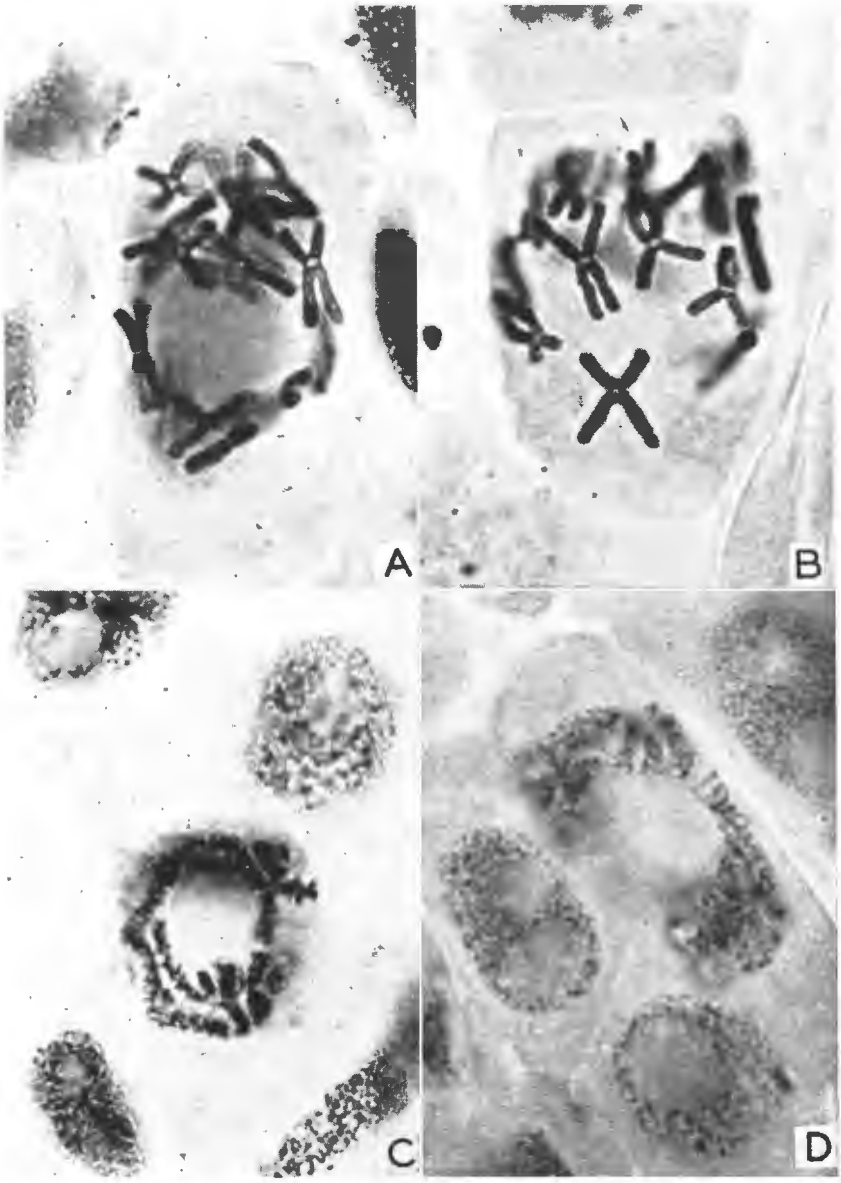


Fig. 3.7—**Allium** root cells treated with colchicine. **A.** Cruciform c-pairs associated around the spindle substance. At bottom of group one pair is completely separated in c-anaphase. The timing of separation is upset as well as delayed. **B.** C-pairs with arms fully repulsed. A light, unstained area surrounds the chromosome. **C.** Chromosome reverting to interphase; dechromatization has occurred. Chromosomal framework associated with the central substance. **D.** An amoeboid restitution nucleus around the pseudospindle or achromatic sphere. The end of at least one c-mitosis. (Photomicrographs furnished by courtesy of Dr. C. A. Berger, Fordham University, N. Y. After Berger and Witkus, 1943)

c-pairs. Polar groupings of c-pairs typify the distributed metaphase, whereas exploded metaphases are nonpolar. Unquestionably, the distributed c-metaphase was clearly illustrated in pollen tubes.[33] *The distributions were equal and unequal. They were not conceived as a somatic meiosis. In root tips, naphthalene acetic acid and colchicine increased the number of distributed c-mitoses compared with either chemical alone. Other chemicals increase this type even more than* colchicine.

3.6: Spindle Disturbance and Cytological Standards

Spindle disturbances in plants may be classified in three categories:[73] (1) full inactivation, stathmokinesis,[28] (2) partial inactivation, merostathmokinesis,[38] (3) slight disturbance in orientation, tropokinesis.[38, 25] All these types are produced by colchicine, as already pointed out. If one wishes to make comparative studies with other chemicals known to influence mitosis, well-defined cytological standards of judgment are needed to classify reactions as either disturbed or normal. If the reaction is disturbed, it is important to distinguish the type according to velocity or strength of reaction. The most reliable criteria appear to be those based upon tests at telophase, rather than at earlier stages.[73]

Abnormal chromosomal distributions may be caused by spindle disturbances in three degrees: first, multipolar; second, apolar; and third, unipolar. When three or more groups of chromosomes join so as to form discrete groups, partial spindle disturbances are obvious. These were carefully noted under the general type, merostathmokinesis,[38] or under the present classification as multipolars. However, complete destruction or inactivation leaves one single group, or there may be two groups with no evidence of spindle function. This is the apolar distribution. Another specialized disturbance is the close gathering at one focal point described before as the star metaphase; this type becomes unipolar at telophase.[73]

Colchicine (0.005 per cent) applied to *Allium* root tips for 46 hours, increases the percentage of tropokineses. The controls may show as many as 10.5 per cent, but treated root tips raised the frequency to 21.3 per cent. These disturbances are the first-order changes occurring at threshold concentration,[25] and are the first signs of spindle disturbance.

3.7: Cytoplasmic Division

Nuclear mitosis and the completed process of cell division are not synonymous, because the nuclear processes and cytoplasmic processes taken together make up cell division. Truly, karyokinesis (nuclear mitosis) and cytokinesis (cytoplasmic processes) are very highly inte-

grated, and are closely coordinated processes.[6] One cannot always mark the separation between the processes. For this reason and perhaps others, biologists use the term *mitosis* as completely synonymous with cell division, when mitosis is only one aspect of a dividing cell.[46]

When colchicine acts during a division, the significance of what has been noted for mitosis and cell division becomes apparent. The multiplication of chromosomes continues in the presence of the drug at a certain concentration, whereas the total absence of spindle fibers prevents the movement of chromosomes to the respective poles. Inhibition of fibers has one drastic effect on the cytoplasmic phases of cell division: the cytokinetic processes are completely eliminated. Among animal cells the cleavage processes are somewhat specific and respond to colchicine in a unique fashion. These aspects are discussed in the next section. In plants no cell plate is formed, and phragmoplasts are prevented. For organization purposes these are discussed separately from animal cells.

3.7—1: Cleavage processes in animals. Marine eggs have been subjects for studying the mechanism of cell division since the pioneering work of Hertwig, Boveri, and Wilson. The sea urchin, *Arbacia punctulata*, was therefore a logical selection for Nebel and Ruttle[72] when, in 1937, they wanted to analyze more completely the activity of colchicine. They established that $10^{-4}M$ concentrations block cleavage. Even a concentration of 0.0002 *M* inhibits cytoplasmic division[5] if applied 22 minutes after fertilization at 22° to 24.4°C. At this time eggs are in prophase, metaphase, or early anaphase, and spindle mechanisms are inhibited or destroyed by colchicine (Fig. 3.3).

If nuclear mitosis passes a certain stage, cleavage is not stopped by these concentrations. Therefore, a critical point is reached beyond which destruction of spindle apparently has no effect. These points emphasize a close integration between nuclear mitosis and cytokinesis.[20, 97, 98]

Specific objectives were outlined to determine precisely up to what stage or stages in the mitotic cycle treatment was effective in blocking cleavage and at which stage colchicine was no longer effective. The results showed that suppression of cleavage by colchicine follows a particular course on the basis of fertilized eggs of *Arbacia punctulata*.[5] The eggs were allowed to stand 10 minutes after fertilization; then different lots were placed in colchicine at 2-minute intervals during a 60-minute period. By this test, a lapse of 22 minutes between fertilization (22° to 24.4°C.) and the addition of colchicine was found as the critical period, because cleavages were not blocked after that time (Fig. 3.3). The mitotic stages most generally present at this time were prophase, metaphase, and possibly early anaphase, each of which was affected by colchicine. These stages regularly precede the

usual furrowing process by about 10 to 14 minutes. Therefore, after the critical mitotic stage, anaphase was passed, the furrowing process started, and after that point colchicine did not inhibit cleavage of the cell into two parts.

Similar results were obtained from tests[20] using the starfish, *Asterias forbesii;* the sea urchin, *Arbacia punctulata;* sea urchins from Bermuda, *Tripneustes esculentus* and *Lytechinus variegatus;* and the sea slug, *Chromodoris* sp. In all cases, the key for inhibiting cleavage was anaphase. The concentrations varied, but otherwise the general plan was very similar for all tests. Once the eggs passed metaphase, cleavage could not be altered by dosages of colchicine that destroyed the mitotic spindle. If threshold concentrations were used at metaphase, furrowing almost divided the egg, and a regression then set in. This showed that the final closing of cytoplasm is distinctly a process dependent upon the spindle. Cases such as these emphasize the interdependence between karyokinesis and cytokinesis as processes of cell division that involve nucleus and cytoplasm.

Cytological evidence for action by colchicine is obtained from the lakelike bodies appearing where astral rays and spindle fibers normally should be found[5] (Fig. 3.3). One lake body indicates prophase; two, one on either side of a clumped mass of chromosomes, point to action at metaphase; and two clusters of chromosomes can be taken as evidence for disturbed anaphase. All these prevented cleavage.

Furrowing is dependent upon viscosity changes, and once processes begin, apparently colchicine does not stop cleavage. In an effort to correlate such changes with the cleavage process, centrifugal experiments were run, but not all results are in agreement.[7] The additional evidence [97] for viscosity or rigidity relationships and nuclear mitosis as well as cytoplasmic division are discussed under the mechanisms in the last chapter.

A demonstrated fact emerges that cleavage is averted if achromatic figures are destroyed before a certain mitotic stage has been reached. Of course, concentration variabilities are important, but the blocking process appears to be an "all-or-nothing" effect; therefore, either nuclei divide and there follows a cytoplasmic division, or an arrested mitosis precludes daughter cell formation. For example, chromosomes, scattered as a result of colchicine, form micronuclei, and no cytoplasmic division takes place.[5, 16, 14] On the other hand, recovery among a number of star metaphases may eventually lead to the cytoplasmic division, because spindle inactivation is not complete.

Depending upon concentration, cleavages may be retarded or stopped (Fig. 3.3). The germ cell of *Triturus helveticus* L. does not cleave if a 1:500 colchicine solution is used.[83] Regeneration of

the spindle may determine the course of cytokinesis. These data have been limited mostly to eggs, where the principles of cytokinesis in relation to the mitotic mechanism are better observed than among other animal cells. Further data on the action of colchicine on eggs are to be found later (cf. Chapter 8).

In those cases where a lowered viscosity is related to mitosis, it is assumed that the gelation-solation phases are influenced.[4] If solation conditions destroy spindles, then lowered viscosity acts accordingly. Spindles are inhibited because colchicine acts upon a mechanism that changes the solation conditions. But viscosity changes may be secondary effects while other mechanisms operate before cytoplasmic changes take place.[97]

Birefringence tests show that the normal variations of the cortical layer of eggs of the sea urchin, *Psammechinus miliaris,* presumably sychronized with spindle and monaster expansion, are entirely independent.[70] The spindle and viscosity changes in the cortical layers may go on simultaneously, yet remain independent. Rhythmical surface changes of eggs of *Tubifex* were not modified by arrest with colchicine. This further substantiates the premise that cytoplasmic processes are not entirely controlled when the mitosis is controlled.

In the neuroblastic cell, lowering of cytoplasmic viscosity was visible through the increased activity of mitochondria.[39] Brownian movements were used to indicate the changes. Chromosomes settled to the lower half of the cell when spindles were completely destroyed. Disappearance of the spindle and a more rapid Brownian movement were correlated. The notable decrease in viscosity was suggested as a consequence of a decrease in the content of ribonucleic acid and phosphorus at the time colchicine acts upon mitosis.[39]

3.7—2: *Cell plate formation in plants.* The continuous fibers form the spindle of cytokinesis upon which the cell plate forms. Between the spindle and cell wall a phragmoplast completes the fibrous structure and the cell plate across the cell.[6, 95] Since colchicine destroys or prevents continuous fibers, there is no spindle of cytokinesis or phragmoplast.

During recovery and regeneration of the spindle, various abnormalities may be seen, but these processes are characteristic only in relation to recovery and reversible effects of which the cells are capable after colchicine.

By the special techniques for applying colchicine at certain stages, the phragmoplast has been tested specifically with regard to the role of the drug acting upon such structures already formed.[95] If the phragmoplast is in formation, colchicine can reverse the process, changing the fibers back to a fluid stage, a kind of cytoplasmatization. Even rudimentary cell plates and the beginnings of septa from each

side are arrested. Under these conditions further development is arrested, and chromosomal bridges extend between the cells.[95]

Direct destructive action upon cell plates was recorded also in wheat root tip cells. Generally, the absence of spindle determines the formation of a restitution nucleus precluding any form of cytokinesis as well as daughter nuclei.[33, 55, 89, 61, 62] The interrelation between cytokinesis and mitosis is shown by the effects of colchicine.

By centrifuging root tips treated with colchicine, a much greater displacement of chromosomes against the centrifugal wall was found among treated cells than among the controls. The action of the drug was interpreted as an effective lowering of cytoplasmic viscosity.

Allium root tips treated with colchicine at varying exposures were centrifuged to determine changes in structural viscosity of the achromatic figure. The decrease in viscosity was indicated. Moreover, there was a low viscosity at eight hours, when c-mitosis was at a peak. After return to normal bipolar mitosis the viscosity showed increases paralleling these recovery processes.

Another view somewhat opposed to that expressed above has been presented. Since the spindle fibers are inhibited and no achromatic figure is present to hold the chromosomes in position, greater displacement may take place regardless of viscosity change. The centrifuge tests merely show that the spindle fibers are lacking. Supporting this view are the observations on cyclosis in *Elodea,* which does not seem to be changed by colchicine.

Additional tests showing changes in viscosity among plant cells are reviewed in Chapter 4.

3.7—3: Cytoplasmic constituents and cell organites. The centrosome, a self-perpetuating body outside the nucleus, becomes involved with spindle destruction. Its activities are depressed along with those of the spindle mechanism. Several centrosomes may accumulate within a cell treated with colchicine, hence the formation of multiple stars. Each star probably represents a centrosomic body. These were carefully demonstrated in *Triturus viridescens.*

A confusion arises from the mitochondrial picture and colchicine. Some say these bodies are affected by the drug;[10] others report no change.[24] The concentrations as well as materials vary widely, but it would seem that some consistent reaction might be obtained. However, until now we can only review the pro and con. Modifications involving fragmentation, dispersion, reduction, as well as minor morphological changes have been seen after colchicine treatments directed to: (1) Flexner-Jobling carcinoma of rat, (2) liver cells of rat,[41, 42] (3) cells of certain orthoptera, *Gyrllus assimilis* and *Melanoplus differentialis.*[26] No mitochondrial modifications are reported for neuroblasts in *Chortophaga viridifasiata,*[31] an observa-

tion coinciding with a phase contrast observation of *Siredon* erythroblastic prophase-metaphases made by the junior author (unpublished).

Root meristematic mitochondria tended toward constrictions and fragmentations after exposures to colchicine for more than 25 hours (0.005 *M* colchicine) (Fig. 3.9). Shorter exposures, 13 hours, were less effective. The relation between viscosity and mitochondrial shapes was believed valid.[79] The mitochondria were demonstrated in *Allium* (Fig. 3.9) in which cases mitochondria did not penetrate the achromatic sphere (Fig. 3.9) (pseudospindle) about which the c-pairs seemed to collect.[61]

While the Golgi bodies have not received the attention given other cytoplasmic organites,[36] fragmentation and scattering of these bodies were induced in adult mice by 0.1-mg. colchicine injections.[43]

Metabolic aspects of cytoplasm were demonstrated among tissue cultures by differential staining with methylene blue (1:10,000). The arrested mitoses remained colorless while the cytoplasm of resting cells was diffusely stained. Untreated cells in division are also colorless because methylene blue is reduced more rapidly when cells are dividing.[59] This suggests that arrested metaphase reduces methylene blue like a regularly dividing cell. This metabolic activity may provide an explanation for the eventual destruction of arrested mitoses in animal cells[59] (cf. Chapter 2).

"Bleb" formation occurred at cellular surfaces among grasshopper neuroblasts[37] when mitosis was arrested. Also, notable cytoplasmic agitations were seen among fibroblasts treated with colchicine and studied by cinematographic projection.[17] These observations call attention to an unusual activity when cytoplasmic division is prevented by colchicine. This agitation has been described by others using treated tissue cultures.[59, 68] Changes at cell surfaces can also be induced by many other substances, such as mustard gas and ultraviolet radiations.[59]

Some observed cases do not indicate direct action by colchicine. The marine eggs of *Psammechinus miliaris* observed for birefringence characteristics indicated that actions in the cortical layers were independent of mitotic arrest.[70] *Tubifex* eggs provided additional cases for observing the relation between changes in cytoplasmic viscosity and mitotic cycles.[98]

3.8: Reversible Characteristics of the Spindle

Let us summarize what has been detailed from Chapter 2 up to this point. If we compare a colchicine-mitosis (c-mitosis) with a regular mitosis, our first impressions might well be the following: c-mitosis is mitosis without metaphase, anaphase, and telophase;

c-mitosis precludes cytokinesis; c-mitosis leads to a restitution nucleus; c-mitosis prevents daughter nuclear formations; c-mitosis stops the formations of daughter cells from a mother cell. Our summary implies — and similar implications can be found in the literature[64] — that, whereas during c-mitosis the notable stages of a normal mitosis are omitted, whereas a single nucleus is formed instead of two, and whereas one cell begets one cell, the whole c-mitotic process appears to be a quicker and shorter one. Seemingly, the reason for this is that the arrested metaphase is a bypass method ultimately short-circuiting, by the influence of colchicine, true division of a cell. But in reality, these apparent abbreviations that would seem to shorten c-mitosis, require more time than a regular mitosis covering similar chromosomal transformations. For example, one c-mitosis takes 430 minutes compared with 155 minutes for a normal mitosis.[95] Furthermore, during the 155 minutes, chromosomes become involved in metaphase, anaphase, and telophase. During the 155 minutes, two cells each with a nucleus are derived from a mother cell and one nucleus. In other words, a c-mitosis (430 minutes) that gives an impression of a shorter procedure by omissions, actually takes 2.8 times longer than the corresponding control (155 minutes).

These comparative figures are accurate measurements from continuously recorded cases of individual living cells, passing through the entire cycles of c-mitosis and mitosis, respectively. Contrary to these time sequences, *Ephedra* pollen cells showed no difference between treated and untreated cells.[64] However, changes may have influenced these time sequences, so that transformations from prophase to interphase took place without a delayed metaphase.[64]

As pointed out in Chapter 2 and summarily stated above, a time scale comparison between c-mitosis and normal mitosis is like projecting a moving picture in slow motion. Action for 155 minutes is stretched out to 430 minutes. Now, most of this extra time is taken up while the chromosomes appear to lie scattered in the cytoplasm, unoriented because colchicine inactivated the spindle fibers, in contrast to the metaphase-anaphase stages that are oriented and activated by spindle mechanisms. We may refer to this phase as the "intactness period" of the chromosomes. Chromosomes retain an individuality, an intactness, ten times longer under colchicine than do those of the control culture, because, out of 430 minutes, 249 are relegated to an intactness period, against 23 out of the 155 in a control cell. Remembering that such data are taken from living cells continuously observed and recorded, these facts are significant.

After a c-mitosis is accomplished, the restitution nucleus forms a single unit that combines the chromosomes which regularly become distributed equally among two daughter nuclei.[55] Of course, a "*pre-*

cocious reversion" from c-metaphase or earlier arrested stages as well as a recovery in due course of time, often true for animals[58, 16, 24, 76, 78, 91, 83] but not limited to them, creates a restitution nucleus or daughter nuclei with diploid numbers of chromosomes (centromeres), because in these cases a c-anaphase does not obtain, under conditions of *reversion* or recovery, from an arrested stage. However, doubling of chromosomes can and does take place among animal cells.[51, 76, 86, 11, 3, 4, 2, 83, 22, 74, 65, 81, 48] Although this process of duplication is more common to plants treated with colchicine, neither situation should be regarded as typical for one group or the other. Such generalizations lead to false conclusions.

Three statements concisely express the primary concepts: (1) c-mitosis creates a polyploid restitution nucleus via c-metaphase-c-anaphase-c-telophase processes; (2) c-mitosis by *precocious reversion* from c-metaphase, or earlier arrested stage, may with exceptions, lead to a nonpolyploid restitution nucleus; (3) c-mitosis may after due time recover from the arrested stage and develop regular anaphase, instead of the c-anaphase, thus leading to diploid daughter nuclei.

Greater than all these remarkable features is the underlying biological principle of reversibility. When the cell, in contact with the drug for a given time, is removed from the influence of colchicine, either by actual transfer or by allowing dissipation of chemical during a recovery period, the characteristics of reversibility come into focus.[55]

Cells treated with optimal dosages that induce a c-mitosis creating the polyploid nucleus, recover so that a normal mitosis may follow with a fully functional bipolar spindle. That is, a restitution nucleus can regenerate a bipolar spindle after the effects of colchicine are removed.[28]

Regeneration among the restitution cells is permanent, and cells develop spindle mechanisms in each succeeding division with metaphase, anaphase, telophase, and, of course, the doubled number of chromosomes. This new divisional process continues thus, as long as the cell lineage retains power to divide. Polyploidy is thereby maintained and continued without attending cytogenetic changes, except for those effects related to an increasing number of chromosomes per cell.[55] No one has demonstrated by careful cytogenetic methods that colchicine at optimal doses for a c-mitosis leading to polyploidy, also increases the frequencies of mutations or chromosomal changes.[33, 92] Caution at this point is advised because mutations and chromosomal changes may occur independently of colchicine but simultaneously with a treatment.[33]

The capacity of the cell to recover after a treatment, to regenerate a bipolar spindle following a c-mitosis, to reverse the inactivating effects of colchicine upon spindle; these are, in our opinion, the most

striking and significant biological characteristics demonstrated when dividing cells of animals and plants come in contact with optimal doses of colchicine.

3.8—1: *Recovery in plants. Allium* root tips transferred to pure water after specific exposures to colchicine are excellent materials for tracing recovery of the spindle mechanism. Very slight toxicity, if any, results from an exposure sufficient to inactivate the spindle completely. Usually 12 to 24 hours in water give adequate time for first recovery stages.[55, 75, 38, 61, 74, 21, 25]

The regeneration of spindle runs a characteristic course, probably representative of many plant cells. But most work has been done with *Allium cepa* L. specifically, and with root tips rather than stem tips, generally. By a *characteristic course* is meant the sequence of chromosomal groups from full c-mitosis to partial c-mitosis, then to bipolar spindles. During this course the obvious abnormalities appear in terms of normal mitosis.[33, 40, 55, 27, 75, 38, 61, 45, 7, 21, 56] First, the chromosomes group into what may be called multiple star formations (Figs. 3.6 and 3.8). There is no connection between the various stars of a single cell. The chromosomes may be somewhat clumped together. Shortly thereafter, asymmetrical and loose spindles appear.

Cells with unusually high numbers are followed in the transition to normal mitosis. Extremely large cells with high numbers appeared in tissue cultures of plant cells.[62] The first hint that a cell is on the road to recovery shows in the telophasic stage. Chromosomes are not condensed into one nucleus when first observed. Later each nucleus becomes perforated and filled with canals. Next the grouping of nuclei of a large cell is like a multiple cell,[61] containing as many as twenty stars.[55] Perhaps each star represents a regenerating spindle area. When telophase sets in, fibers running between each group lead to cell wall formation (Fig. 3.9). Thus, the large restitution nucleus containing many chromosomes, becomes divided into as many as 20 small cells.[61, 62]

The obvious reduction to many small units means reduced chromosomal numbers. While this is "somatic reduction," it does not correspond to reduction through meiosis, except in the numerical changes. Certainly no qualitative genetic reduction takes place such as occurs in meiotic processes.[56]

After 36 hours most cells have run their normal course. A diagram correlating length of exposure to time for regeneration and completed recovery, has been constructed.[55] The exposures, covering 7 to 30 minutes, require between 12 to 24 hours for the first spindle regeneration, and 36 hours for regular spindle. An increasing exposure, 2 to 72 hours, retards spindle regeneration to 24 hours, and delays complete recovery to 36 and 48 hours. This means that the longer the exposure, the longer the time for recovery.

Another view is obtained from the 1-hour and 5-hour treatments with *Spinacia* root tips. In these cases metaphases were plotted during recovery. Complete recovery occurred within 48 hours if exposure was 1 hour, but 63 to 66 hours were required for a 5-hour exposure.[7]

Cytological consequences in relation to treatment have been analyzed. The first tetraploid cell begins a second cycle after 30 hours,[45]

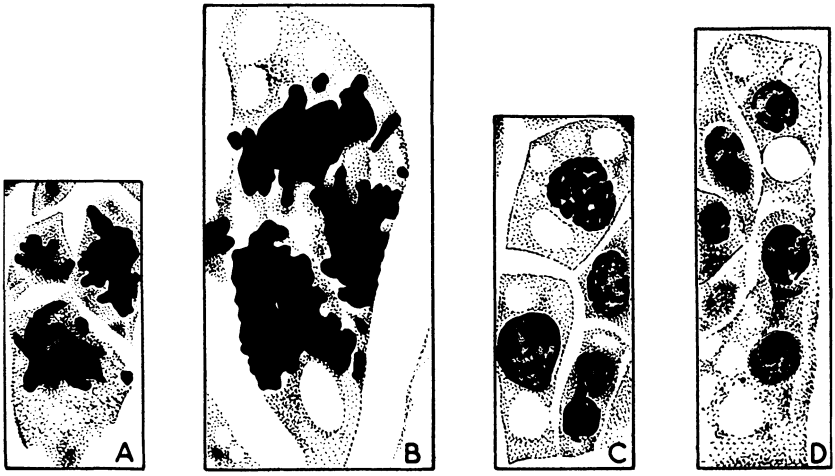


Fig. 3.8—Recovery stages in cells of roots of **Triticum** treated with colchicine. **A.** Multipolar groups of chromosomes, unequal numbers. **B.** Cell with a larger number of chromosomes showing that several cycles of c-mitosis had been accomplished. Upon recovery, cell plates may form between groups. **C.** A large cell cut into several smaller ones, a characteristic recovery pattern. **D.** One cell divided into at least six cells upon recovery from the effects of colchicine. These cells do not survive but are replaced by diploid, tetraploid, or octoploid cells. (Drawings adapted from photomicrographs of Beans and King, 1938. Their Figures, 31, 32, 34, 35)

octoploids at 72 hours,[61] and after 96 hours, 16-ploid cells, or 128 chromosomes, were in division.[61]

If one studies the entire root, some new facts come to our attention that are more meaningful than any absolute ratio between time and number. Euploid numbers, multiples of 8, predominate so that usually the count reads 16, 32, 64, 128, etc. There are very few polyploid cells near the root tip; in fact, after 72 hours diploid cells persist a little farther from the tip. Tetraploid and octoploid cells persist in even larger numbers. At the region farthest from the tip, where lateral root initials are found, giant lobed nuclei were plentiful.[61] These cells were crowded with chromosomes having as high as 1000 c-pairs.[55, 56] In these cases no regeneration of the cell took place. As a rule, the nearer the root tip, the lower the chromosome number. Or in other words, a greater percentage of cells with high numbers is found in older portions of the root.

Just how far this accumulation can continue with hope for reversibility to normal was answered by an elaborate test that required a series extending over a long time. About 500 chromosomes is the upper limit beyond which no recovery can be expected, but 128 and 64 make the most rapid recovery to bipolar spindle.[55]

Lethal or toxic effects have been disregarded, but the drug has a growth-depressing influence if shoot growth is the index. The effects

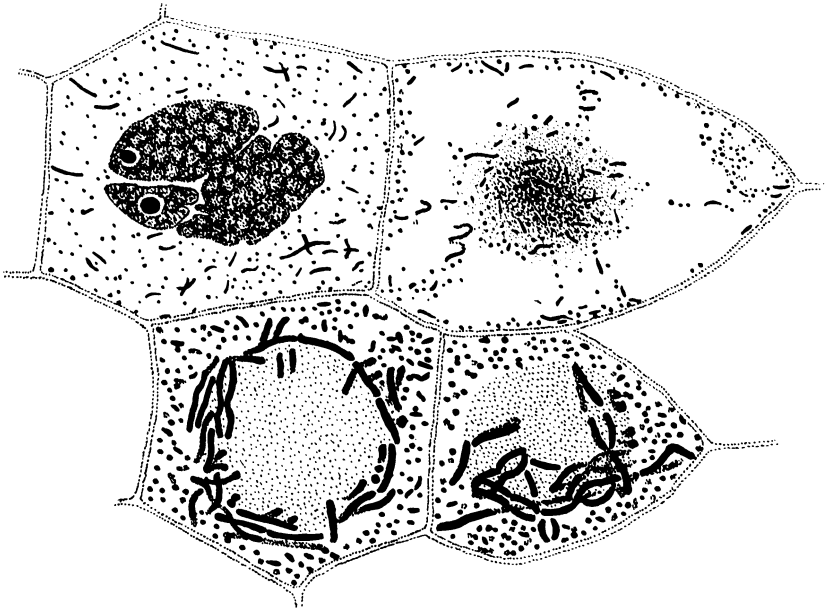


Fig. 3.9—Allium root cell treated with 0.05 per cent colchicine 32 hours, then fixed and stained with iron alum haemotoxylin. The lower cells show chromosomes around the pseudospindle. Shortened mitochondria do not penetrate the area of the pseudospindle. Large restitution amoeboid nucleate cell not in c-mitosis. (Adapted from Mangenot, 1942)

of the poison may be expressed in growth differences between treated and control plants. Controls had leaf shoots 34 cm. long on the seventh day; .01 per cent of the treated plants grew to 15 cm. (about one-half), and 0.1 per cent of the plants were reduced one-fourth, to 8 cm.[55]

3.8—2: Recovery in animals. Recovery analyses in animals present difficulties not met in plant cells because animal cells are not able to survive as long.[28, 98, 58] A c-mitotic dose frequently becomes lethal to the animal, an effect that precludes recovery. Another difficulty is the variation in toxicity between animals as well as the differences when dealing with warm-blooded and cold-blooded animals, and/or tissue cultures.[53, 93]

Among the first experiments at Brussels, 24 hours was considered a recovery time in mammals, and at 48 hours [28, 58, 31] normally dividing cells were in abundance. Many cells degenerated before 24 hours. Results with *Siredon* and *Xenopus* have been discussed in Chapter 2.

Generally, 5 to 10 hours represented the duration of arrested mammalian mitoses, while in cold-blooded vertebrates mitoses may remain arrested for several days.

Certain trends are seen not only in the recovery figures with *Triturus viridescens*,[74] but also in the recovery frequencies in corneal tissues.[18, 50] A cornea is treated and then allowed to recover. The maximum arrested metaphases observed at the first fixation (8 hours) are an unoriented type (92 per cent) which means that both continuous and chromosomal fibers are inactivated. Only 5 per cent of the figures are stars and 2 per cent bipolar mitoses. The next fixation shows a drop in unoriented metaphases and an increase in stars, 69 per cent and 20 per cent, respectively. Bipolar mitoses increase to 8 per cent. Finally at 72 hours, only 5 per cent of the figures are unoriented while the stars maintain their numbers up to 16 per cent, and most remarkable is the increase in bipolar mitoses to 80 per cent. The picture at 72 hours is a reversal compared to the 8-hour fixation.

Diploid, tetraploid, and octoploid mitoses definitely show that animal cells can be made to double the number of chromosomes.[74, 24, 2] A few anaphase bridges, fragments, as well as chromosomes were found outside the nucleus.[74] As late as 168 hours, some bimetaphases, or the "distributed" c-mitoses, were found in *Triturus*, also some trimetaphases that present a multipolar picture.[74]

Conclusions drawn from studies of the recovery pattern are that (1) chromosomal fibers recover first – otherwise stars would not be first to rise and fall; (2) the continuous fibers follow the chromosomal in recovery; (3) the interaction between two kinds of spindle fibers and the centromeres determines the metaphasic type to be expected; and (4) animal cells may develop into polyploid cells capable of dividing upon recovery.

The nuclear figures were followed during recovery in rats having received single injections following partial hepatectomy.[16] The regenerating liver offered special advantages for the tracing of these stages; a definite series was noticed.[16]

At 12 hours, there were two changes: (1) the chromosomes thickened and shortened, while (2) a gradual clumping was seen. At 18 hours, the cells were full of miniature nuclei, the micronuclei. Some swelling accompanied the clumping.

Between 18 and 48 hours, some amoeboid patterns emerged. These were obviously a result of fusing micronuclei.[14, 15, 16] Perhaps the related and progressive stages were the binuclear and trinuclear stages.

First signs of partial spindles were seen at 48 hours. This is evidence that recovery or reversibility was taking full effect, so that by 72 hours a complete spindle was reformed.

Reversibility is seen in animal cells, but the recovery is complicated by other effects in addition to arrested mitosis. This is particularly true in mammals, where considerable destruction of arrested metaphases takes place not giving time for the spindle to recover before the chromosomes are irreversibly altered.

3.9: Summary

In this chapter and in the preceding one, selected works were correlated to describe, first, the action upon nuclear mitosis as observed through chromosomal patterns and, second, the spindle mechanisms fundamental to arrest by various techniques. We are aware that little attention was given to the mechanism of action, theoretical aspects, and problems of c-mitosis, all of which are suggested by the data.

The action of colchicine involves the cell as a whole and, for animals, the correlated activity of tissues. Before a discussion of the problems can be made most effectively, other aspects must be viewed. Therefore the mechanisms of action as well as the very important problem of mitotic poisons are grouped together in Chapter 17. Here it is hoped that some of the important issues raised by the action of colchicine on plant and animal cells can be brought into a synthesis, the *problems of c-mitosis.*

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