CHAPTER 2

Nucleus and Chromosomes

2.1: Original Concepts

A basic and far-reaching discovery in biology emerged from the activities[29, 33] of the Laboratories of Pathological Anatomy, Faculty of Medicine, University of Brussels, under the direction of Professor Albert-Pierre Dustin: *Colchicine induced metaphasic arrest (stathmokinesis)*. Nuclear mitoses were studied experimentally at Brussels for more than a decade, 1924–1934, chemicals being applied by several methods. After colchicine was suggested,[61] evaluation of its mitotic activity came quickly, and showed that a powerful agent had been discovered. Comparative tests for mitotic poisons proved that colchicine was one thousand times more potent than sodium cacodylate, which they had studied previously.[30] Pure substance, in minute quantity, caused metaphasic stages to accumulate in a treated tissue far beyond the percentages found in untreated sarcomas. These original tests with colchicine, coupled with previous experience with other mitotic poisons, helped to frame the idea of metaphasic arrest by colchicine.[29]

The original slides preserving the tissues treated with colchicine were re-examined by the authors when they worked together in 1949.[35] From these impressive sections, new photomicrographs were made for this book (animal cells, cf. Chapter 10, Fig. 10.1; plant tissues, Fig. 2.1*C*). The total effectiveness displayed by the drug acting upon mitosis is re-emphasized by these pictures. Microscopic inspection reveals an unusual sight. Similar impressions of this totally different mitotic picture had been formed earlier when the senior author,[34] in 1937, saw animal cells treated with colchicine and placed beneath the microscope (cf. Chapter 1). The power to stop mitosis in metaphase was clear to us, and this property has been confirmed by many experimenters.[35] Everyone agrees that the reaction upon nuclear mitosis is specific, selective, and total, under prescribed conditions.[56, 58]

A large bibliography[35] has accumulated since 1934, but one of the original conclusions, metaphasic arrest, conceived by Professor A. P.

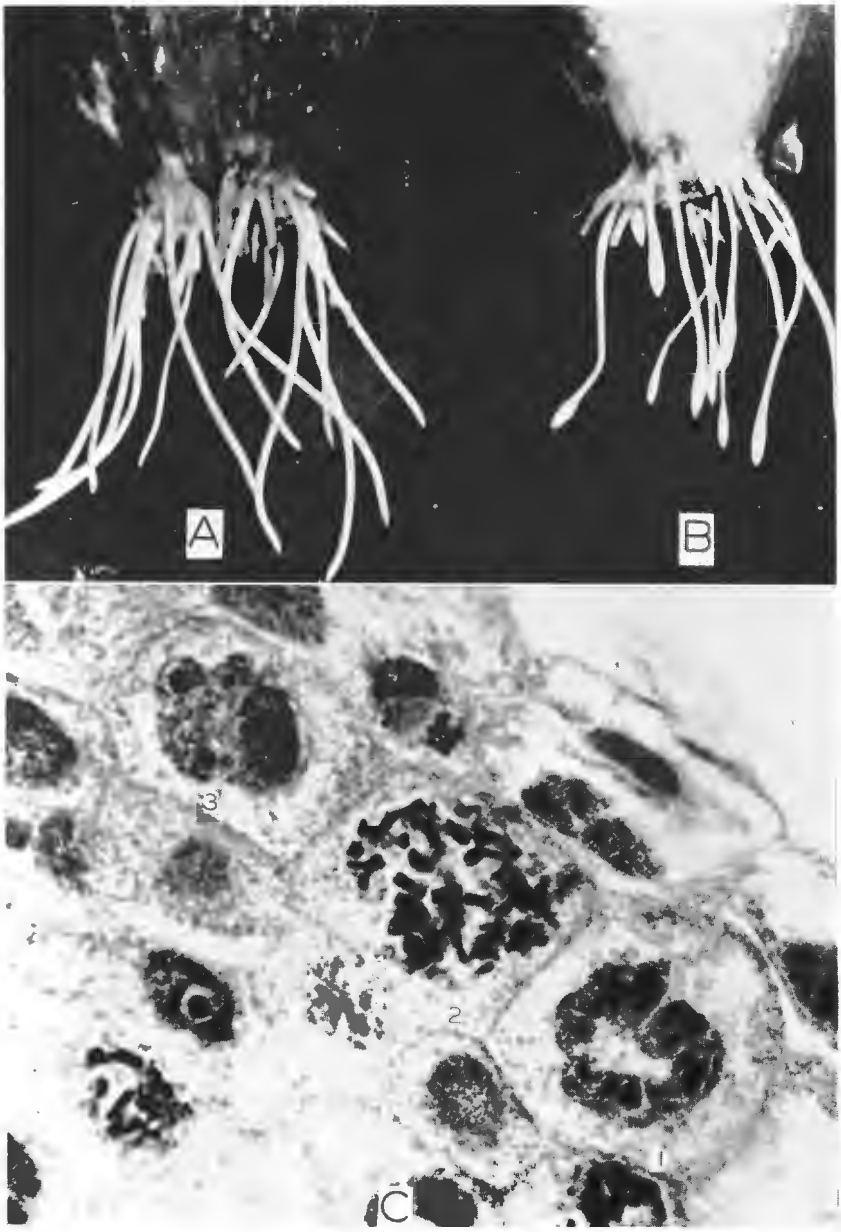


Fig. 2.1—**Allium** roots. **A**, untreated; **B**, treated; and **C**, photomicrograph of section from treated root. **A.** Roots grown in tap water do not show enlargement. **B.** Colchicine solution of 0.01 per cent causes spears, or colchicine-tumors. This group was one of the original tests run in 1937 at Cold Spring Harbor, Long Island, N. Y., by Eigsti. **C.** A photomicrograph prepared specifically for this monograph, from a slide of sectioned root tip made in the Brussels' laboratory, 1934 to 1937, and presently with the A. P. Dustin Collection, University of Brussels. The polyploid numbers can be seen, as well as large multinucleate cells, amoeboid nucleate cells, and pseudospindle. Similar views were illustrated by Havas, Dustin, and Lits in 1937.

Dustin, Sr., stands correct.[29] Almost universally, living cells respond to colchicine after one basic pattern, and new tests extend knowledge into other areas of science. The "colchicine-mitosis"[56] (abbreviated, c-mitosis) is built upon the principle of an arrested metaphase. A c-mitosis was conceived from experiments with plants after the idea had been developed from animal cells.[12, 15, 61, 62] Undoubtedly, the interest in colchicine by the biologist has stimulated an extensive research in the chemistry of this substance.[21]

Metaphasic arrest implies control over dividing cells; seemingly then, control over cancer might be obtained from the use of this chemical or others. This discovery raised hopes and new questions about the problem. However, biological problems being as complex as they are – and cancer is a major one – the answers have not come to us as definitely as might have been hoped or expected. Nevertheless, basic contributions to knowledge such as the idea of metaphasic arrest opened new frontiers in research,[59] even though magic cures have not been produced.

Chromosomal numbers in plant cells are frequently doubled after treatment with colchicine; polyploidy is a consequence of contact with the drug.[25] Since many species, including those important economically, i.e., wheat, cotton, oats, and tobacco, are natural polyploids, the suggestion was frequently made that this tool would help create new "synthetic" plants according to man's desires.[52] A revolution in agriculture was predicted when colchicine became known for its capacity to induce polyploidy. But many were disappointed as the heralded magic did not appear with each newly created tetraploid plant.[37] Informed geneticists, acquainted with polyploidy as a plant-breeding method,[82] did not underestimate the difficulties, nor did they fail to appreciate the opportunities provided by this new tool. Unfortunately, some practical agronomists[64] have condemned the use of colchicine for its failure to produce practical results within a short time; therefore, such research using induced polyploidy has been discouraged. Nevertheless, the technique is valuable for those able to direct such plant breeding, harmonizing theoretical and practical knowledge. For by these methods, mankind's food and fiber supply can be increased (cf. Chapters 12 and 13).

2.2: The Original Statements

When nuclear mitoses in the grafted sarcoma of the mouse were treated with colchicine,[29] deviations from normal division gave the observer a picture of an arrested mitosis. In 1934, Professor A. P. Dustin made the following description:

> . . . after a very short prophase, the nuclear membrane disappears, the cytoplasm swells, and the chromosomes clump together in a strongly basophilic mass. The mitoses remain arrested in that state for about twenty-four hours.

During that period, a certain number of nuclei undergo degeneration. . . . After that period . . . cells . . . complete their division. . . . The achromatic figure becomes visible. . . . Chromosomes move toward the poles. . . . Cytoplasmic division is completed. . . . Some mitotic figures of too great size . . . and some pluricentric divisions remain as a testimony of the nucleotoxic effect. . . .*

These basic statements require no change today even though knowledge has expanded in many directions. Admittedly, as the basic idea becomes extended and broadened, additional points are added. For example, the c-mitosis illustrates enlargement of the original explanation, but no radical changes in concept are necessary.[56]

The Dustin school did not limit their work to animal cells. A Hungarian scientist, the late Dr. L. Havas, treated *Allium* root tips with colchicine.[31] His slides were a part of the Dustin collection available to the authors in 1949. Since the arrested metaphase or c-mitosis was so clearly preserved, new photomicrographs were made (Fig. 2.1*C*), showing the increase in numbers of chromosomes, large restitution nuclei, and "achromatic spheres."[86, 7] But the original text by the Brussels investigators did not mention the polyploid conditions of these cells.[31]

Independently, in 1937, the senior author tested cells from treated root tips (Fig. 2.1*A* and *B*) with acetocarmine methods; the tests showed that polyploidy was created in many different areas of the *Allium* root. The Brussels material and that used at Cold Spring Harbor (cf. Chapter 1) were, in every respect, similar.[34]

A third and independently conducted test with *Allium* roots and colchicine was reported by Dr. Pierre Gavaudan and associates. They published the first account of polyploidy induced by colchicine in June, 1937. Their report stated:[41]

It is evident that in these cases there is a separation of pairs of chromosomes, the number of chromosomes of a restitution nucleus is *double* the normal number. The chromosome list of Gaiser indicates that 2n-16 occurs in *Allium cepa*. Our results show "pseudomitoses" with more than thirty pairs.†

This original report and its significance were not mentioned in reviews[25, 52] or papers[56] in the period immediately following its publication. The more dramatic demonstrations that dealt with induction of polyploidy in plants overshadowed the original and what is now realized as a classic publication by the Gavaudan school.

As soon as Dr. Albert Levan returned to Sweden from America in the autumn of 1937,[56] experiments with *Allium* roots and colchicine were started. This material formed the basis for his concept of an arrested metaphase, as a colchicine-mitosis.[56] Remarkable simi-

* A translation of pertinent comments from the article cited in Reference No. 12, Chap. 1.

† Translated from paper written in French by authors cited in Reference No. 20, Chap. 1, and Reference No. 41 of this chapter.

larity exists between the separate descriptions with animal cells[29] by Professor Dustin and the plant work by Professor Levan. A colchicine-mitosis was described by him as follows:[56]

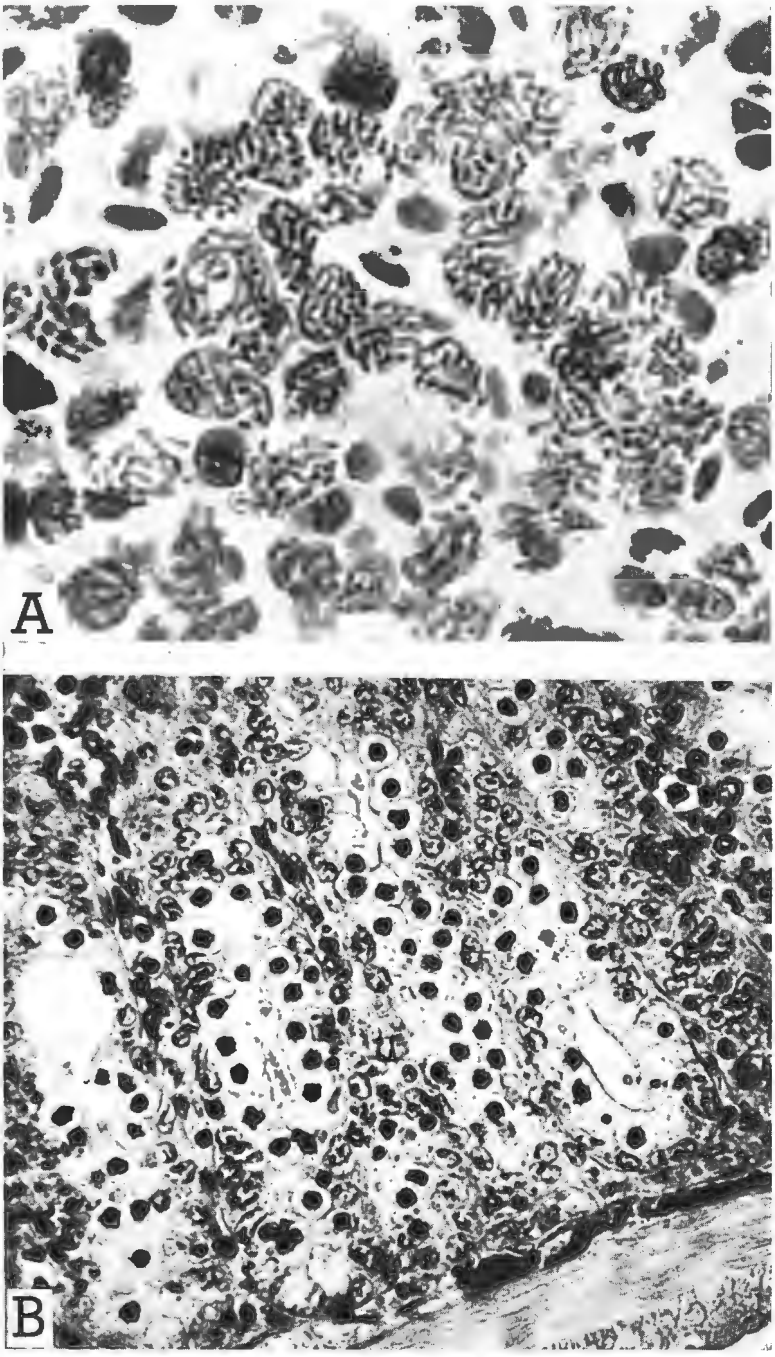
The effect of colchicine on the course of mitosis is entirely specific. . . . Modification in mitotic behavior . . . will be abbreviated "c-mitosis." . . . Prophase stages take place normally: the chromosomes divide, condense, and assume metaphase appearance. . . . They are scattered over the cell. . . . This condition (c-metaphase) lasts . . . long . . . after the disappearance of the nuclear membrane. . . . Formation of "c-pairs" is peculiar to material treated with colchicine. . . . Their origin is evidently due to a delay of the division of the centromere. . . . After a few hours . . . the two daughter chromosomes are straightened out . . . like "pairs of skis." . . . Centromeres are placed opposite one another in each pair. . . . During the c-anaphase . . . division of the centromeres does not take place quite simultaneously within one cell. . . . Inactivation of the spindle . . . is reversible. . . . After a period of 12–24 hours in pure water the spindle begins to regenerate. . . . In the course of the transition to normal spindle all kinds of abnormalities are seen. . . . After 36 hours the mitoses run their normal course. At a certain moment after transfer from colchicine . . . frequent diploid mitoses are seen. . . . Highly polyploid giant nuclei still linger in the prophase stages. . . . Numbers as high as five hundred were not rare.*

Summarily, these are the interesting points covered thus far. An unusual sight appears in a microscopic field focused upon tissues treated with colchicine; the nuclear mitoses are halted at metaphase, and converted into c-mitoses.[36, 78, 2] This power to induce c-mitosis belongs to select chemical and physical agents,[58, 33] of which the most potent, in this respect, is colchicine. It acts upon mitosis with great efficiency,[77] high specificity, and total selectivity. The obvious difference between normal nuclear mitosis and c-mitosis is the tremendous accumulation of chromosomes within a given area (Fig. 2.2) where numerous cells adjacent to each other are arrested in metaphase, a primary feature of c-mitosis activity.

Now the total or partial reaction from this drug depends upon the interaction of (1) a specific concentration, (2) given exposure period, (3) particular mitotic stage when chemical contacts nucleus, (4) cellular type, and (5) environment favorable to mitosis. Under these conditions metaphases are arrested. Consequently metaphasic

* A condensation of the concept of a c-mitosis taken from Levan, 1938, Reference No. 26, Chap. 1.

Fig. 2.2—Accumulation of arrested mitoses in animals injected with colchicine and sodium cacodylate, both spindle poisons. A. Spleen of Siredon five days after a single injection of colchicine. The organ has increased in size, and many arrested prophase-metaphases can be observed. These belong mainly to young red blood cells. The longitudinal splitting of chromosomes can be noticed at some places. (From an unpublished photomicrograph by Delcourt) B. Accumulation of arrested metaphases of the "ball" type in the intestinal crypts of the small intestine of a mouse. This condition follows injection of sodium cacodylate and is identical to that observed 6 hours after injection of colchicine. Cf. Chapter 17. (From an unpublished photomicrograph from the work of Piton and A. P. Dustin)


A
B

chromosomes accumulate in pairs, "colchicine-pairs," [56] in cytoplasm. Their distribution then is not the usual equatorial plate arrangement. Furthermore, an arrest at metaphase reduces the number of anaphases or telophases (Fig. 2.3) thus adding to the apparent increases in this one particular stage, the c-metaphase. That is why the observer is struck by a totally different mitotic pattern as he looks at treated tissues through the microscope. Usually tissues have a few metaphases, some anaphases, some telophases, but mostly non-dividing cells. Even a meristematic tissue in plants or a sarcoma of animals,[13]

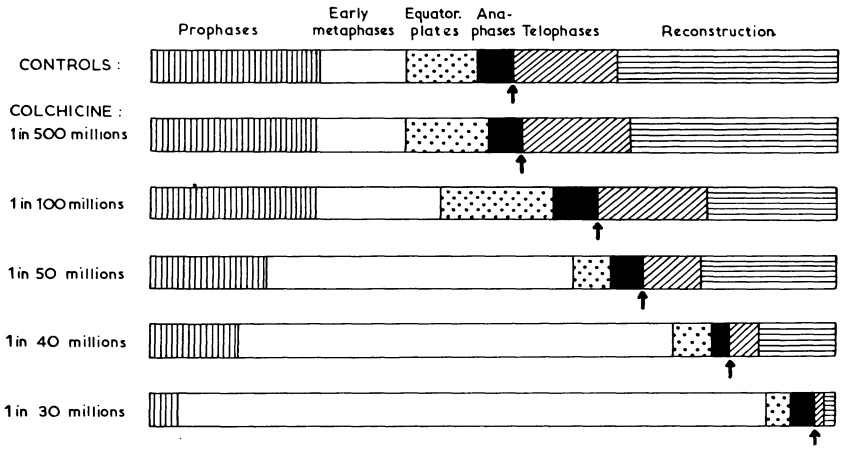


Fig. 2.3—Graphic representation of the percentages of mitotic stages in fibroblast cultures exposed for ten hours to solutions of colchicine. With increasing concentration, the percentage of metaphases with unoriented chromosomes increases. The displacement to the right of the arrow, indicating the end of anaphase, demonstrates that in the most concentrated solutions, nearly all mitoses remain arrested and do not proceed to telophase. This effect is clearly related to concentration. (After Bucher, 1947)

each noted for cell division, has only a limited number of cells showing chromosomes at a particular moment. It is not surprising that the accumulation of metaphases impressed one pioneering investigator who described this reaction by colchicine as "an explosion of mitoses."[61]

Ultimately, exclusive of recovery, the restitution nucleus is formed when the chromosomes transform[22] to interphase without forming the daughter nuclei. This transformation may start from an arrested metaphase, thus by-passing the c-anaphase. Or, the changes[22] may begin after the chromosomes of each c-pair have fallen apart in the c-anaphase[56] — a transition involving separate chromosomes. Sometimes the uncoiling begins as early as prophase.[93] These different points of origin mark three routes taken when the chromosomes "unravel" and undergo transformations to interphase. If the number of centromeres has doubled, a feature clearly seen at c-anaphase, then

the chromosomal number in the restitution nucleus will be twice that of the nucleus before a c-mitosis began. One important consequence of the c-mitosis in contrast to the normal nuclear mitosis is the induction of polyploidy.[41, 56] But not all restitution nuclei become polyploid, since the changes[22] may start from a prophase or metaphase.[84] In fact, many animal cells treated with colchicine are arrested at metaphase. The transformation from this stage does not lead to a restitutional polyploid nucleus, for in these instances other changes occur.[29, 61]

Finally, the most significant biological feature basic to all these changes is *reversibility*.[56] After the colchicine in concentrations creating arrest becomes dissipated, the cell may recover; that is, a bipolar nuclear mitosis again proceeds in the same manner as before an arrest was induced. Such recovered cells will continue to divide thus as long as the cell lineage retains that power. No permanent damage, with few exceptions,[91] to spindle mechanisms or chromosomes is acquired from the arrested metaphase. Of course, the arrest may have been so severe that changes in metabolism cause the cell to degenerate and ultimately die, but our concepts of reversibility now refer to those cases where there is complete recovery, a reversibility to the bipolar mitosis. These can take place among plant and animal cells. The recovery pattern like the whole c-mitotic sequence is unique and notably uniform for many subjects.

Since there is the reversibility potential, a restitution nucleus with twice the number of chromosomes may regenerate its new spindle mechanism. From a genetic view this is a most significant aspect of reversibility, since the restitution nucleus with twice the number of chromosomes gives rise thereafter to daughter cells, each with a polyploid condition.

By this procedure of metaphasic arrest – c-anaphase, restitutional polyploid nucleus, and recovery – the induced polyploidy is transmitted to succeeding generations. This discovery has had important ramifications in agricultural research. Whereas control over cell division would appear to be desirable for treating certain diseases, this same control over cell division has entirely different, broad applications in agriculture. That is why a basic discovery in science can be so widely used in other fields.

2.3: Prophase

First reports said that colchicine had no influence upon prophase.[56, 29] Later by cinematographic record, no modification at prophase was noticed.[15] A general belief developed that this portion of nuclear mitosis was not changed by the drug, for data obtained by new methods from fixed and stained cells appeared the same for treated and untreated cases.

In animal cells the prophase stages were thought to be non-susceptible to colchicine because the drug did not penetrate the nuclear membrane.[62] Therefore chromosomes remained as usual until the membrane disappeared. Then the chromosomes came in contact with the drug present in the cytoplasm. After this period, contraction might take place.[71, 7, 77, 63, 83]

From plant tissues, fixed and stained, three important changes were compared at prophase.[65] First, chromatin threads developed the minor spiral in both instances. Second, the major spiralization proceeded along usual patterns. Third, chromosomes condensed into proportioned prophasic structures as this stage ended. The two distinct chromatids were strongly cleaved, appearing as longitudinal pairs twisted about each other in a relational coil (Fig. 2.10*A*). On these three points no noticeable differences among fixed and stained cells, treated and untreated, were observed.[65] But such opinions about the action of colchicine at prophase required modification as new techniques[93, 34, 39] replaced traditional cytological methods, and a wide range of concentrations was included.

Living cells were observed continuously from prophase through all mitotic stages in *Tradescantia* staminal hair cells.[93] By this method colchicine could be applied at any stage chosen by the investigator, who then followed the effects from that particular stage on through subsequent ones.

Strong concentrations (2 per cent) admitted during mid-prophase at the stage when chromosomes were condensing, caused the process to revert back to an interphasic dispersion of chromatin.[93] The time schedule for this reversion showed that a metaphasic arrest had not been reached, but the restitution nucleus was formed from a mid-prophase stage. In some cases the restitution nucleus appeared to be doubled for chromosomal number. Similar cases were reported for *Siredon* (Fig. 2.9A–D).[24, 84] This is one type of transformation from prophase to interphase.

Time schedules for the formation of chromosomes in prophase have been made with *Tradescantia*. This phase is called the *anachromasis*[93] period of chromosomes. Untreated cells require 97 minutes from early prophase to the polar cap stage. Longer time is taken in the presence of 0.05 per cent (121 min.), but a minimum time in 0.1 per cent (84 min.) is less than control. These concentrations permit the chromosomes to move into the arrested metaphase, whereas a stronger solution induces interphase. Colchicine slows down the process of anachromasis as it occurs in prophase. To contrast these developmental processes, new methods had to be developed.

The neuroblastic cells of grasshopper are used in another technique[39] with unusual possibilities for a different inspection of c-

mitosis, particularly at prophase. Like the *Tradescantia* staminal hair cell method, the drug can be administered when mitosis reaches a certain stage; thus a new approach is made with animal cells. Time, gross changes, and unusual developmental sequences can be charted.

By this critical method the action of colchicine upon prophase was manifested in three distinct ways.[39] First, strong concentrations (50 and 25 $\times$ 10^{-6} *M* col.), applied at late and very late prophase, caused the chromosomes already partially formed to revert to an earlier dispersed phase. Second, lowering the concentration (2.5 $\times$ 10^{-6} *M*) induced precocious reduction in the relational coiling and an unusual contraction of the chromosomes before the nuclear membrane disappeared. At this concentration, prophase chromosomes, normally fixed with centromeres at the polar side of the nucleus, were disoriented. By microdissection methods, the polar fixation at prophase was tested.[39] Colchicine, in proper concentration, destroys some factor associated with this fixed position. Third, additional decrease in concentration (1.9 $\times$ 10^{-6} *M*) applied at prophase disposes the chromosomes into the "star" formation as soon as the nuclear membrane disappears. These stages may develop into a multiple-star phase, and from this formation chromosomes settle out to the bottom of the cell. These three conditions show that colchicine induces changes at prophase when certain concentrations are used. These changes are revealed when continuous records can be made.[39]

Thus colchicine may act upon chromosomes at prophase, causing interphase loss in relational coiling, contraction, destruction of intranuclear orientation, and predisposing the chromosomes to a star formation. These comparisons required a special technique able to focus attention upon specific stages, using a wide range of concentrations, and then following the successive development from one stage to the next.[39]

Pollen grains planted in colchicine sucrose-agar[34, 89] provide a special method for observing the effects of strong concentrations (1 per cent) upon prophasic stages. Each grain at the time a culture starts, begins with a nucleus in prophase. Pollen tubes grow and the cell lives for a time, but the prophase goes into interphase and does not move into an arrested metaphase. These unpublished data were collected from treated and untreated cells fixed and stained at given intervals.

Analyzing percentages of prophases, treated and untreated, there is noted a proportional decrease in the relative percentage of prophase as the experiments continue.[65] Inhibition of prophase is indicated with concentrations that cause arrest at metaphase (0.01 per cent). This decrease for *Allium* begins after twenty-four hours[60] (Table 2.1). At this period the c-metaphases have reached a peak.[60]

TABLE 2.1
PERCENTAGE OF C-MITOSES FOR ONE HUNDRED FIGURES
(After Mangenot, 1942)

Root Tips of Germinating Onion Seedlings—Colchicine 0.05%

	Control	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Resting stage	85.0	85.0	86.2	90.0	96.6
Prophase	6.6	3.2	2.8	1.6	0.6
Meta-anaphase	4.2	9.6	7.2	6.4	2.0
Telophase	3.4	2.2	3.8	2.0	0.8

Onion Bulb Root Tips—Colchicine 0.05%

	Control	18 hrs.	40 hrs.	93 hrs.	184 hrs.
Resting stage	88.42	77.22	77.30	88.61	95.76
Prophase	8.21	7.18	7.53	1.84	0.69
Meta-anaphase	1.57	14.30	13.84	8.46	3.00
Telophase	1.78	1.30	1.30	1.07	0.53

Onion Bulb Root Tips—Combined Test—Heteroauxin 0.0001%—Colchicine 0.05%

	Control	24 hrs.	40 hrs.	67 hrs.	91 hrs.	139 hrs.
Resting stage	88.42	80.5	84.50	89.20	90.70	97.30
Prophase	8.21	4.6	4.50	2.60	1.50	0.4
Meta-anaphase	1.58	13.10	8.00	5.30	4.80	1.40
Telophase	1.78	1.80	3.00	1.90	3.00	0.90

A similar inhibition was seen in neuroblastic cells[39] but expressed in somewhat different manner. Cells subjected to colchicine in late prophase remained arrested in prophase for 150 minutes before developing a metaphase stage.[39] This process at late prophase, a transition from prophase to metaphase, requires 32 minutes.[39]

Critical time-dose relationships must be observed to produce maximum arrested metaphases in regenerating liver of rat.[11, 12, 13] This dose is one microgram per gram of body weight. Above this concentration, colchicine causes reduction in the mitotic stages in metaphase. Even before any supralethal dose kills the animal, the inhibiting action upon mitosis is observed. That is, the prophases do not seem

to move into the arrested metaphase. This would seem to be an inhibition at prophase. Under optimum conditions for dose-time relations, the maximum metaphasic arrest is obtained in mammals at 8 to 10 hours following the injection of colchicine.[61]

Amoeba sphaeronucleus may grow in colchicine without noticeable changes. When colchicine is injected into the cytoplasm by micropipette, action upon mitosis occurs. Amounts injected when the nucleus is in prophase cause return to interphase. Continuous photographic records verified this process. About 1 per cent strengths are needed to induce such chromosomal changes.[20]

Different cells in *Allium* root tips show variation in degree of polyploidy. Pericycle cells may contain several hundred chromosomes, yet the cells at the tip, a meristematic area, will have the diploid number. Seventy-two hours of treatment with adequate concentrations do not induce polyploidy among restricted groups of cells.[65, 67] This has been called a prophase "resistance," characteristic of younger cells.[86] Practical significance becomes attached to this feature if polyploids are to be induced without any diploid cells accompanying the new tissues. Prophase stages are more involved than was formerly accepted.

Two terms might be useful in discussing prophase influences by colchicine and other chemicals: (1) the pre-prophase poison which prevents resting cells from entering the prophase, and (2) the prophase poison, as described above, that inhibits the normal prophase development and in exceptional cases causes a change to interphase. Plants and animals differ with respect to the relative toxic action of colchicine and these make a great difference in the inhibitions not only of metaphase but of prophase as well.

Prophasic arrangements that are held over from the previous telophase are not disturbed in plants by concentrations that induce c-mitosis, e.g., *Dipcadi*.[56] Yet this arrangement is upset in neuroblast cells with concentrations that give typical arrested mitosis,[39] while in mammals, prophase appears to be the most resistant period.[15, 29, 61, 63]

Earlier opinion regarding prophase as always normal in the presence of colchicine must be modified. More information is needed at this critical and difficult stage. Depending upon concentration and the particular material treated, prophase stages are influenced by colchicine.

2.4: Colchicine Metaphase

Again and again, after experiments with animals and with plant cells, the same conclusions were reached: colchicine changed the nuclear processes at metaphase. With few exceptions, agreement is unanimous, and the opinions are usually formed around the follow-

ing explanations: (1) The metaphasic arrest arises when the spindle fiber mechanisms are partially or totally destroyed.[62, 56, 25, 27, 4, 80, 58, 77, 75, 39] (2) Chromosomes lose their metaphasic orientation when the spindle fibers become disengaged from the chromosomes.[34, 87, 90, 85, 7, 26, 22] (3) The spindle mechanisms are inhibited by colchicine; therefore, nuclear mitoses are arrested at metaphase.[29, 5, 93, 33, 40, 1, 79, 39] While three similar cases are presented, each thesis leads to the same general conclusion: the metaphasic arrest. That is why agreement in the final analysis is so excellent considering the many different biological specimens studied. Universally every one's attention is directed first to the chromosomal pattern at metaphase arrested by colchicine (Fig. 2.1*C*, 2.4*D*, and 2.8*A*) that is quite different from the normal metaphasic orientation (Fig. 2.4*A*). Spindle mechanisms enter the discussion only after the first impressions of chromosomal patterns have been obtained. Accordingly, our discussion is first directed to the chromosomal patterns of arrested metaphase. After these have been compared, it would appear consistent to discuss and analyze the spindle mechanisms that must operate in the production of c-mitosis. The spindle mechanism will be considered in Chapter 3.

2.4–1: Types of arrested metaphases. The regular metaphasic figures and equatorial plate orientations are replaced by different chromosomal patterns (Figs. 2.7*A*, 2.8*A*, and 2.4*D*). Such distributions are induced by colchicine, and these arrangements are not wholly random ones.[1, 79] Characteristic stages repeat often enough that a classification (Fig. 2.5) is possible.[1] If we disregard spindle action for the moment, the arrested metaphases may be grouped into two major categories: (1) the *oriented* metaphase (Fig. 2.5, *above*), (2) the *unoriented* metaphase (Fig. 2.5, *below*). There are subtypes for each group which will be considered under the special headings that follow.

Analysis of the pattern will be made on the basis of interacting factors that create the special type of arrested metaphase, while direct reference to spindle mechanisms will be deferred for the moment. The classification shown in Figure 2.5 was made from stained cells by cytological methods not thoroughly reliable in differentiating the fibers.[1] For this reason, criticism[79] has been made regarding assumptions involving spindle mechanisms, specifically with reference to the distorted star metaphase. Even though this classification was developed by a chromosomal pattern, an insight into c-mitosis and the arrested metaphasic types can be gained by such comparisons.

Colchicine penetrates the cell very rapidly. Effects may be noticed within seconds after the drug contacts the nucleus. C-mitosis in *Allium* develops permanently and completely within fifteen minutes.[88] Rate of penetration, as well as concentration, is very important. The

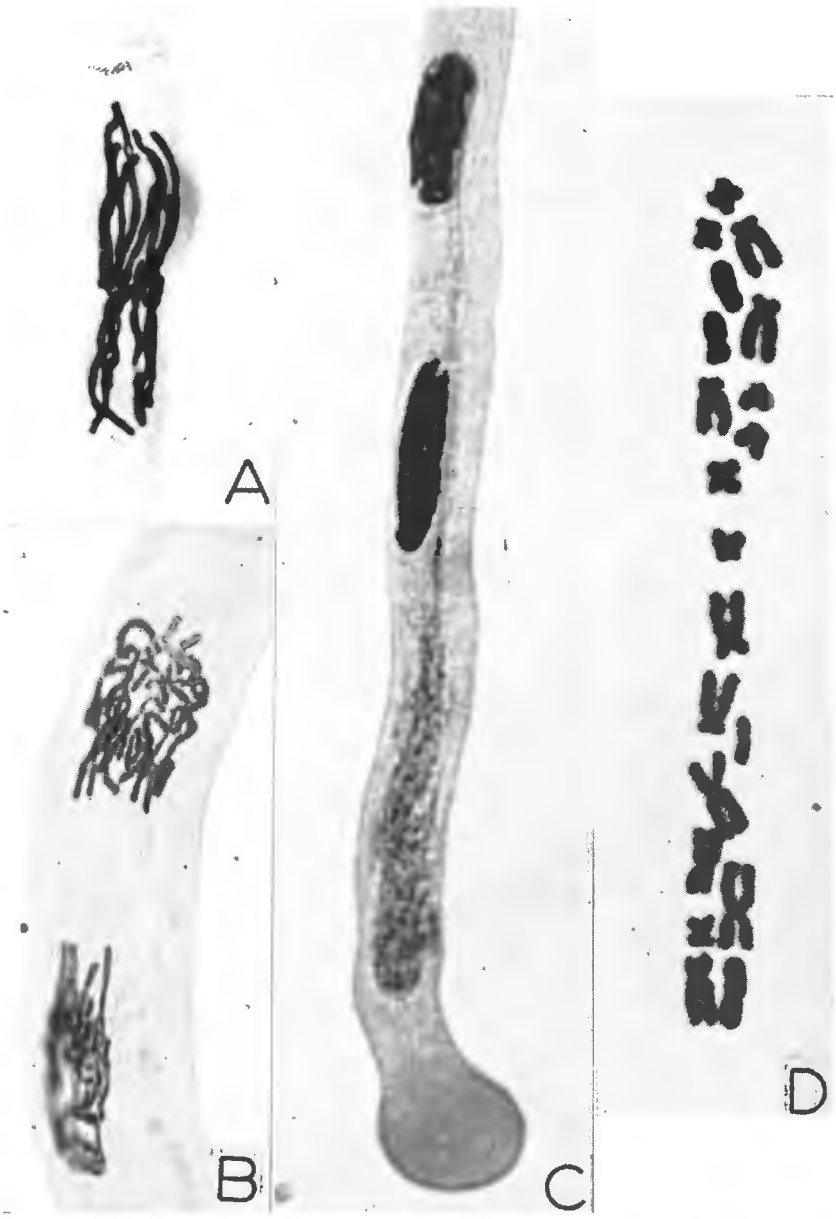


Fig. 2.4—Pollen tube cultures treated and untreated. **A.** A metaphase of generative cell of **Lilium michiganensis** without treatment. One per cent agar and 7 per cent sucrose, stained with iron alum haemotoxylin. **B.** Anaphase, **Polygonatum commutatum** untreated. Stained with acetocarmine. **C.** Two microgametes and tube nucleus. **D.** Arrested metaphase, c-pairs, caused by adding 0.01 per cent colchicine to culture media. The duplications among c-pairs indicate polyploidy. There are 20 c-pairs but only 10 types for the entire group. Centromeric locus shown by incision along chromosomes. Stained with acetocarmine. (Eigsti, 1940)

mitotic stage on hand when colchicine reaches the nucleus may determine the metaphasic type.

Since the action is reversible,[5,6] cells may recover from the action of the drug. Arrested types appearing during the recovery sequence[7,9] on the way to complete bipolar mitosis are as significant as those showing up when the drug is acting upon the mitosis.[1]

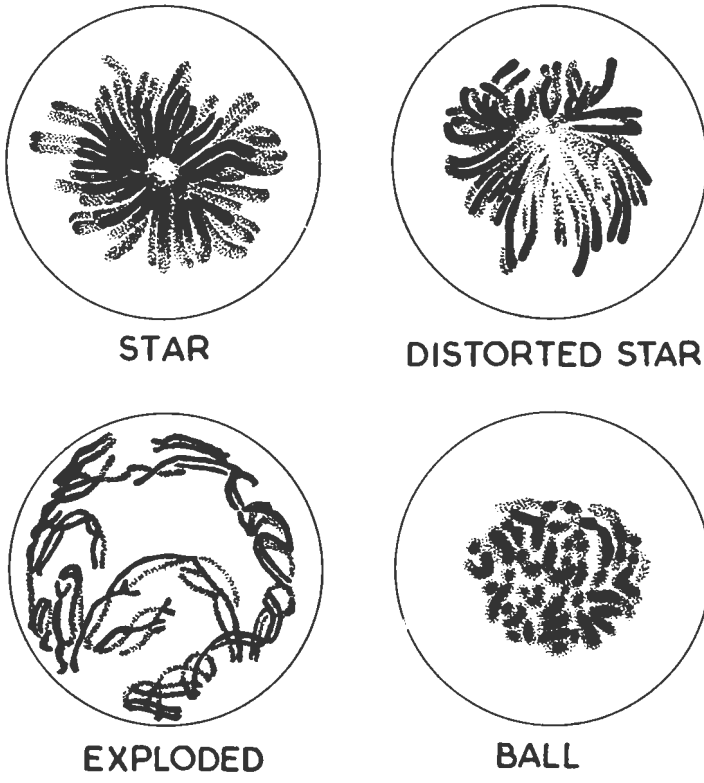


Fig. 2.5—Schematic representations of the main types of arrested metaphases. (After Barber and Callan)

Length of exposure and concentration are directly related to the pattern that will develop.[7] A given situation must be noted with reference to these two factors.

Then, as was mentioned before, concentration, exposure, mitotic stage, kind of cell, recovery, active treatment, and general growth conditions become critical to the formation of an arrested metaphasic pattern whether oriented or unoriented.[1] Even though the interacting factors are several, the number of metaphasic types is surprisingly

limited. In light of the complex interaction, it would seem that the kinds of metaphase that could develop would be more extensive.

2.4–2: The oriented arrested metaphase. In 1889, Pernice[78] sketched the first star metaphase, a distinctive oriented type induced by colchicine.[36] Next, these were reported in 1936[61] among tissues of mice and carcinomatous tissue cultures,[62] and since then the oriented star metaphase has been published many times, from a great variety of biological specimens.

The frequency of star metaphases is far too regular to be ascribed to a random occurrence.[1, 79] The chromosomes are all drawn to one focal point with the proximal portions extended outward resembling a star, and the type was named accordingly. The centromeric portions of the chromosomes are congregated at this one focal point[8] (Figs. 2.5, *upper left,* and 2.7*B–F*).

Two sets of data from similar materials, *Triton vulgaris*[1] and *Triturus viridescens*,[79] respectively, are pertinent to the matter of origin of the star. Larval cells of *Triton* were kept in solutions and were then removed from time to time, fixed, and stained for chromosomal pictures. The star, or oriented, metaphases, exceeded the unoriented types in the first fixations, at three hours (Table 2.2). The *Triturus* corneal cells, fixed and stained at intervals during recovery from the effects of drug, do not show the star metaphases at their peak until twenty-four hours have elapsed (Table 2.2).

Two critical experiments performed with neuroblastic cells in the grasshopper explain some of these differences.[39] Strong concentrations applied when the cell was at metaphase led to a star metaphase (cf. Chapter 3; Fig. 3.20). This action occurred after a particular mitotic stage had been reached. Another route was used to produce the star in neuroblastic cells, viz., application of lower dosage (1.9×10^{-6} *M*) at late prophase. Two sets of factors were operating: the concentration and the mitotic stage. In one instance a metaphasic stage was used, and in the other, prophase. Each required a different concentration. In the *Triton* materials, strong concentrations acted early, yet in *Triturus,* the stars accumulated later as cells were recovering from a previous strong dose. We shall return to this problem again under the subject of spindle mechanisms.

Multiple stars in single cells are commonly found in *Allium* root tips when cells recover.[56, 65] In similar instances, the "multiple" stars (Fig. 2.6) are to be seen in the *Tubifex* eggs.[95] Among the *Triturus,* recovery stages at six days show multiple stars (Fig. 2.7). Multiple stars are formed in connection with transition stages from the full c-mitosis to the complete recovery of the bipolar mitosis.[56]

Distorted star metaphases[1] are asymmetrical figures (Fig. 2.5). The origin of distorted star metaphase is controversial, and although they

TABLE 2.2

ARRESTED METAPHASES—TREATMENT AND RECOVERY

I. COLCHICINE TREATMENT STUDY: *Triton vulgaris*; EPIDERMAL CELLS OF LARVAE

(After Barber and Callan, 1943)

Frequency of Different Types of Cell (Means of Counts From 3 Larvae)

Duration of Treatment (*hours*)	Prophase	Bipolar Meta-phase	Star Meta-phase	Un-oriented Meta-phase	Total Meta-phase	Anaphase
0	22.3	25.0	.....	.....	25.0	30.7
3	24.0	15.7	7.7	6.3	29.7	20.0
6	20.3	15.0	16.3	10.7	42.0	15.7
12	27.0	12.3	20.7	66.3	99.3	8.3
24	17.7	5.0	6.7	175.3	186.0	6.7
48	12.0	0.3	1.7	83.3	85.3	4.3
72	2.3	.....	.....	9.7	9.7	1.0

II. COLCHICINE RECOVERY STUDY: *Triturus viridescens*; CORNEAL TISSUES

(After Peters, 1946)

Differential Count Expressing Percentage of Mitotic Types During Recovery

Recovery Time (*hours*)	Metaphase, Anaphase, Telophase	Unoriented Metaphases	Star Metaphases
8	2+	92+	5+
24	8+	69+	20+
72	79+	5+	16+

were among the first cases known,[24] less exact knowledge of their formation is at hand than for the star metaphase.

Outside the star or the distorted star, isolated chromosomes are regularly observed. This formation accounts for "lost" chromosomes frequently described in plant and animal tissue-culture cells.[15, 70]

2.4–3: Unoriented metaphases. Chromosomes scattered in the cytoplasm after a nuclear membrane disappears have been thoroughly described in plants[72, 34, 25, 73, 56, 76, 86, 27, 80, 40, 65, 77, 75, 22, 83] and animals.[29, 61, 62, 13, 24, 32, 87, 90, 1, 79, 28, 53, 39] The descriptive expression *exploded metaphase* is appropriate (Figs. 2.4*D*, 2.7*A*, and 2.8*A*). There

is a complete lack of the usual equatorial metaphase orientation, hence the epithet *unoriented* (Fig. 2.1*C*, 2.4*D*, and 2.8*A*).

The exploded metaphases were described from cells of mice treated with strong doses of sodium cacodylate.[30] Therefore, a reappearance with colchicine tended to call attention to similarities between the two substances.[33]

Among regenerating liver cells following hepatectomy, the exploded metaphase is very characteristic (Fig. 2.8*A*). The investi-

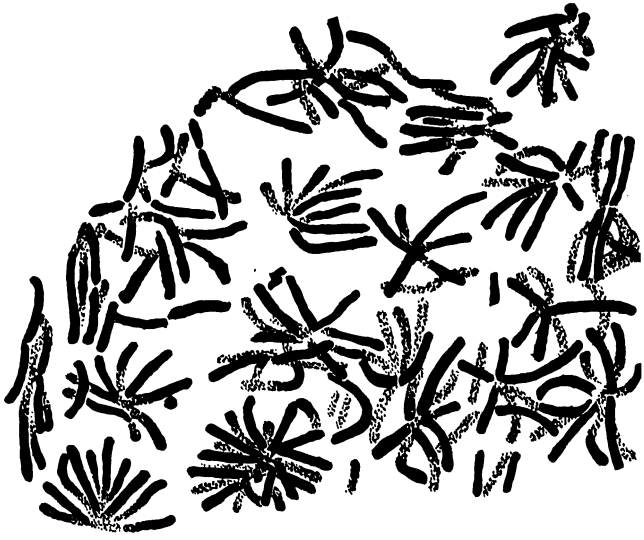


Fig. 2.6—Cell of **Allium** root tip with an excessive number of chromosomes. Fixed after treatment for 208 hours, with 0.05 per cent colchicine in nutrient solution. The cells are beginning recovery; multiple star metaphases are present. Later cell plates form between the groups reducing one large cell to a number of smaller cells. Cf. Chapter 3. (After Mangenot)

gators[12, 13] described the unusual arrangement as though the individual chromosomes "repulsed one another." These widely scattered chromosomes in a single cell were equally impressive from other animals, the tissue cultures, and special cases, e.g., *Siredon*,[24] *Triton*,[1] *Triturus*,[79] and *Orthoptera*.[87] With plants, *Allium* root tips have been a favorite source for these types, but pollen tubes show unusual scattering of the c-pairs through the length of a single tube (Fig. 2.4*D*).

A specific concentration (2.5×10^{-6} *M*) applied at late prophase created the exploded metaphase in grasshopper neuroblastic cells. Similarly, critical dose-time requirements were necessary to produce an arrested exploded metaphase in the regenerating cells of liver

(hepatectomized rats).[11, 13] Supralethal doses did not induce maximum arrested metaphases or exploded metaphases. There is then an optimum dose required for this type. Apparently this same rule holds for pollen tubes, because maximum scattering throughout the tube occurred only under given conditions of concentration and favorable pollen tube growth.[34] There are other cases bearing on this point.

Prophase-metaphase arrangements of chromosomes as an unoriented type are frequently observed (Fig. 2.2*B*). The spleen of *Siredon* yielded these types among the first colchicine-arrested mitoses ever studied (Fig. 2.2).[24, 84] Perhaps a more logical descriptive term would be *arrested prophase,* since the prophase orientation is maintained as the nuclear membrane disappears. No sign of spindle movement is detected. The chromosomes may revert to the interphase from a prophase-metaphase. During periods as long as five days after injection, the prophase-metaphase appears in *Siredon* (Fig. 2.9). Representative cases in animals are noted for this type.[16, 92] Following anaphasic treatment the intermingling of two sets of chromosomes leads to a similar prophase-metaphase grouping,[39] so that treatment at prophase or at anaphase might give this unoriented association.[39]

Ball metaphases[1] are distinctly clumped types (Figs. 2.2, 2.5). In fact, the clumped c-mitosis observed in *Spinacia,*[7] *Lepidium,* and *Petroselinum*[83] are typically ball metaphases. A toxic action is undoubtedly responsible for the particular apparent fusion of unoriented chromosomes. The next step in progressive development is either the degeneration after pycnosis or recovery to an interphasic stage. *Triton* material was represented with more ball metaphases than any other unoriented type. Even though chromosomes appear clumped, an individuality may be maintained as was pictured for cells of mice by the lacmoid-acetic method applied to a ball metaphase.[33] Many of these mitoses undergo destruction eventually in warm-blooded animals.[61] Lysis or degeneration after a ball metaphase may account for the destruction noticed in *Tubifex.*[53, 54, 55]

Ball metaphases are regularly produced in pollen tube cultures when the concentrations exceed .01 per cent in culturing media.[34] Clumping at the early stages followed by pycnosis and eventual lysis forms the regular course taken by the ball metaphase in pollen tube cells. Similar degeneration and settling of chromosomes in neuroblastic cells indicate destructive action as accompanying this particular unoriented type.

Much discussion has been directed to the distributed c-mitosis, a type that can be clearly demonstrated in pollen tubes when the c-pairs group into two clumps (Fig. 2.4*D*). The chromosomes are c-pairs, and separation may or may not be equal in number. The

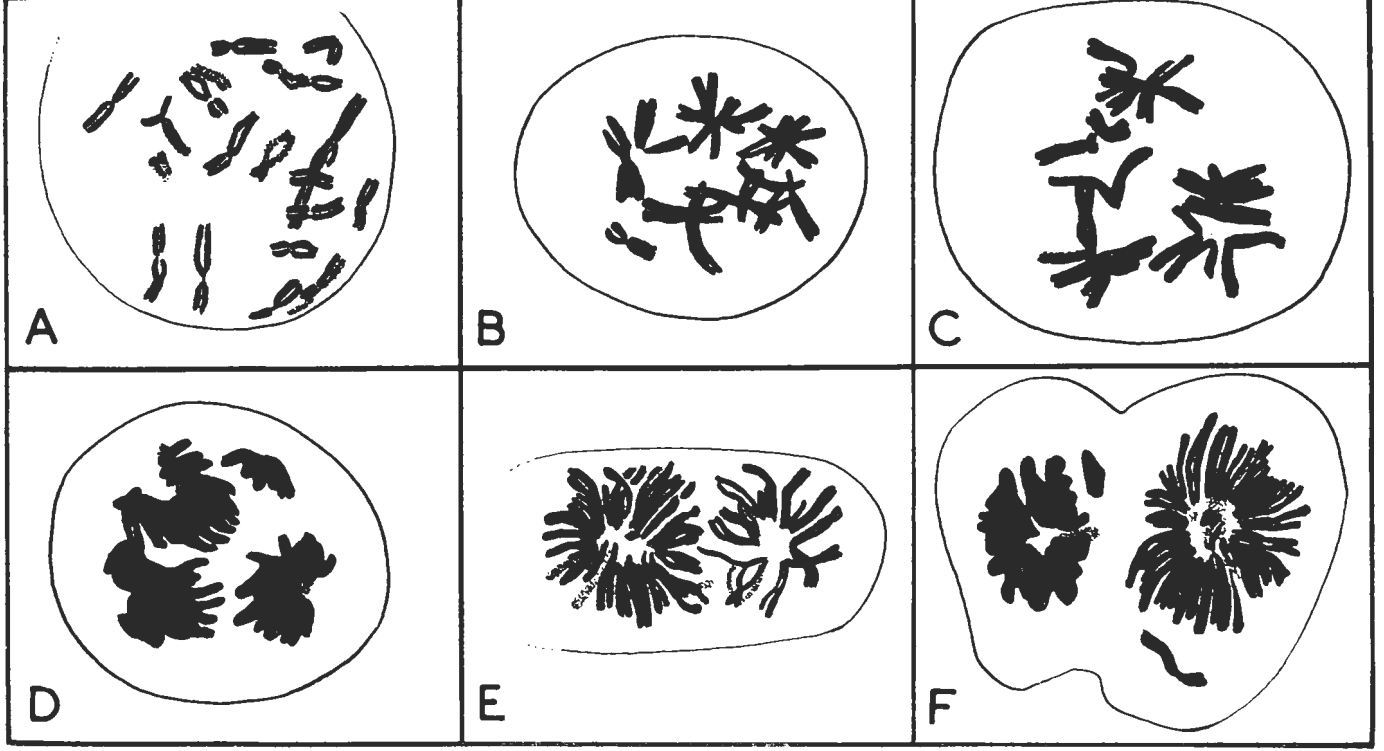


Fig. 2.7—Redrawn from photomicrographs of whole mounts from the cornea of **Triturus viridescens**. Cells treated with colchicine according to a schedule, then allowed to recover. Views show cells in various stages of recovery after treatment. **A.** Six days after recovery. An exploded metaphase, c-pairs similar to pollen tube and **Allium** root tip figures. **B.** Multiple stars six days after recovery, some c-pairs isolated from stars. **C.** Two centers of focus; some evidence of fibers observed on slides but only the position shows spindle action. Diploid number of chromosomes could be determined. **D.** Polyploid cell, five days after recovery; several anaphasic groups, multipolar spindle. **E.** Two metaphase groups, a distributed c-metaphase would give rise to such numbers. **F.** A diploid and tetroploid cell; each has a single chromosome outside the main group, six days after recovery. (After Peters)

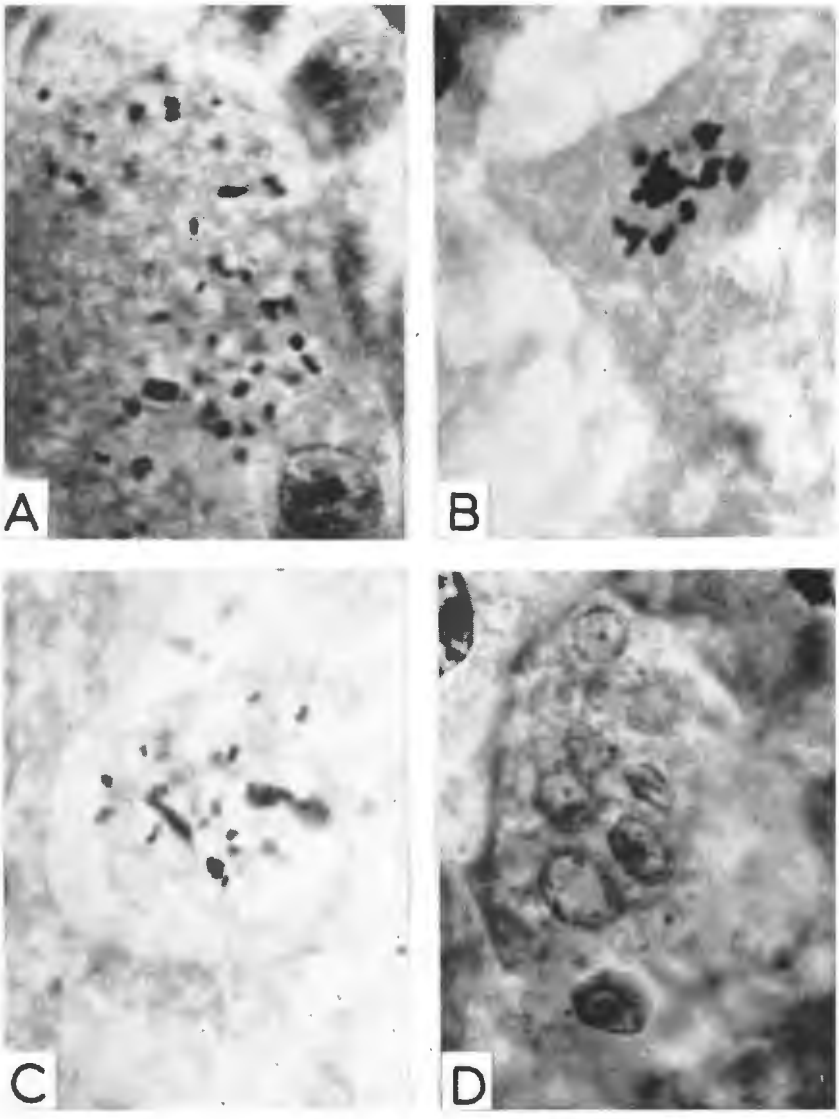


Fig. 2.8—Stages of restitution in exploded metaphases in the regenerating liver of rats injected with colchicine. Feulgen-fast green staining. A. Eight hours after colchicine. Typical exploded metaphase, without spindle. Scattered and shortened chromosomes. B. Sixteen hours. Chromosome agglutination and lengthening. C. Sixteen hours. Some suggestion of catachromatic changes. D. Thirty hours. Formation of large micronuclei; these originate by the catachromatic changes of agglutinated groups of chromosomes. (Original photomicrographs. Courtesy of A. M. Brues, Univ. of Chicago)

best classification for the distributed c-mitosis, or bi-metaphase,[79] is a subtype of the exploded metaphase. A somatic meiosis is not conceivable for the pollen tube, yet the distributed c-mitosis is like the cases upon which evidence for somatic meiosis has been built.

Seven years after the distributed c-mitosis was first published and illustrated[34] the term was coined.[75] This is preferable to *somatic meiosis*.[94] An unfortunate confusion in terms arises because one word has been used in two different instances to describe entirely different processes: The word *pseudoanaphase*[7] is used for the distributed, so-called bipolar arrangement of the c-pairs. In another instance, *pseudoanaphase* is synonomous with colchicine-anaphase.[65] The word should

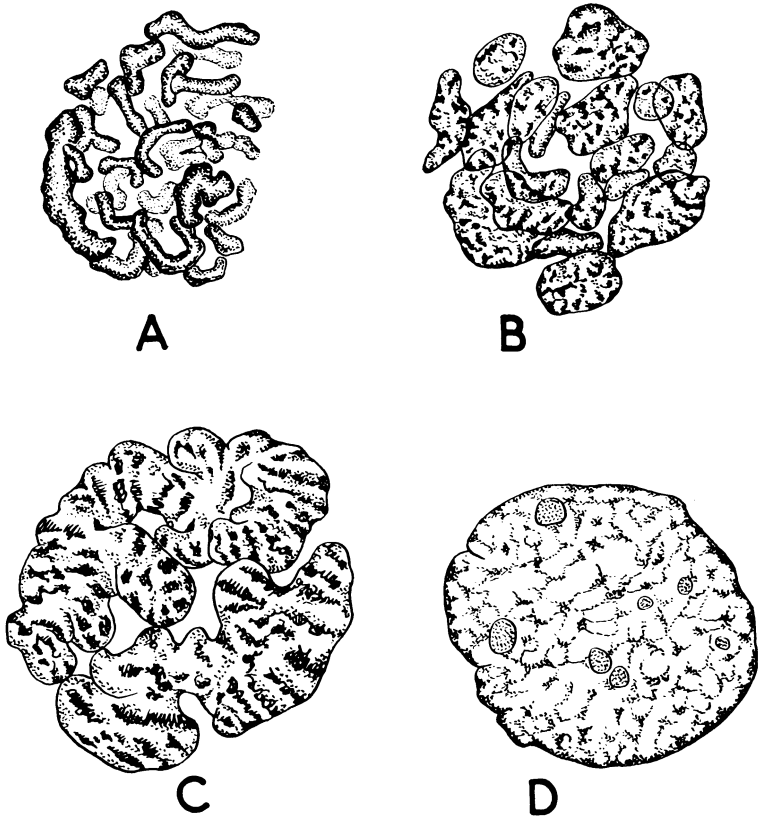


Fig. 2.9—Stages of recovery of arrested prophases in epidermal cells of **Siredon** after colchicine treatment. (Compare with Fig. 2.2A). Acetocarmine smear. **A.** Slight swelling of the chromosomes which have retained their prophasic disposition. **B, C.** Gradual loosening of the chromatic material of similar chromosomes: catachromasis. **D.** Restitution nucleus, formed by the fusion of the swollen chromosomes, which is already noticeable in **C.** (After Ries)

be dropped in favor of (1) *distributed c-mitosis,* and (2) *colchicine-anaphase.* Our preference for distributed c-mitosis instead of somatic meiosis has already been given. Since all factors related to the distributing action cannot be logically considered here, they will be reviewed later.

2.4–4: Chromosomal evolution in plants. Chromosomes persist individually ten times longer when colchicine is present than during ordinary mitosis.[93] Their intactness as measured in *Tradescantia* is maintained for 23 minutes normally, but treated cases extend this intactness period to 249 minutes. Of course, concentration plays an important role; however, optimum doses give this extensive period of intactness. A comparative estimate of metaphasic delay is gathered from inspection of records that show total time chromosomes remain intact.[93]

Estimated time given for neuroblastic cells also indicates a delay, but the extent of retardation is calculated in a different manner. The interval is seven to nine times longer with colchicine. Again the concentrations are all-important for any calculation.[39]

Specific measurements for pollen tube cultures, with colchicine in sucrose-agar, are from five to seven times that of the control. Treated and untreated populations were compared for the total period of chromosomal intactness.[34]

An analogy may be drawn with normal-speed motion pictures that are slowed down five to ten times their regular speed. Chromosomes normally go through metaphase, anaphase, and telophase at a speed of 20 minutes. With colchicine, this process is drawn out to 200 minutes. Such delay affects the sequence of chromosomal evolution. The number of chromosomal changes from prophase through telophase is not different, but the span of time which is longer, 200 rather than 20 minutes, accentuates the changes made in the longer period. Now one begins to realize how impressive a definite sequence of chromosomal forms becomes; this is characteristic enough to be outlined.

This extension in time is the reason for a comparison that is usually made between chromosomal evolution under colchicine in plants and the "terminalization of chiasmata" at meiosis.[56]

During a regular nuclear mitosis the process of chromosomal change is so rapid that one loses sight of the uncoiling and the straightening or evolution of the chromosome. There is a threshold for chromosome contraction that is independent of the c-mitosis. The contraction is related to c-mitosis but is autonomous.[77] Some studies indicated that the longer time allowed a greater contraction since super-contraction was caused by excessive coiling.[7]

The first sequence in chromosomal evolution is seen at the late prophase and early metaphase, while chromosomes are strongly cleft, and two chromatids are coiled about each other in a relational coil (Fig. 2.10). The entire chromosome is straightened so that relational coiling is easily perceived. Through the whole process of uncoiling, the delayed metaphase permits observation at each stage. Since both arms are held at one point, the centromere, the description of uncoiling is made easier. Uncoiling, then, is the first step and begins when the nuclear membrane disappears, unless action takes place earlier in a precocious uncoiling, as was reported in the section above under actions during prophase. The first step in the evolution toward a c-pair is passed when the major relational coiling has been removed (Fig. 2.10).

Next, the further reduction is similar to the terminalization of the chiasmata. The contacts of chromatids occurring originally at several points, finally slip off at the end (Fig. 2.10*B*). The movement begins at the centromere and proceeds to the end of each chromosome. The last contact is at the very end of each chromosome. If both ends are in contact, the characteristic figure-8 obtains (Fig. 2.10*B*). Should one end lose contact, and the other remain attached, a forceps type develops (Fig. 2.10*C*). All the while uncoiling takes place, the chromosomes are shortening. Usually the reduction is to one and one-half times the regular length.[77] In one instance, actual measurements for chromosomes of *Petroselinum* were 4.0 microns for control and 1.5 microns for colchicine-treated chromosomes at c-metaphase.[83]

Finally the last stage is reached, when both ends separate and move out as if there were actual repulsion of the two arms (Fig. 2.10*C*). The cruciform type has been seen a number of times in plant,[56] insect,[87] and mammalian cells cultured *in vitro*.[90] Mammals receiving colchicine via injection have not generally shown cells with the cruciform type. A maximum contraction is attained and the c-pair is held together only at the centromere (Figs. 2.4*D* and 2.10*C*). Thus the two chromatids starting from pro-metaphase as a cleft structure relationally coiled, are reduced until only the ends are in contact. After these are released, there develops the typical X-shaped structures (Fig. 2.10*C*). This sequence has taken a longer time than the control because an intactness period is ten times longer than untreated mitosis.

A stickiness of chromosomes prevents the X-shapes, or cruciforms. Such physical changes are important to the falling apart of the c-pairs.[77]

Straightened chromosomes that are clearly marked at the centromere (Fig. 2.4*D*) improve the cytological and morphological studies

of chromosomes. Not only the comparative sizes of chromosomes within a set can be judged (Fig. 2.4*D*), but the relative differences between the two arms of a chromosome can be estimated.[34] For these reasons the pretreatment of chromosomes by colchicine was suggested[76] and there followed an important advancement in cytological technique which now makes it possible to study chromosomes, particularly among root tips, with much greater accuracy.[17, 9, 74, 69] Scattered chromosomes in the pollen tube led to the discovery of the natural polyploid *Polygonatum commutatum*.[34] If the chromosome pairs are studied, duplication of a haploid set is obvious (Fig. 2.4*D*). Since the generative nucleus is haploid, there should theoretically be only one of each chromosomal type. But each type was repeated, typical of tetraploids (Fig. 2.4*D*). Then any related diploid should have only one of each type. This was found by extending the study to other representatives of the genus. The colchicine technique was useful for this cyto-taxonomic study.[34].

2.4–5: Duration of colchicine-mitosis in animal cells. Degenerative changes are frequent in arrested metaphases of animal cells, especially in mammals. Their mechanism, which may be of some importance when colchicine is utilized in the treatment of abnormal growth (cf. Chapter 10) is not clearly understood. As explained in further chapters, colchicine has been extensively used as a tool for the study of growth. It is impossible to reach precise conclusions if the duration of a given c-mitosis is not known. Direct observations can be made only in limited cases excluding all sectioning materials. From the study of sections, it appeared from the early work that within 24 hours or less, an arrested metaphase either recovered, or underwent destruction.[29, 61]

In cold-blooded animals, colchicine is probably metabolized much more slowly (cf. Chapter 7). In *Siredon*, after a single injection, a great number of arrested mitoses could be seen in the spleen (Fig. 2.2). This was apparent five days after the injection, and lasted for about ten days.[24] In *Triturus,* seven days after colchicine had been applied to the cornea, abnormal mitoses with scattered contracted and unoriented chromosomes have been reported (Fig. 2.7).[79]

However, a precise study of the duration of colchicine-mitoses in the larva of *Xenopus* led to the conclusion that destruction took place much sooner. This was calculated by an indirect method.[63] From data of short treatments with colchicine and from direct observation, it was found that epidermal mitoses lasted about 100 minutes. It was further assumed that the normal prophase duration of about 25 minutes was not modified by colchicine. In colchicinized animals the relations between the numbers of prophases and colchicine-metaphases and the average duration of each should be equal.

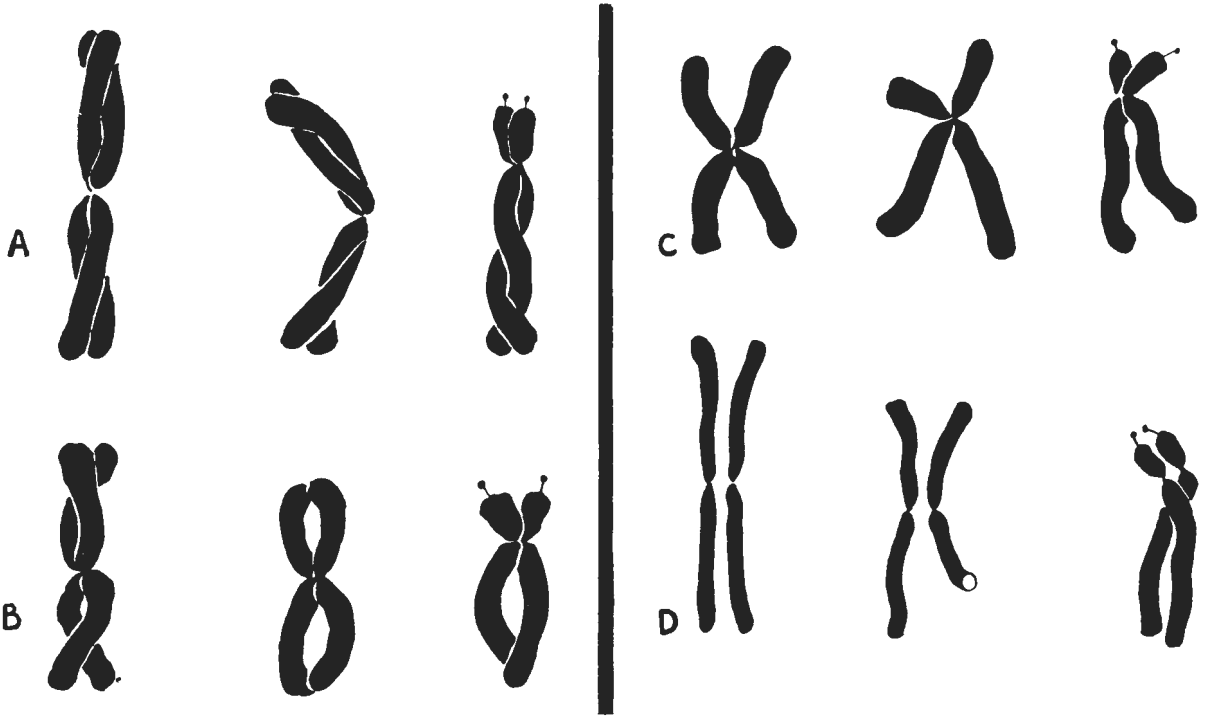


Fig. 2.10—Evolution of c-pairs in **Allium.** **A.** Relational coiling of the cleft chromosomes. **B.** Uncoiling has reduced the number of turns for each segment giving a figure-8 and a forceps type. **C.** Cruciform c-pairs held only at centromere. Arms expanded or spread apart. **D.** A colchicine anaphase marked by the chromosomes lying like "a pair of skis." (After Levan)

It was found that the arrested mitoses lasted from 5 hrs. 26 min. to 14 hrs. 20 min., and later were destroyed.

The spleen of Siredon was crammed with arrested mitoses five days after colchicine treatment. If the figures given above are accepted, the correlation of the two sets of data — (1) duration of c-mitoses and (2) the appearance of large numbers five days after treatment — naturally raises some questions that appear important. In *Xenopus,* while cellular degeneration may be rapid, the percentage of metaphases remains very high as long as three days after colchicine. In *Siredon,* it is possible that in the spleen only the intact cells remain visible, the others being washed away by the blood stream, so the results are not as contradictory as they seem at a first glance.

It is thus most probable, from what is known about the pharmacology of colchicine (cf. Chapter 7), that in warm-blooded animals, *and particularly in mammals, arrested metaphases are destroyed in less than ten hours. This is in agreement with the histological evidence* of nuclear degeneration,[29, 61] and must be kept in mind when colchicine is used as a tool for the study of growth.

2.5: Processes Leading to Interphase

Chromosomal formation is not stopped by colchicine. Under certain conditions the process is slowed down or the delay is so pronounced that there is an appearance of its formation being stopped. For example, many prophase-metaphase types are essentially arrested prophases. Also we pointed out how colchicine might stop chromosomal formation during prophase and turn the process back to interphase.[93, 39]

There are three ways in which chromosomes change to interphasic dispersal under the influence of colchicine — exclusive of recovery, which we will discuss in a subsequent section. They are: (1) the just-mentioned prophase reversal to interphase;[39, 93] (2) the changes from any of the arrested metaphases,[1, 22, 34] i.e., prophase-metaphase, ball metaphase, exploded metaphase, star and distorted star metaphases; and (3) a full c-mitosis through c-anaphase and c-telophase transformations.[56, 65]

Basically, the physical change that takes place in the chromosome does not differ much in either of the three routes taken. Therefore a general description of this process shall include the changes[22] common to plants and animals. Moreover, the process is not very different from a regular telophasic transformation found in a normal nuclear mitosis.[93] In all probability the unraveling, loss of chromaticity, and general physical changes are very similar.[7] Colchicine does not prevent the return of chromosomes to interphase and similarly

it does not prevent chromosomal formation.[7] But colchicine does one thing important at this stage; it desynchronizes the separation of the chromosomes.[34, 56, 76, 46, 23] Or we may say the coordinated processes of anaphasic separation of all chromosomes at one particular moment are very badly upset.

Colchicine does not inhibit the uncoiling or the stage of *katachromasis*,[93] the return to interphase. The drug in certain concentration does slow down the uncoiling process in *Tradescantia* since it takes 60 minutes for uncoiling with 0.05 per cent colchicine and 77 minutes in 0.1 per cent contrasted with 35 minutes among untreated cells. There is one other relation of interest: The ratio of time for chromosomal formation, *anachromasis*, to chromosome uncoiling, *katachromasis*, is about 2:1 in regular mitosis. Colchicine-treated mitoses maintain this 2:1 ratio, i.e., 121:60 in colchicine and 97:35 for untreated cells. The significance of these corresponding figures is not understood.

The loss of chromatin, despiralization, and vesiculating stages[34] in the presence of colchicine are much the same as in normal plant cells. A solid chromosome becomes perforated, and two twisted coils appear. The chromosome is reduced to a zigzag thread. There is a fusion of chromosomes that lie close by and the final stages appear as a reticulated network with nucleoli[3] and a membrane surrounding the chromatin. Whether the change begins (1) from prophase, or (2) from arrested metaphase, or (3) through c-anaphase, the general despiralization, sometimes called unraveling, dechromatization, or katachromasis, is similar (cf. Chapter 3).[34, 56, 93, 65, 7]

A full c-mitosis implies that the c-pairs of chromosomes "fall apart" like "pairs of skis"[73, 72] in the cytoplasm (cf. Chapter 3; Fig. 2.10). *Allium* root tips (Fig. 2.10*D*), particularly, demonstrate this stage except when stickiness holds them together. Thus the c-anaphase can be observed without question.[56, 65, 1, 79] Such separation is evidence that the restitution nucleus shall carry the tetraploid number of centromeres.

Desynchronization is most easily observed if the chromosomes can be compared at a given moment. For example, Figure 3.7 shows a c-anaphase pair at the bottom, whereas above, c-pairs are clearly in X's and held together.[7] This has been shown over and over, from plants and animals, at arrested metaphase.[56, 88, 65] Within one set, single chromosomes, and others in c-pairs, have been noticed to revert[22] to interphase.

C-anaphase is more distinct in some plants, but the distinction is by no means valid for differentiating animals from plants.[87, 85, 3, 2, 1, 79, 56] Tetraploid restitution nuclei have been observed for many kinds of animal cells treated with colchicine.

Tetraploid numbers would also develop in animals if colchicine hit a cell in regular anaphase, because the two groups of chromosomes intermingle, fuse, and form a restitution nucleus.[39] This was demonstrated in grasshopper neuroblastic cells. This is basic to the development of triploid animals by treating egg cells at second maturation anaphase.[68]

Pycnotic changes are very common when chromosomes revert to the interphase. This is especially so in mammals where destruction is the fate of most arrested metaphases.[29, 33, 61] Toxic or strong concentration induces pycnosis. What structural changes occur are difficult to determine. Such changes are discussed under the section of chromosomal alteration.[29, 33]

2.6: Alterations of Chromosome Structure

The most frequent change of the chromosomes in arrested animal mitoses is an abnormal thickness and shortness.[79] This is especially evident in arrested and exploded metaphases of mammalian cells. The shortening may be the consequence of an excessive coiling. Very often these chromosomes degenerate, losing all visible structure; only irregular clumps of basophilic material remain scattered in the cytoplasm, and these in turn fall to pieces.[33] Agglutination and fusion are also quite frequent (Fig. 2.8*B*, 2.8*C*).[29, 61, 12, 13, 24, 15] These have been observed in cells where the colchicine action was incomplete and where the spindle was apparent,[15] a fact suggesting that the alkaloid modifies the chromosomes themselves.

In mammals, the colchicine-mitoses with short and clumped chromosomes are more frequent when the dose of alkaloid is high.[61] Animals injected with colchicine show mitotic abnormalities that vary from cell to cell. As an example, the tubules of the kidney contain cells with exploded metaphases and shortened chromosomes, while the cells of the renal pelvis show ball metaphases.[32] Short chromosomes are seen in cells of regenerating liver[12] when treated with colchicine according to specific schedules of time and concentration. Similar shortening also appears following bile duct ligature.[28] and in carbon tetrachloride poisoning.[18] Such changes were also observed in cells of human tissues poisoned with colchicine.[33] The *junior author had the unique experience of following the successive changes in cells of the human body in a clinical case. This occurred when an individual suffering from an overdose of colchicine was brought to the hospital in which the junior author was a staff member.* These effects are described in detail in Chapter 7.

There is no clear evidence that their structure is damaged. In mammalian cells, pycnotic, ball, or star metaphases may often proceed to normal telophase, although many degenerate, the whole cell being then rapidly destroyed.[61] There is no clear indication that the

chromosomes are the first to be involved in the cellular death. Their eventual disintegration is probably a consequence of cytoplasmic or metabolic changes. A better understanding of these would be of great physiological interest, for it appears that among the warm-blooded species of vertebrates the chromosomes are unable to remain for more than a few hours in a cell with arrested mitosis. Quantitative data on this problem have been given in a preceding paragraph; it would be necessary to know what the biochemical changes are which lead to the destruction of the nuclear structures, and in what way this is related to the prolongation of metaphase.

Breakages such as transverse division of chromosomes in plants have been reported.[51] A number of other observations have been made along this line, but no tests have been performed to demonstrate that colchicine increases their frequency. Broken chromosomes and fragments are observed in untreated cells.

2.6–1: *The destruction of chromosomes in Tubifex.* Colchicine is regarded as a destructive mitotic poison, leading to degenerative changes of the nucleus in *Tubifex*,[53, 54, 55] as opposed to the inhibitive mitotic poisons which prevent cell division mainly by disturbing the spindle mechanism. *Tubifex* is very favorable for the study of early development and cytoplasmic division, but the "numerous and very small chromosomes are unfavorable for cytological analysis,"[95] so this may explain the great discrepancies between these findings and those of workers using different cells.

When the egg of *Tubifex* is treated by colchicine during its first cleavage, the spindle gradually fades away as it does in other objects. Then the chromosomes become progressively pycnotic and lose all visible structure. In the second cleavage, or after longer colchicine treatments, a total disappearance of the chromosomes was observed.[53, 54, 55, 95] The cells became empty; no more nuclear material could be stained by any method. More than seventy per cent of the eggs, twelve hours after colchicine, had such empty cells. But a few hours later, new nuclear structure appeared. First were seen protoplasmic condensations which did not stain with the Feulgen reaction. Then scattered Feulgen-positive masses appeared in the cytoplasm (Fig. 2.11). They seemed structureless but bore some resemblance to the small nuclei which are found in the control eggs. It is suggested that some synthesis of thymonucleic acid takes place in the cytoplasm.

The accompanying Figure 2.11 shows pseudonuclei in *Tubifex*. Among *Amphibia* after colchicine, podophylline, and benzanthracenequinone, evidence has been presented of a "multiplication of nuclear material without mitosis."[54]

One may, nevertheless, conclude that in animal cells other than *Tubifex*, chromosomes disintegrate only when extensive degenerative changes alter the whole cell. Contrary to plant cells, which may

undergo subsequently several colchicine-mitoses, animal cells either remain arrested at prophase-metaphase or metaphase, or recover from the action of the drug and, exceptionally, become polyploid. This is true whether in protozoa, invertebrates, amphibians, or mammals; tissue cultures show that colchicine is no more a chromatin poison in animals than in plants. Nor does it appear to affect other nuclear

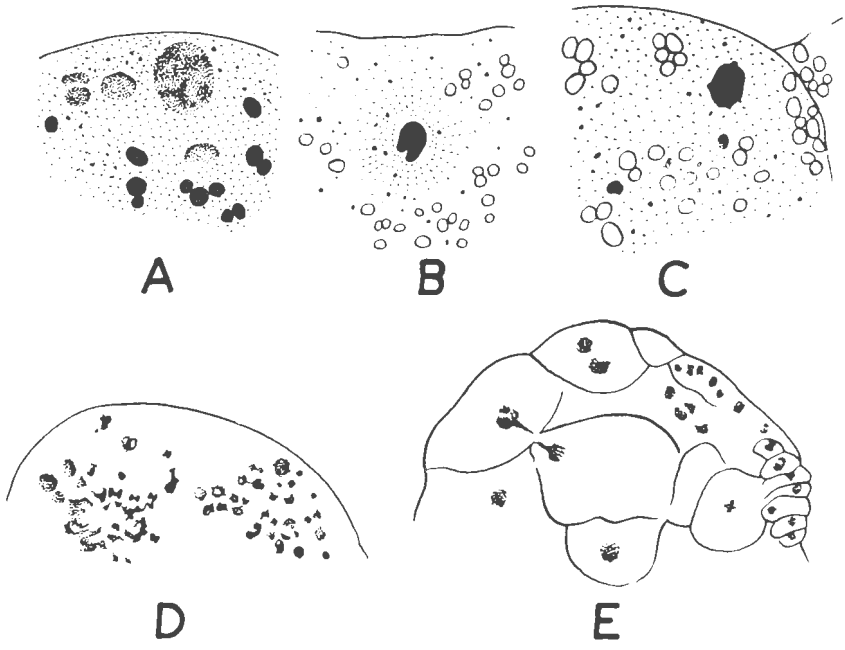


Fig. 2.11—Action of colchicine on the nuclei of developing eggs of **Tubifex**. **A.** After 44 hours, no nucleus is visible. Several cytoplasmic condensations (stippled) are noticeable. Yolk platelets are black. **B, C.** Formation of "pseudonuclei" (black). These are Feulgen-positive, apparently unstructured masses. **D.** Numerous pseudonuclei in an egg treated for 70 hours with colchicine. **E.** Control egg at the same stage as **D**. Note that colchicine has suppressed the cleavage clearly visible in **E**. (After Woker)

structures; there is no mention of any nucleolar changes apart from their possible multiplication in relation to polyploidy. Changes in the nuclear sap will be discussed later.

2.6–2: *Colchicine and X-ray combined.* Neoplastic tissues have been subjected to X-ray and colchicine,[59] but small attention was *given to the relation between c-mitosis and the pretreatments that influence the effect of X-ray in normal cells (cf. Chapter 10).*

Allium root tips pretreated with 0.05 per cent colchicine and then subjected to irradiation showed one-third as many chromatid aberrations among colchicinized root tip cells as the controls.[14]

The mutation process[23] was measured by pretreating barley seed twenty-four hours before irradiation. A series of solutions (0.1, 0.05, 0.01, 0.005, 0.001 per cent) of colchicine were used just prior to treatment with 5000, 10,000, 15,000 r units, respectively.[43] A treatment with colchicine prior to irradiation causes a decrease in the viridis mutants, but an increase in the rare and very rare mutations. There was no significant change in the albinos.[23]

It was concluded that the mutation process is considerably altered by the application of colchicine to the seedlings previous to irradiations according to the schedules given above.[43]

2.7: Reiteration of the C-mitosis

Cells of *Allium* with sixteen chromosomes as the diploid number accumulate chromosomes in hundreds, even more than a thousand per cell. These large numbers are striking. Obviously more than one doubling has taken place. If we plot the progression, it becomes clear how such high numbers accumulate. If the number of basic sets in a somatic cell is 2, then the chromosome number is 2 $\times$ the haploid number per set, i.e., 2 $\times$ 8 = 16 for *Allium*. When one c-mitosis has been completed, the doubling produces 32, or four sets of 8 each. The second c-mitosis doubling 32, creates a cell with 64 chromosomes, or 8 sets of 8 chromosomes per set. We may let n equal the number of c-mitoses completed. Then $2^{(n+1)}$ represents the number of basic sets. Multiply these factors by the number of chromosomes per set. If cell A has completed 6 c-mitoses, then $n = 6$ and the number of sets of chromosomes becomes $2^{(6+1)}$ or 2^7, or 128 $\times$ 8 = 1024 chromosomes after 6 c-mitoses. Therefore, the c-mitotic cycles occur in a definite order.[56]

The number of chromosomes that may be packed into one cell is an interesting question. When the total exceeds 500 per cell, recovery of the bipolar mitosis does not occur.[56] Divisions of 64 may recover regularly, but numbers over 100 often show twisted spindles among recovering cells. The high numbers are found most generally in the embryonic vascular cells, notably the area where lateral root initials develop.[57, 65]

Short exposures of seven minutes to one hour permit one c-mitosis while more cycles follow in the longer exposure, i.e., 24- and 72-hour treatments.[56] A tetraploid cell begins the second c-mitosis after 30 hours and an octaploid c-mitosis at 72 hours.[46]

There is a correlation between the number of c-mitoses per cell and the region of the root.[56, 65, 40, 57] If an *Allium* root is divided into five or six regions and chromosome numbers tabulated, the greater percentage of cells with increased numbers occurs in the older parts of the root while cells very near the tip retain diploid numbers.

A distribution study for seven root tips showed that the regions away from the tip contained largest number of polyploid cells.

Reiteration of the c-mitosis in animals is limited by other factors, such as toxicity to cells exposed over a long time. Also the balance may be upset by increase in chromosomes per cell, so that only cells with tetraploidy or octoploidy may survive. High numbers per cell in animals have not been found as a consequence of c-mitosis.

2.7–1: *Recovery in plants.* One remarkable feature about colchicine is the ability of cells once stepped up to higher chromosome numbers, to recover and thereafter produce new cells with the increased number.[56, 65, 40] In other words, tetraploid cells induced by colchicine, if removed to water, will resume nuclear mitosis with the new increased numbers.

A second notable point in the recovery process is the change taking place when cells with high chromosome numbers begin the renewal of the regular mitosis. If one hundred or more chromosomes have aggregated in one cell and colchicine is removed, soon the chromosomes gather into small groups giving the effect of many star metaphases. Each of these groups may be the focal point around which a new cell is formed (Fig. 2.6). By a process of multipolar divisions the large numbers in a cell become reduced to smaller numbers.[66]

The length of treatment at a given concentration determines the speed of recovery based upon the types of metaphase chromosome formations observed. A one-hour treatment of *Spinacia* in 0.25 per cent shows complete recovery in 48 hours. A five-hour treatment at 0.25 per cent requires 63 hours for recovery.[7]

2.7–2: *Recovery in animals.* Interphase from star metaphase without an anaphasic movement took place in corneal epithelial cells as these tissues recovered from a strong dosage under a short exposure period.[79] Multiple stars appeared after five and six days from the time of the last application of colchicine.

Siredon cells show another phenomenon reported many times in other material, the swelling of chromosomes and cytoplasm. The immobile chromosomes seem to swell while in a scattered arrangement.[84] This is similar to reversal of prophase; later the chromosomes fuse into an interphasic nucleus (Fig. 2.9). Similar reconstructions during recovery are to be found in regenerating liver cells of the rat (Fig. 2.12).[13] A progressive fusion of micronuclei reduces the number until trinucleate and binucleate cells develop. Tissue cultures show comparatively the same micronuclear development.[15, 90]

Partial c-mitoses and multiple stars are common during recovery as observed in neuroblasts.[79] The multiple stars are evidence that recovery processes are underway.

2.7–3: Consequences of c-mitoses: polyploidy in plants. The artificial induction of polyploidy by colchicine was not a new discovery in plant science. Doubling of chromosomes was demonstrated in plant cells as early as 1904.[82] During a long and successful teaching career, Professor C. F. Hottes, University of Illinois, repeatedly outlined cytophysiological methods for inducing polyploidy in root tip

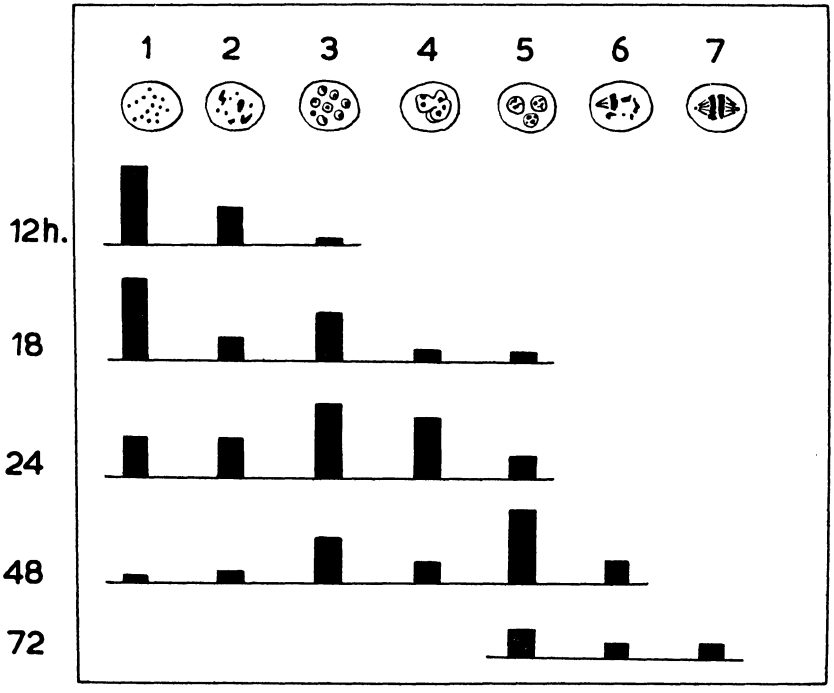


Fig. 2.12—Regenerating liver of the rat, after a single injection of colchicine. Schematic drawings of the various types of restitution nuclei: (1) exploded metaphase with scattered chromosomes, (2) fusion of some of these chromosomes, (3) micronuclei, (4) fusion of the micronuclei (compare with Fig. 2.4), (5) three nuclei, (6) abnormal mitosis with partially inactive spindle, (7) normal mitosis. The percentages of these types of cellular changes at various intervals after colchicine are expressed by the black rectangles. Normal mitoses are only found 72 hours after the injection, and restitution appears to proceed by the fusion of the micronuclei. (After Brues and Jackson)

cells. Specific polyploid plants were induced by regeneration techniques with mosses in 1908 by the Marchals. Later, polyploids were created among the flowering plants by Winkler in 1916 and similar work was continued by Wettstein, Jorgensen, Lindstrom and Koos, and Greenleaf from 1924 to 1934. An early suggestion for inducing polyploidy by temperature change was made by John Belling in 1925.[6] The temperature shock technique was later standardized suc-

cessfully for maize in 1932,[82] after which time other laboratories followed Randolph's general method. This is a brief history of polyploidy through artificial means before the colchicine era began. That important period made work with colchicine more fruitful than it otherwise would have been. Sudden attention to colchicine almost blotted out the facts that polyploidy induced by several techniques had been well developed before 1937.

The vast literature[35] dealing with polyploidy in plants is discussed in subsequent chapters.

2.7–4: Polyploidy in animals. Polyploidy in animals has also received attention for a long time but success with artificial induction has been limited. The introduction of colchicine did not achieve the success found among many projects with plants.

Temperature shock-cold treatments with newly fertilized eggs of *Triturus viridescens*[38] were more successful than the application of colchicine to these animals. The procedures with colchicine were not efficient, at least when compared with treatment of plants; much was to be desired for work with animals.

Newly fertilized eggs of rabbits were treated with weak solutions of colchicine.[81] Other animals, frogs,[44, 50] *Triturus*,[79] *Triton*,[1] *Xenopus*,[63] *Artemia*,[3] silkworm,[48] *Habrobracon*,[49] *Drosophila*,[42, 10] chickens,[47] *Amoeba*,[20] were tested with colchicine for polyploidy. Generally colchicine has failed in comparison with the induction of polyploidy in plants.[38]

One remarkable series of experiments demonstrated in *Amoeba sphaeronucleus* how polyploid unicellulars could be created by colchicine.[20] This had no effect unless injected into the cytoplasm at metaphase, with a micropipette. Actual counting of chromosomes was not possible but there resulted larger cells with a larger nucleus. These, however, at each division built one normal and one abnormal nucleus, a fact suggesting triploidy. Supposedly polyploid nuclei were transplanted into enucleated fragments of normal amoebae and vice versa. It was observed that the size of the unicellular was directly related to the size of nucleus. The opposite was also true, and a normal nucleus grafted in a "polyploid" cytoplasm was observed to swell considerably. Cytoplasm and nucleus underwent several divisions and then recovered their normal volume of the original species. If the normal nucleus was grafted into a fragment of a polyploid cell, growth was resumed normally. These experiments have been illustrated by a remarkable series of cinemicrographic documents. They have provided new insight on nuclear-cytoplasmic relationship and the possibility of observing colchicine effects in cells, the membranes of which are impermeable to the drug.

A different attack was tried by taking advantage of the fact that colchicine coming in contact with egg cells in the second maturation division would arrest the anaphase stage thereby creating a diploid egg cell. If this cell united with a haploid sperm, it could give rise to a triploid individual.[19] The reasoning was logical enough and colchicine could be introduced at the proper moment through the admittance of sperm and colchicine by artificial insemination methods. Whether sufficient dosage of drug was given shrouds these tests with doubt.

Experiments with frogs in 1947[44] encouraged the trial of introducing colchicine at the time of fertilization, since larvae from eggs treated at fertilization seemed to be polyploid judging from the size of cells and nucleus. The idea was extended to other animals, notably rabbits and pigs.[45, 68] Certain principles were substantiated by these tests, viz., that the application of colchicine at the precise moment of fertilization would bring triploidy in the zygote, because a doubled egg cell would unite with a haploid sperm.

Techniques were developed to inseminate artificially rabbits and pigs,[45] by adding colchicine to sperm material. Proper concentrations were determined by laboratory tests. Suspected triploid offspring were studied cytologically and a conclusion was reached that egg cells were doubled by this procedure. One rabbit that deviated from diploids showed 66 chromosomes among certain mitotic cells of testicles.[68] There were other diploid cells in this test with 44 chromosomes. Thus the individual may have started as a triploid zygote with reduction as development proceeded. These results were, however, by no means conclusive. Previous accounts as well as these above have been criticized and not without some basis.

Similar experiments were done with pigs.[44, 45] Among 31 offspring from artificial inseminations, one differed from the rest as well as from diploid pigs. This male animal showed consistent mitotic figures with 47 chromosomes,[68] a good triploid, that originated when a diploid egg of 32 chromosomes and a haploid sperm carrying 15 chromosomes united. These techniques are new and merit further attention for theoretical studies of polyploidy among animals.[45]

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