

THE EFFECTS OF COLCHICINE UPON THE MECHANISM OF MITOSIS

By

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AN ABSTRACT

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The Pisum test was used for the purpose of studying the effects of colchicine upon the mitotic mechanism and an attempt was made to gain some clues as to the nature of the forces involved in mitosis.

Root tip meristems of Pisum sativum seedlings were exposed to a solution of 1000 ppm colchicine for fifteen minutes. They were then washed and returned to recover in a nutrient solution until normal mitosis had been resumed. Cuttings of the root tips were made before treatment, at the end of treatment, and at fifteen and thirty minutes, one hour, and at hourly intervals thereafter, during the recovery period. Twenty-four and forty-eight hour recovery cuttings were also made. Feulgen stained squash preparations of the root tips were used in making the cytological observations.

The cuttings made before treatment (zero hour controls) furnished the data for normal mitosis. The chromosome movement cycle was shown to consist of four distinctive stages; namely: the movements from early to midprophase, midprophase to prometaphase, prometaphase to metaphase, and the anaphase movements. Mitotic indices, representing the frequency of cells in division, and the frequencies of the several stages of mitosis were calculated. The kinds and relative proportions of abnormalities were recorded. These data served as a basis of comparison in determining the effect colchicine had upon mitosis.

It was found that colchicine increased the duration of the mitotic cycle, especially at metaphase, during the first four hours after treatment. At from five to six hours after treatment an

increase in the absolute number of prophases was determined. The latter observation was interpreted to mean that colchicine has a stimulating effect upon the frequency of division.

The immediate effect of colchicine was a stalling of chromosome movement at the prometaphase clump. Metaphase, anaphase and telophase occurred in situ and tetraploid interphases were formed. Early anaphases were arrested and formed into polyploid nuclei. The arrest in movement of later anaphases and the failure of cytokinesis resulted in the production of binucleate cells, many of which were bridged. The majority of mitoses for four hours after the end of treatment were blocked in movement at the prometaphase clump. From five to eight hours there was a rapid decrease in the number of clumped mitoses. Scattered mitosis became common and resulted largely in the formation of multinucleate cells. At the same time there was a gradual return to normal mitosis and by eight hours of recovery few abnormal divisions were seen. At twenty-four and forty-eight hours mitosis was normal. Polyploid cells were seen in interphase and in division. Numerous binucleate and multinucleate cells were in evidence. Essentially the same effects were observed in Allium as in Pisum although no quantitative determinations with the former were made.

It was concluded that the blocking of chromosome movement at prometaphase was the major cause of polyploidy and that it resulted, largely, from the destruction by colchicine of the achromatic component of the spindle. It was determined that all cells which were in division at the time of colchicine treatment had been eliminated as telophases by the end of four hours. Therefore, scattered mitosis

resulted from an effect by colchicine upon interphase nuclei at the stage known as antephase. The stimulation of division frequency occurred at the same time.

The preprophase effect of colchicine paralyzes the kinetochores and centers so that they have no control over the movement of prophase chromosomes. The forces inherent in the chromosomes themselves account for the scattered distribution of the metaphase chromosomes. Although colchicine inactivates the kinetochores, as far as chromosome movement is concerned, it does not destroy the mutual attraction that exists between them. After an initial repulsion between sister chromatids following kinetochore cleavage the kinetochores of two or more chromatids exhibit a mutual attraction for each other and result in the formation of multinucleate cells.

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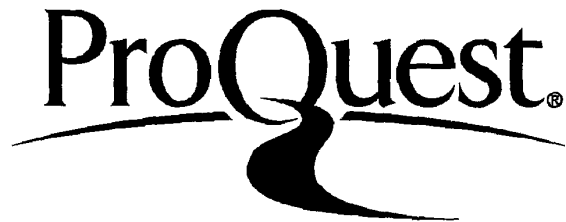
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INTRODUCTION

During the long and seemingly endless search for new facts and clues about the process of mitosis the cytologist has made use of many tools. Among these tools are antimitotic agents of both chemical and physical nature. Their damaging effects upon mitosis often result in the breakdown of the process and lead to observable variations in mitotic behavior. The value of antimitotic agents to the cytologist lies in the assumption that, by observing the collapse of normal mitosis and noting the variations and deviations that arise, new information might be obtained about the mitotic mechanism either in terms of the forces involved or in respect to the mitotic functions of the chromosomes themselves.

Of all the chemical substances used in the realm of experimental cytology, colchicine has probably been more widely employed than any other single substance. Likewise the results obtained from its use have contributed more to the present knowledge about mitosis than similar experiments with any other single chemical.

To the casual observer it would appear that the effect of colchicine is well known. Its peculiar effect upon the spindle and its specific action in upsetting the interrelated forces involved in the mechanism of mitosis have served to make the "colchicine effect" a kind of cytological standard against which the effects of many other substances have been judged. Experiments have been conducted in an effort to determine its mode of action upon the cellular components.

In the process a colchicine literature of impressive size and scope has been developed. Indeed, it might seem as if colchicine were one drug whose cytological effects were well-known and convincingly documented.

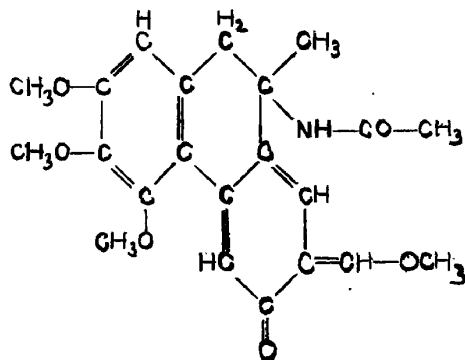
There has been, however, a growing tendency in recent years for careful workers to demand a more precise definition of the drug's effect. The need has become especially urgent as more and more substances have been compared and contrasted in one way or another with the effects of colchicine.

The present studies were undertaken for the purpose of re-investigating some aspects of colchicine and its cytological significance. The objectives of the studies were three in number. First, a careful review of the pertinent literature to determine how the concept of "colchicine mitosis" has developed. Second, actual experiments with colchicine were to be conducted in order to determine, if possible, how it affected the mechanism of mitosis from the beginning of treatment until the tissues had recovered from its effect. The final purpose was to see what clues to the mechanism of mitosis itself might be made apparent by the destructive effects of the drug.

AN HISTORICAL SKETCH OF COLCHICINE

During the review of the literature the writer came upon many interesting bits of information concerning colchicine that are not directly related to its cytological past. For the sake of general interest and to provide a little background it seems worthwhile to record a few of the anecdotes concerning the drug before it became a tool of the geneticist and cytologist.

Colchicine is an alkaloid found in some members of the Liliaceae, especially in species of Colchicum. The genus is well named, for the ancient kingdom of Colchis was the home of the sorceress, Medea, who is famed in mythology for the evil potions she was alleged to have brewed. Colchicine U.S.P. is obtained from the corms and seeds of Colchicum autumnale L. Its structural formula has been determined to be (Muldoon 1950):



Colchicum autumnale, meadow saffron or autumn crocus, is native to the Old World. It occurs in southern, central and western Europe, east to the Balkans, southern Russia and Kashmir; it is also found in North Africa. The plant is widely grown as an ornamental for it flowers late in the summer and early fall. Meadow saffron is considered

a dangerous poisonous plant; all its parts contain colchicine. Generally cattle and horses will not eat the plant in either pasture or hay. Young stock are very susceptible to its effect and die soon after eating it. Sheep and goats are said to be able to tolerate considerable quantities of meadow saffron without ill effects. Milk from cows feeding on the plant may contain sufficient quantities of the alkaloid to be fatal to calves and infants (Hutchinson 1948). An epidemic of colchicine poisoning is reported to have occurred in Rome and is said to have been caused by colchicine-contaminated goat milk (Hegi 1909). A woman is reported to have died after mistakenly eating meadow saffron corms (Hutchinson l. c.).

Colchicine seed was first used as an official drug in 1820. Its use in folk-medicine goes much farther back than that (Hegi l. c.). For example decoctions made from the flowers and corms of Colchicum autumnale were used to eradicate lice from children and cattle. A corm, carried in the pocket, was assumed to ward off dysentery and toothache and to serve as a protection against the plague. Meadow saffron has been eaten as a remedy for jaundice and a tincture of saffron and saffron wine have been used for asthma, gout, rheumatism and dropsy.

In modern times colchicine is still the standard remedy for gout and is sometimes used in treating rheumatism. In the case of gout, according to Olsol and Farrar (1947), it is administered, " . . . until pain is relieved or gastrointestinal symptoms appear. . . ." Fortunately for the patient, relief is usually obtained before diarrhea, ". . . necessitates discontinuance . . ." of the medication.

Colchicine must be used with great caution because of its highly poisonous nature. Overdoses of the drug result in death.

LITERATURE REVIEW

The first observations on the cytological effects of colchicine were made in 1889 by Pernice (Eigsti, Dustin, Jr. and Gay-Winn 1949). He is reported to have been impressed by the abundance of cells in division and by the great number of abnormalities they displayed. Apparently Pernice was also the first observer to note a blocking action on mitosis by colchicine, for he claimed that the late stages of mitosis were rarely seen. Dixon (1905) also reported on the disturbing effect of the drug upon the mitosis of leucocytes. Later Dixon and Malden (1908) stated that colchicine caused the appearance of erythroblasts and leucocytes with diffuse and punctate basophilia. The initial action of the colchicine was to markedly reduce the number of leucocytes in the blood. After a period of an hour there was an abnormal increase of leucocytes and they were of the poly-morphonuclear type. Dustin (1934) and Lits (1934), both working with mouse tissue treated with colchicine, thought that the increased numbers of dividing cells they saw indicated a stimulation of mitosis by the alkaloid. Ludford (1936), however, came to the conclusion that the apparent increase was caused by an "accumulation of arrested mitoses" and that this accumulation was the result of the, ". . . failure of the mitotic spindle to form and function in the normal manner. . . ." Brues and Jackson (1937), working with hepatic cells of rats, confirmed the opinion of Ludford by showing that colchicine stopped mitosis at the beginning of metaphase. They recorded the abnormalities that appeared at successive intervals after treatment and attempted to show the

stages that a "single arrested mitosis" might pass through before it recovered normal spindle function.

Colchicine research became very popular after the announcements by Blakeslee (1937), Blakeslee and Avery (1937) and Dustin, Havas and Lits (1937) that the drug was capable of inducing polyploidy in plant cells. The bibliographies prepared by Eigsti (1947) and Eigsti and Dustin, Jr. (1949) well illustrate the widespread interest colchicine has received from many quarters since 1937. It was, of course, quite natural that cytologists should become concerned with the cytological action of the drug and attempt to determine how it caused polyploidy. Nebel (1937) and Nebel and Ruttle (1938), in studies made on living stamen hair cells of Tradescantia reflexa, reported that colchicine treatment inhibited the formation of the spindle while all the other nuclear developments were allowed to continue. Thus, with the absence of anaphase separation of the chromatids a single telophase nucleus was formed and it was tetraploid. Dermen (1938) arrived at similar conclusions after studying the colchicine effects upon the stamen hairs of Rhoeo discolor. Numerous other workers (e. g., Beams and King 1938, Eigsti 1938, Walker 1938), also working with plant cells, soon presented results which confirmed the first observations and conclusions. In addition to the production of polyploid cells, Beams and King and Eigsti showed that micronucleate and binucleate cells were also formed. Beams and King concluded that colchicine lowered the viscosity of the cytoplasm and prevented the normal viscosity changes that accompany mitosis from occurring. Therefore, the polar centers and the spindle, which are cytoplasmic in origin, were not

formed and the nuclear processes continued while the cytoplasmic processes were halted.

Levan (1938) established the precedent for most subsequent colchicine work by introducing the so-called "Allium test." Onions were rooted in water and the vigorously growing root tips were exposed to various concentrations of colchicine solution for specified periods of time. Cuttings of root tips were made at regular intervals during treatment and after the bulbs were returned to water. Levan considered the effect of colchicine to be a specific one and he called its modification of the mitotic cycle, "c-mitosis". C-mitosis was defined as, ". . . an inactivation of the spindle apparatus connected with a delay of the centromere. The effect thus produced may be expressed as a completion of the chromosome mitosis without nuclear or cellular mitosis. . . ." Levan observed no effects upon prophase chromosomes; they reached a metaphase appearance without the normal arrangement at an equatorial plate. He described these metaphase chromosomes as being, ". . . scattered over the cell in a diakinesis-like manner. . . ." Because the division of kinetochores was so long delayed there was an accumulation of highly contracted, cross-shaped metaphases which Levan called "c-pairs". After some hours the kinetochores would finally divide, though not always synchronously. No separation of chromatids occurred and with their passing into telophase a single nucleus with twice the somatic number of chromosomes was formed. Levan observed, during recovery experiments, that the inactivation of the spindle by colchicine was reversible. When root tips were returned to water the spindles began to reform. At first irregular-shaped nuclei were formed.

Then multipolar spindle activity was seen and many small nuclei were produced within one cell. Often these micronucleate cells formed walls which separated the nuclei from one another and microcytes were formed. Gradually, increasingly more normal conditions were noted until all cell divisions were bipolar and apparently normal. In later papers Levan (1939, 1940) reiterated his earlier conclusions and emphasized the selective nature of the colchicine effect. While the drug inactivated only the spindle apparatus there was a differential response by the exterior (centrosomic) and the interior (centromeric) parts of the spindle. When the concentration of the colchicine solution was at just above the threshold value the exterior spindle was always inactivated first. The delay in separation of the chromatids at their centromere was associated with the effect on the interior spindle.

A report by O'Mara (1939), while purporting to be a study of the immediate effects of colchicine, merely confirmed observations by others. However, he suggested that colchicine might be useful in studying chromosome morphology because of the high degree of contraction of metaphase chromosomes during treatment.

Shimamura (1939), in an application of the Allium test, presented observations which coincided closely with those of Levan. However, he noted that after the kinetochore separation of the X-shaped metaphase chromosomes the chromatids were arranged around a spherical body which he presumed was "a deformed and degenerated spindle substance." Shimamura also saw numerous binucleate cells in which the two nuclei appeared to be fusing to form a tetraploid nucleus.

Bhaduri (1939) studied the effects of different forms of colchicine upon the root tips of Vicia faba. He attempted to follow the progressive development of the colchicine effect and the subsequent recovery of the treated tissues until normal mitosis was re-established. His results showed that the inactivation of the spindle led to blocked metaphase and the result after telophase was the formation of a tetraploid nucleus. Anaphases that had been organized before treatment appeared to collapse and a failure of cytokinesis allowed the telophase nuclei to fuse and form into a tetraploid nucleus. Upon recovery from treatment, and during prolonged continuous treatments, multinucleate cells and micronuclei were found. Bhaduri believed that colchicine could not affect resting nuclei because of the "resistive" nature of their nuclear membrane. On the other hand he claimed that polyploid resting nuclei resulting from treatment had no nuclear membrane and were therefore more susceptible to further colchicine effect. Bhaduri hypothesized that the role of colchicine was as a catalyst in a chemical reaction which brought about physical changes in the colloidal condition of the nucleus; it, ". . . increased the fluidity of the nuclear material. . ." and inhibited spindle formation.

Shimamura (1940) offered evidence to the effect that no "atractosome" or spindle fibers were formed in materials treated with colchicine. He said that, "The atractosome is an indispensable medium in nuclear division, and chromosomes do not lie directly in the cytoplasm, but in the atractoplasm." Wada (1940) also concluded that the effect of colchicine was specific upon the "Atraktoplasma" and caused a decrease in its surface tension; therefore, the movement of chromosomes into metaphase and anaphase was stopped. He stated that the

atractoplasm forms a boundary between the attractosome and the cytoplasm. When the atractoplasm was affected by colchicine it was destroyed and changed into cytoplasm.

Hawkes (1942) studied the effects of colchicine upon the root tips of Allium cepa seedlings. His observations agreed with those of Levan except in one detail: he found that the centromeres of the c-pairs did not divide until a restitution nucleus was formed. Cornman (1942), in a study of the effects of colchicine upon the excised roots of Colchicum byzantinum, arrived at the same general conclusions as others had with Allium. However, he made the observation that binucleate cells and cells with bridged nuclei were the result of the cessation of anaphase movement and the return to interphase of the nuclei without cytokinesis. He did not speak of the nuclei fusing to form a tetraploid nucleus.

The next major effort to study the effects of colchicine upon plant meristems was made by Berger and Witkus (1943). Their observations were made on root tips of Allium cepa and Spinacia oleracea. Their results with Allium were consistent with those made by earlier workers. They, like Shimamura, noted the presence of an "achromatic sphere" formed from the spindle substance. Quite different results were seen in Spinacia. The chromosomes remained scattered at prometaphase and entered into a metaphase of long duration. Then the scattered "diplo-chromosomes" formed into a dense clump; occasionally their kinetochores would divide before clumping. No sign of an achromatic sphere was seen. A reversion phase followed and a tetraploid restitution nucleus was formed. The restitution process consisted, essentially, of the single chromosomes separating from each

other and a nuclear membrane being formed. The process was likened, in general appearance, to, ". . . the preleptotene prochromosome stage described in insect spermatogenesis (Wilson 1925). . . ."

Soon after the discovery of the cytological effects of colchicine it became apparent that many other chemical substances had a similar effect. (Acenaphthene, for example, was found to behave in a manner quite similar to colchicine (Kostoff 1938). An immediate burst of activity produced numerous accounts of the "c-mitotic" effects of various unrelated substances. Often the studies were of such a nature that direct comparative effects between colchicine and other colchicine-like compounds were investigated. New information on the mechanism of "c-mitosis" was brought to light. In the course of these investigations colchicine was considered the type substance and the vocabulary used to describe its effects was applied to the similar and superficially similar effects of many other substances.

Levan and Östergren (1943), in a comparative study of the effects of colchicine and a series of naphthalene derivatives, arrived at the conclusion that the mechanism of c-mitotic action was dependent on the physical properties of the compounds rather than on any specific chemical properties. This was suggested by the negative correlation between c-mitotic activity and water solubility of the naphthalenes. They believed that the lipoidal phases of the cell were affected and suspected that the seat of action might be in the mitochondria or the centromeres. Similar experiments with the benzenes (Östergren and Levan 1943) supported their conclusions. In a continuation of the experiments Östergren (1944) pointed out that the colchicine type of

mitosis was only one of several related narcotic effects that the c-mitotic substances possessed in common. In the higher plants these were as follows: c-mitosis, c-tumour formation, chromosome contraction and a poison effect. Östergren presented a new hypothesis concerning the mechanism of colchicine action. He thought that the substances became ". . . associated with the lipophilic side chains of the protein polypeptide chains . . ." and, by disturbing the degree of folding of the polypeptide chains, the fibrous protein molecules were changed ". . . into a more or less corpuscular shape" Although he related chromosome contraction to the contraction of the protein chains, Östergren reported observing contraction in prophase chromosomes. In other cases where spindle activity was normal or near normal he noted a high degree of contraction in the chromosomes.

Steinegger and Levan (1947) found that c-mitotic activity could also be related to thermodynamic activity of the substances. They found that colchicine had a specific, or chemical, activity while one of its isomers, iso-colchicine, proved to be unspecifically, or physically, active.

While the greater number of important studies dealing with the cytological effects of colchicine have been made on plant material, numerous experiments have also been performed with animal tissues. An important investigation, using newt larvae, was reported on by Barber and Callan (1943). They found that colchicine caused several kinds of metaphase abnormalities, which in the light of Bernal's tactoid theory of the spindle, they related to an inactivation of the centromere or centrosome or both. "Ball" and "prophase" types of

metaphase represented an inactivation of both the centrosome and the centromere; the movement of the chromosomes resulted from the coiling and uncoiling of the chromosomes and the lack of any chromatid attraction. In the "exploded" type of metaphase the centromere alone was inactivated and the "passive" chromosomes were pushed about the cell by the centrosomic spindle. "Star" metaphases were formed when centromeres in close proximity formed spindles which united to form a monaster. "Distorted star" metaphases resulted from partial cooperation between centrosome and centromere spindle forming efforts.

The studies of Peters (1946) were made upon the cornea of the newt, Triturus viridescens. His results were similar to those of Barber and Callan. Peters classified the abnormalities he found as 1) those in which there was no orientation of the spindle attachment regions and the chromosomes were either clumped or scattered, and 2) those in which spindle attachment regions were oriented to one another and the chromosomes formed either one or more stars or a normal metaphase. Peters then determined that in the unoriented metaphases there were no chromosomal fibers; in the star patterns, chromosomal fibers were visible but there were no continuous fibers. He concluded that the star formations were due to the action of chromosomal fibers and the centriole, both of which acted independently of the continuous fibers and that chromosome fibers recovered more rapidly from colchicine effects than did continuous fibers.

The war years saw a waning interest in colchicine and its cytological significance. This was in part due to the urgent need of cytologists in other war-time occupations and in part to the growing interest

in the antimitotic and mutagenic properties of many new organic compounds used in medicine, agriculture, industry and war. Although the study of colchicine was out of fashion its effects were still used as a control for assaying the effects of other substances.

The renewed interest in colchicine after the war began with some observations made by D'Amato (1948) on its cytological effects. He found binucleate cells and cells with bridged nuclei and surmised that they arose by stickiness of the chromosomes at anaphase as well as from spindle disturbances. He noted also that many micronuclei were formed. In addition to scattered c-pairs, which he said led to tetraploidy, he found "distributed c-mitosis" similar to that caused by methylnaphthoquinone (Nybom and Knutsson 1947) and "exploded metaphases" (Barber and Callan 1943). He also noted "precocious reversion" during both partial and full c-mitosis. D'Amato attributed the latter aberration to timing and physiological factors which were related to spindle inactivation, stickiness of the chromosomes and disturbances in the water metabolism of the cells.

Sigenaga (1949) concluded that the action of colchicine was due to its injurious effect upon the protoplasm and led to a breakdown of the visco-regulating mechanism of the chromosomes and spindle. Wada (1949) advanced the theory that a disturbance of the submicroscopic structure of the atractoplasm was the primary effect of colchicine. Gaulden and Carlson (1951), in a series of observations made on living cells of the grasshopper, showed that a completely organized spindle could be destroyed by colchicine and the altered spindle material formed into an "inert hyaline globule." They also found that

prophases showed colchicine effects by reverting to interphase or by losing the centromere orientation they normally maintained. Inoué (1952) presented some observations made on spindles in living cells of Chaetopterus with a polarizing microscope. He found that colchicine caused the spindles to contract and concluded that a disorientation of the spindle micelles had occurred, probably due to an antagonizing action by colchicine upon some cellular component which was responsible for the polymerization and maintenance of the spindle substance.

Coincident with the revived interest in the effects of colchicine there has recently appeared a real need to distinguish between "c-mitosis" in its restricted sense (i.e., spindle inactivation leading to polyploidy) and the cytological effects that only casually resemble the effects caused by colchicine. D'Amato (1948b) called attention to the fact that many substances that possessed a c-mitotic activity were actually "prophase poisons". Later (D'Amato 1949) he pointed out that, ". . . the spindle inhibiting effect manifested by typical C-mitotic poisons may be of quite another nature than that by which preprophase poisons induce C-mitosis. . . ." He attributed a "massive toxic action" by the latter in producing their c-mitotic effect. D'Amato and Avanzi (1949a) defined typical c-mitotic poisons as ". . . only those which induce C-mitosis without interfering with the entry of the resting nuclei into prophase. . . ." Typical cell poisons caused prophase poisoning and inhibited the initiation of mitosis (D'Amato and Avanzi 1949b).

Other voices were raised in both protest and caution since D'Amato distinguished between c-mitotic and prophase poisons. By interpreting the comparative effects of colchicine and the salts of

nucleic acids, Allen, Wilson and Powell (1950) came to the conclusion that "reductional groups" (Huskins 1948) found after sodium nucleate treatment represented a different nuclear response than did the "distributive c-mitoses" formed during colchicine (D'Amato 1948) and vitamin K (Nybom and Knutsson 1947) treatment. Levan and Lotfy (1949) had previously proposed that "reductional groupings" were merely another example of c-mitosis. Hindmarsh (1951), while studying the effects of nitrophenols, likewise, concluded that the term "c-mitotic" was often loosely applied to many substances. She opined, "Many authors either assume without justification that substances which suppress spindle formation have a common mode of action, or do not recognize that, by use of this term, they imply a common mode of action, for all such substances. . . ." Miss Hindmarsh suggested that ". . . the term c-mitotic be confined to substances which have true colchicine action. . . ." Guttman (1952) warned that ". . . one or a combination of factors may alter the course of mitosis, resulting in an almost identical cytological expression. . . ."

In 1951 Levan grouped the cytological reactions caused by chemical substances ". . . according to their morphological type. . . ." into three main categories. They were the lethal and toxic reactions, the reversible physiological reactions and the mutagenic reactions. The action of colchicine typified the reversible physiological reaction; which included ". . . c-mitosis, i.e., the reversible inactivation of the mechanism of movement of the chromosomes. . . ."

The activities of the cytology group at Michigan State College over the past several years have disclosed the need for a "new look"

at colchicine. The studies on many antibiotics (Wilson 1950a, Wilson and Bowen 1951, Hawthorne 1951, Hawthorne and Wilson 1952, Huston 1952, Bowen 1953, Bowen and Wilson 1954 and the unpublished research of Miss S. Wilson) have shown that a reanalysis of colchicine is sorely needed. Several agricultural chemicals, including Endothal (Daniel 1953) and Lindane (Tsou 1954), illustrate widely different effects upon the mechanism of mitosis; in the loose sense of the term both might be called "c-mitotic agents". The interest of Wilson and his associates (Wilson, Hawthorne and Tsou 1951, Wilson, Tsou and Hyypio 1952) in mitotic variations and their relation to the mitotic mechanism has served as a further stimulus for the present research.

Bowen (1953) and Bowen and Wilson (1954), while studying the cytological effects of some antibiotics, made some comparative studies with colchicine. Their results showed an increase in absolute numbers of prophases over postprophases during treatment and suggested that colchicine might stimulate mitosis. They found that during colchicine treatment the chromosomes arrived at a typical prometaphase clump where they were blocked from further movement. Bowen stated ". . . that the impairment of spindle function is not necessarily an all or none process. . .", as evidenced by the appearance of disorganized postmetaphase figures and micronuclei in treatments with threshold dosages. He discussed at some length the implications of colchicine effect and the mechanism of mitosis.

While the present studies were being brought to a close two recent papers on colchicine effects came to the writer's attention. The first was a study by Hindmarsh (1952) on the spindle of onion root tip cells

after acid fixation. Miss Hindmarsh concluded that colchicine destroyed spindles that were already present at the beginning of treatment as well as inhibiting the formation of new spindles. She was of the opinion that, when anaphase movement was stopped by spindle impairment, the two groups of chromatids reversed their direction of movement and became incorporated into a single, though often irregular-shaped, tetraploid nucleus. In order to account for the duration of colchicine effect Miss Hindmarsh postulated that late interphases must have been affected.

The second paper was a study by Levan (1954) on the effects of colchicine on mouse ascites tumors. His observations were in general accord with those he had made earlier on Allium (Levan 1938), except that the chromatids of the c-pairs in mouse tumors failed to separate at their kinetochores until the interphase following the c-mitosis. Levan also refined his earlier definition of c-mitosis by distinguishing between "initial c-mitosis" and "scattered c-mitosis". The former included balled and clumped metaphase associations and were found in early treatment while the latter category denoted the highly scattered c-pairs.

Levan defended his concept of c-mitosis in the light of recent criticisms and stated that the term could be applied to describe ". . . all morphologically discernible disturbances of spindle function irrespective of any notions as to the mode of action underlying the effects. . . ."

MATERIALS AND METHODS

Experimental Procedure

The meristematic materials used in these studies were the actively growing root tips of Allium cepa and Pisum sativum. The results of Bowen (1953) clearly demonstrated the advantages of the "Pisum test" over the "Allium test". Therefore, the use of Allium was abandoned in favor of Pisum after a few runs had been made to determine how long a treatment with a 1,000 ppm colchicine solution was required to produce a rapid and uniform colchicine reaction. The data obtained from Pisum form the basis of the quantitative studies. Later observations on Allium were made merely to confirm the results obtained from Pisum.

The onions were furnished by the Michigan State College Agricultural Experiment Station through the courtesy of Dr. B. H. Grigsby. They had been especially grown for cytological purposes and were said to be free from contamination by any organic herbicide or insecticide. The peas were donated by Ferry-Morse Seed Company. They belonged to the commercial variety "Alaska", a disease-free strain of relative genetic homogeneity, which had not been treated with any antifungal agents.

Allium root tips were obtained by the standard method of rooting bulbs of uniform size over jars of aerated distilled water. When the roots were about three centimeters long, and if they possessed sufficient mitotic activity, they were ready for experimental purposes.

Pisum root tips were obtained by following the technique developed by Bowen (1953) with few minor modifications. Essentially, this consisted of germinating peas in moist paper toweling and allowing them to grow until the seedlings had developed roots about three centimeters long. At that time the seedlings were placed on a paraffin-coated metal grid made of hardware cloth with the roots inserted through the openings and suspended in quarter strength modified Hoagland solution. After an acclimatization period of twelve hours root tips were checked for mitotic activity. The collections made at this time were called "zero hour controls". They always possessed a remarkably high and uniform mitotic index. The seeds were germinated at room temperature (about 24° C).

The colchicine employed was a USP preparation of Mallinkrodt Chemical Works bearing the control ZMV. It was dissolved in distilled water to make a 0.1% or 1,000 ppm solution. This concentration was used in all the treatments after it was shown to cause a rapid and uniform expression of the colchicine effect. Unused colchicine solution was stored under refrigeration until needed. Such portions as were required for treatment were brought to room temperature before use in treatment. All experiments were conducted at room temperature.

At the time of treatment the root tips were exposed to the 1,000 ppm solution of colchicine for exactly fifteen minutes. Care was taken that only the roots became exposed. At the end of the treatment the roots were removed from colchicine and carefully and thoroughly washed. In the case of Allium the bulb was returned to a jar containing fresh aerated distilled water. Pisum were returned to fresh

quarter strength mineral solution. The root tips were then allowed to recover until normal mitosis was re-established.

Control runs were carried out simultaneously with the colchicine experiments. They were handled like the colchicine treated materials except that they were not exposed to the drug nor washed.

Collections of root tips were made at regular intervals. Before treatment began "zero hour controls" were collected. Other collections were made at the end of the fifteen minute treatment, at 15, 30 minutes and one hour of recovery; hourly collections thereafter were made until it was established by cytological examination that normal mitosis had been resumed. Cuttings were also made at 24 and 48 hours after treatment. Three root tips were collected at each interval.

Fixation took place in a three to one mixture of absolute alcohol and glacial acetic acid for thirty minutes. After hydrolysis in one normal hydrochloric acid the root tips were stained by the Feulgen reaction. Squash preparations were made and dehydrated in 95% ethyl alcohol overnight. A small amount of fast green was added to the alcohol to act as a counterstain. The slides were made permanent with Diaphane.

Cytological Examination

The slides were examined with a 90X oil immersion objective and 12.5X oculars. A ribbon filament lamp with a type B green filter placed between the lamp and the microscope was used for illumination.

The Pisum slides, from which quantitative data were desired, were carefully examined along several widely separate strips in what amounted

to a random sampling of the more or less uniformly distributed meristematic tissue. The sampling was standardized so that 1,000 meristematic cells were counted on each slide. Usually this count could be obtained by scoring three horizontal strips located at one-quarter, one-half and three-quarters of the way down the cover slip. In counting the 1,000 meristematic cells, an accurate record of the number of all cells in division was made. After 1,000 cells were scored and their rate of division had been determined, scoring of additional divisions continued until 100 dividing cells had been counted. Dividing cells were determined as belonging to one of five general stages of mitosis, viz. prophase, prometaphase, metaphase, anaphase or telophase. After the stage of mitosis had been resolved it was further necessary to determine whether the figure in question was normal or not. If abnormal, it was then a matter of deciding to which of several predetermined mitotic variations it most closely corresponded.

From the data obtained in the above manner it was possible to determine such quantitative information as the mitotic index, the percent of divisions in the various stages of mitosis and the increase in number and kind of abnormalities after treatment. The data obtained in these studies are summarized in Appendix Tables I to VII and form the basis for all quantitative analyses.

OBSERVATIONS

Normal Mitosis in Pisum

It seems highly desirable, before attempting to give an explanation of abnormal mitosis after colchicine treatment, that an account of normal mitosis in Pisum should first be related. The following remarks are based on observations made on numerous slides of zero hour control material. They are presented for the convenience of reference when later observations on the effects of colchicine are considered.

For the sake of classification the process of mitosis has traditionally been divided into several categories or so-called phases. It should be borne in mind, however, that the phases are but moments of association in a continuous series of movement which is climaxed by the production of two nuclei where only one existed before. An established number of chromosomes undergo certain morphological changes and participate in certain maneuvers within the confines of the mother cell. They are the chief performers and play the leading roles. Behind the scenes, unseen and unobserved, are several forces, some recognized and others only presumed, which are closely synchronized with the observable changes in form and position of the chromosomes.

If one considers the mitotic process to be a smoothly moving series of events in which one phase passes without pause into the next, it becomes a matter of little wonder that the observer seldom

sees the "typical" text-book phases. Some stages are well-defined and well-organized only because they have a duration long enough to be often seen. Others, though all dividing nuclei must pass through them, are so transitory that rarely are they observed; indeed, to the casual observer, they appear quite abnormal because of his unfamiliarity with the configurations.

To the keen observer, on the contrary, the "typical" stages become the rarities as he follows the act of mitosis. More often than not, the point at which fixation interrupted mitosis will be intermediate between two accepted stages. One must concede that mitosis can no more be interrupted than a complicated ballet without catching the performers in some awkward and unsuspected poses.

Although the resting stage, or interphase, is not a mitotic stage it deserves some attention. Cells in this condition make up the bulk of any meristem and mitosis is certainly initiated in interphase. The resting nucleus of Pisum is usually more or less diffuse and granular. The chromosomes are said to consist of weakly staining chromonemata and darker staining heterochromatic granules (Hughes 1952). The darkly staining region at the polar cap probably represents the association of the kinetochores that has been maintained from the previous anaphase (Plate I, fig. 1). The chromosomes are said to lie loosely coiled within the nuclear membrane and probably appressed to its inner surface (Wilson 1950). Each nucleus has at least one nucleolus. The nucleolar cycle in Pisum has been studied in detail by Håkansson and Levan (1942).

At the onset of mitosis the chromosomes begin to contract and consequently become thicker and easier to distinguish (Plate I, fig.

2). They appear to become more or less uniformly staining throughout their length. The association of kinetochores at the polar cap is often very obvious; they seem to be arranged in a circular pattern and are probably attached to the inner surface of the nuclear membrane. The volume of the nucleus begins to increase with mitosis.

As prophase continues the chromosomes continue to contract and the circle of kinetochores begins to open up and, simultaneously, move toward the equator of the nucleus (Plate I, fig. 3). It is assumed that during this process the nuclear membrane is still intact. The movement of the chromosomes at this time may be directed by the activity of the centers which appear to control chromosome movement before the nuclear membrane disappears (Schrader 1953).

In the present study some nuclei in prophase have been seen which suggest that during contraction of the chromosomes tautness due to tension between two parts of a chromosome arm may occur. It appears that chromosomes may contract differentially or asynchronously. If this is the case it may well explain why the kinetochore association in mid- and late prophase is often disturbed. It is suggested that if the differential contraction between two parts of a chromosome arm develops sufficient tension the kinetochore might be dislodged from its vestigial position and contact with the nuclear membrane.

While the sliding movement of the kinetochores continues, the chromosomes continue to contract until the prometaphase length is achieved (Plate I, figs. 4, 5, 6, 7). The kinetochores and secondary constrictions become apparent because of the differentially staining regions at those points. Each chromosome is clearly double except

at the kinetochore. The sister chromatids are associated with one another in a loose relational coil which keeps uncoiling until the prometaphase chromatids are held together by relatively few twists. The spindle is said to begin to form at this time (Hindmarsh 1952) but its presence is difficult to demonstrate in Pisum.

Were the sliding movement of the kinetochores to continue uninterrupted during prometaphase the chromosomes would reach their metaphase position without further ado. Seldom, however, is this achieved directly. At some time or another between the initiation of movement by the kinetochores and their approach to the metaphase plate the process is seen to have collapsed. This collapse may be due to the disintegration of the nuclear membrane. The chromosomes lose the organization they had maintained, more or less, since the previous anaphase and form a tangled clump at the center of the cell. This ball or clump stage in prometaphase is not an uncommon prometaphase configuration. Schaede (1938) described "Ballung der Chromosomen um den Nucleolus" from many plants that he and others had studied. Bowen (1953) noted the ball prometaphase to be numerous in the control material of Pisum. Prometaphase clumps are illustrated in Plate I, figs. 5, 8, 11. Not all prometaphase chromosomes appear to form into compact balls. Some seem to reach metaphase simply by moving directly to the equator either under spindle forces or by their own power or both.

After the collapse of organization the prometaphase ball opens up and metaphase orientation begins (Plate I, figs. 10, 11, 12). The untangling is aided, at least in part, by the final maximum contraction and the accompanying gyrations of the chromosome arms. It is

also very likely that at this time the spindle which has been forming becomes functional and has some influence in orienting the chromosomes to their position along the equator. With the expansion of the spindle to its fullest circumference about the equator the chromosomes become disposed along the periphery of the metaphase plate. The random distribution of the chromosome arms on both sides of the equator comes about as a direct result of the haphazard clumping and subsequent untangling of the prometaphase. Not all chromosomes arrive at the equator at the same time (Plate I, figs. 11, 12). Often, at least the shortest chromosomes lie parallel to the equator (Plate I, figs. 13, 14).

The condition of full metaphase is reached when the chromosomes have come to an equilibrium at the equator. It probably exists but a very short time; as soon as the chromatids cleave at their common kinetochore they appear to be mutually repulsed and attain a most haphazard looking appearance (Plate I, fig. 10). However, the chromatids come under the influence of the spindle and anaphase movement begins. During this movement the kinetochores become oriented towards their respective poles and the chromosome arms trail behind (Plate I, fig. 15).

Upon their arrival at the poles the chromosomes begin to lose chromaticity and pass into telophase which represents the reorganization of the chromosomes into new nuclei. A nuclear membrane and nucleoli are formed and the nucleus takes on the appearance of an interphase nucleus. It is interesting to note that when a lagging chromosome is left behind it still retains the appearance of an

anaphase chromosome while the main nuclei are well in telophase (Plate I, fig. 16). The same phenomenon has been described by Gaulden (1954) in cells of grasshopper.

Beginning during late anaphase and continuing into telophase, cytokinesis occurs and the two nuclei become separated from each other as separate cells.

The foregoing treatment of mitosis is highly generalized and treats specifically only those details that have been and can be observed. Schrader (1953) has recently reviewed many of the theories of metaphase mechanics and postmetaphase movements. Most of them are highly theoretical and involve forces which cannot be demonstrated with the present techniques. It therefore seems that not much would be gained by discussing them here. One may well agree with Schrader that little indeed is really known about metaphase and postmetaphase -- prometaphase may as well be included -- except that,

The chromosomes themselves play an active part in the mitotic mechanism. Any movement beyond the initial separation is, however, associated with the presence of some part of the mitotic apparatus, even though the relationship is not always obvious. The forces that are involved in such an active participation of the chromosomes are still obscure, although the proponents of chromosomal autonomy have performed no small service in insisting that the chromosome is not a mere passive body which is transported hither and yon by forces more or less extraneous to it. (Schrader 1953, pp. 111-112.)

Accordingly, the theory followed here was one proposed by Wilson, Hawthorne and Tsou (1951). They suggested that there were at least two components to the mitotic spindle; a dipolar cytoplasmic orientation, and a nuclear component inherent in the chromosome or its kinetochore. The advantage of working with a streamlined theory such

as this is obvious. There are no reasons to encumber oneself with complicated theories of forces that cannot be seen or demonstrated.

The chromosome movement cycle in Pisum sativum may be summarized as consisting of five, more or less distinctive, stages of movement, namely;

- I. Early to midprophase
- II. Midprophase to prometaphase
- III. Prometaphase to metaphase
- IV. Anaphase
 - A. Metaphase to anaphase
 - B. Anaphase to telophase

The movements are diagrammatically illustrated in Text Figure 1.

Analysis of Colchicine Effects in Pisum

Classification of Aberrations

Zero hour controls were scored for abnormalities so as to give some idea of a standard against which abnormalities resulting from colchicine treatment might be compared. It soon became evident that many of the so-called spindle abnormalities found in untreated cells were of little value in judging colchicine effects. The majority of aberrations seen in the controls consisted of tilting or tipping of the metaphase plate and the anaphase spindle, split or segregated figures and unusually clumped or balled prometaphases (Plate I, figs. 5, 8, 11) and metaphases. Minor aberrations, such as lagging chromosomes (Plate I, figs. 9, 16), and micronuclei were so seldom seen that their frequency could not be determined. Only the frequency of the



Text figure 1. The Chromosome Movement Cycle in Pisum.
I to II, early to midprophase; II to III midprophase to
prometaphase; III to IV, prometaphase to metaphase;
IV to V, anaphase movement.

balled and clumped prometaphases and metaphases was analyzed for comparison with later treated material.

Following treatment the dividing cells were similarly scored as being normal or abnormal. The abnormalities were placed into several broad categories depending upon their morphological stage in the mitotic cycle. Since prophases showed no visible effects from colchicine they were considered as being normal.

Prometaphases that were unusually clumped or balled were called abnormal. It should perhaps be pointed out that this particular configuration was found to be quite a common and normal stage in the controls; therefore only its increasing frequency in treated material, and not its presence, was considered abnormal.

Abnormal metaphases were generally considered as belonging to two rather broad categories. The first of these was the clumped and balled category. It included all metaphases in which the chromosomes were located at the center of the cell in a more or less close and unorganized association (Plate II, figs. 1, 2). No metaphase plate was formed and the chromosomes did not come to an equilibrium at the equator. The association may be somewhat loose and haphazard; others show a tendency for kinetochores to become attracted to each other or to the telomeres of other chromosomes (Plate II, fig. 13). The second major category of metaphase aberrations consisted of the scattered type of chromosomes. Scattered metaphases were further divided into two arbitrary groups: "scatter I" and "scatter II". All metaphases which showed some tendency to group themselves in the center of the cell, but were so loosely arranged that they did not

belong to the clumped category, were called scatter I (Plate II, figs. 5, 6, 8). The term scatter II was used for metaphases in which the chromosomes were highly scattered within the cell (Plate II, figs. 9). Sometimes the scattering appeared uniform while at other times there appeared to be one or more centers of association between two or more chromosomes. In addition to being highly scattered these chromosomes are usually highly contracted and resemble the c-pairs described by Levan (1938).

The abnormal anaphases were also classified as being clumped, balled or belonging to scatter I or scatter II by much the same criteria as was used to classify the metaphases. Plate II, figs. 1, 7, 8, 10, and 11 illustrate some of the anaphase abnormalities. It was assumed that a particular metaphase aberration was capable of giving rise to the corresponding anaphase abnormality; thus, ball metaphases produced ball anaphases, and so forth. An additional anaphase aberration was the stalled or arrested type which appeared with the collapse of the spindle (Plate II, fig. 3).

Telophases were called abnormal when they were bridged or showed an unusually close proximity of the nuclei. The quantitatively important telophase abnormalities were designated as "telomorphs". This term should not be confused with the term "telemorphic", used by Beal (1944) to describe the gross effects of certain growth-promoting substances. Telomorphs were found to be either unipolar or multipolar. The unipolar telomorph consists of a nucleus in which chromosomal division has occurred without an accompanying cell division and a tetraploid nucleus has resulted (Plate II, fig. 2). Such telomorphs may be quite uniform in shape when produced from balled or

clumped anaphases but often quite ragged and irregular when arising from somewhat scattered anaphases. The multipolar telomorph is produced from anaphases which are highly scattered and may consist of two or more nuclei arising from the reversion in situ of scattered chromosomes (Plate II, fig. 12).

Interphase nuclei were not scored quantitatively since it was evident that a telophase produced a corresponding type of resting cell. Following the first abnormal telophases, and accompanying them, abundant similar resting nuclei were found. Abnormal interphases were polyploid, micronucleate or binucleate. A concerted attempt to detect further division of affected nuclei was made. At 24 and 48 hours polyploid cells were seen dividing. Often the cells thus seen were not true tetraploids but rather were aneuploids which contained less than the tetraploid number of chromosomes. No cells with less than the diploid number were ever definitely shown to redivide. Binucleate cells were often seen. It was of course impossible to ascertain whether the nuclei were chromosomally equivalent or not since these cells were never observed in mitosis.

Frequency of Division

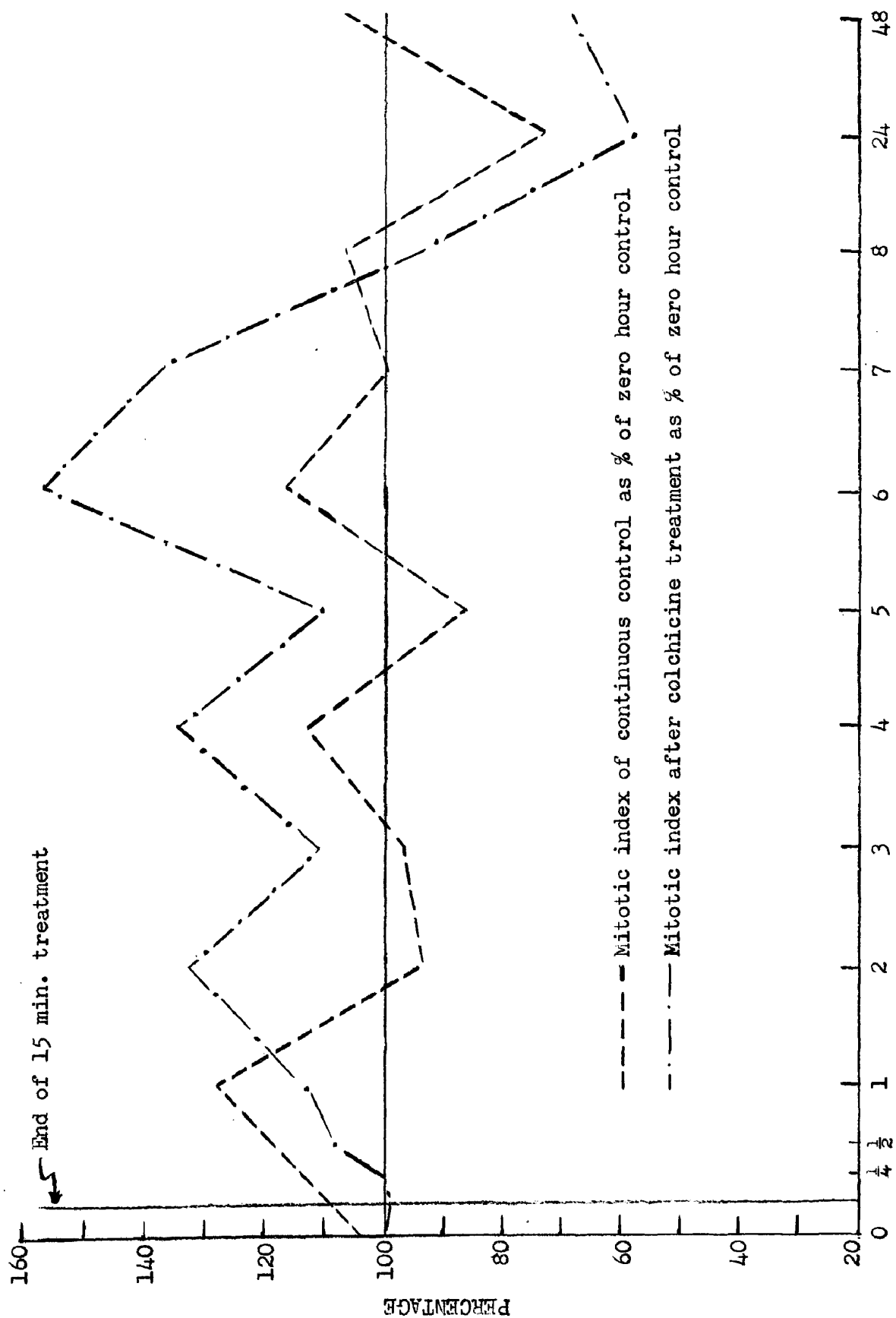
From the analysis of zero hour controls a mitotic index was established for normal mitosis. This index represented the average absolute number of cells in division per 10,000 meristematic cells (Appendix Table I). The apparent increases in the mitotic indices for data grouped as fifteen minutes to four hours and five to eight hours of recovery were found to be insignificant (Appendix Table I).

However, there was a significant difference between the decrease of the mitotic index for twenty-four and forty-eight hours and the zero hour control.

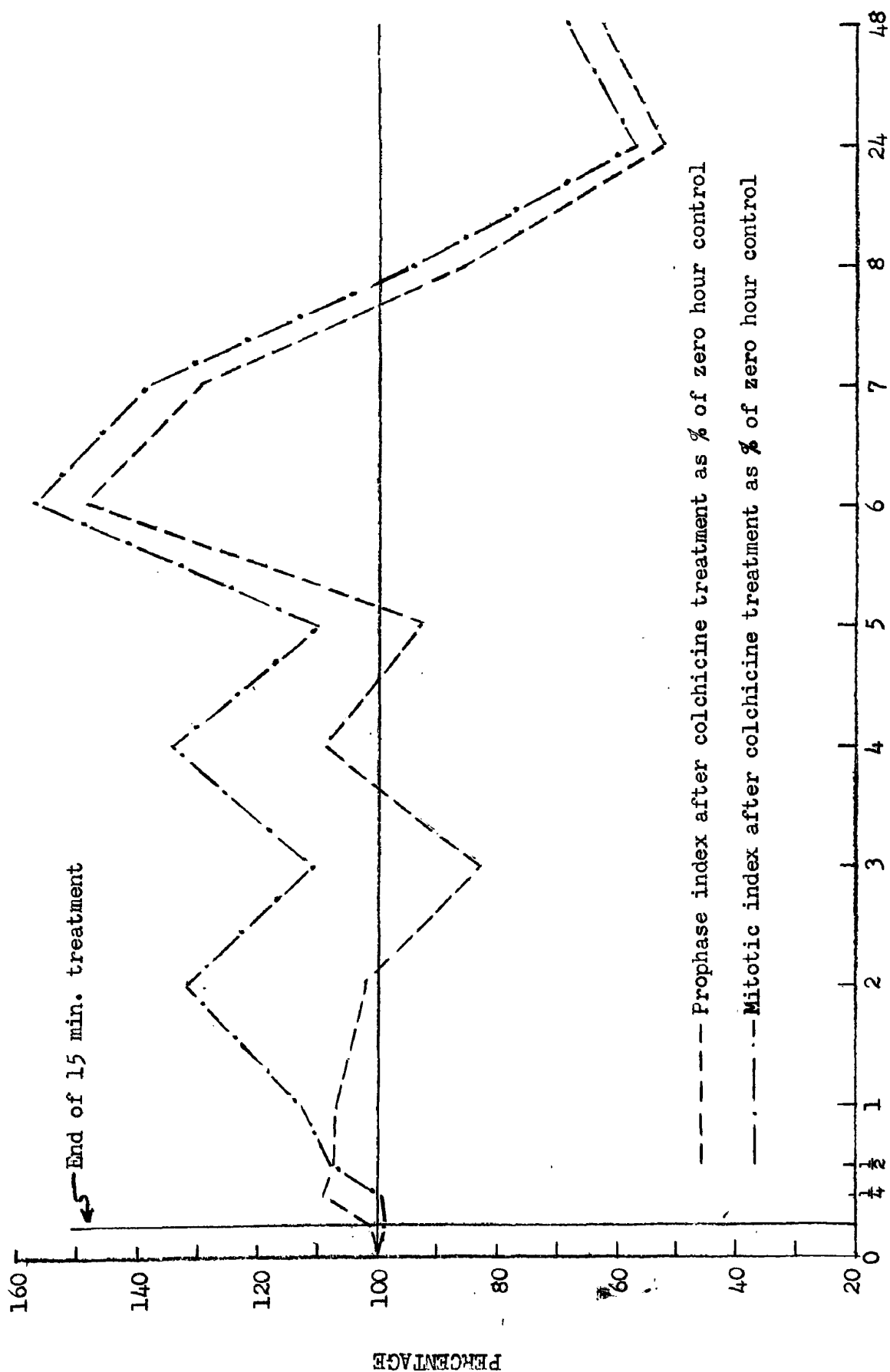
The individual mitotic indices determined at specific intervals after colchicine treatment showed a rather consistent increase of division frequencies over the zero hour controls. The individual mitotic indices are listed in Appendix Table II. The highest index was found to occur at six hours after treatment. After that the frequency of division fell to near normal levels at eight hours and was substantially lower than normal at twenty-four and forty-eight hours. Text Figure 2 illustrates the change of mitotic index after treatment relative to the zero hour control and a continuous control.

Separate indices were determined for prophase, metaphase, anaphase and telophase. It was found that the index for prophase figures after five hours of recovery corresponded very closely to the mitotic index for the same period. This close relationship indicates that the increase in the mitotic index at that time, relative to zero hour controls was due, at least in part, to an increase in the absolute numbers of cells entering into division and suggests that colchicine stimulates mitosis. The relationship of the mitotic index after treatment and the corresponding prophase index is graphically shown in Text Figure 3.

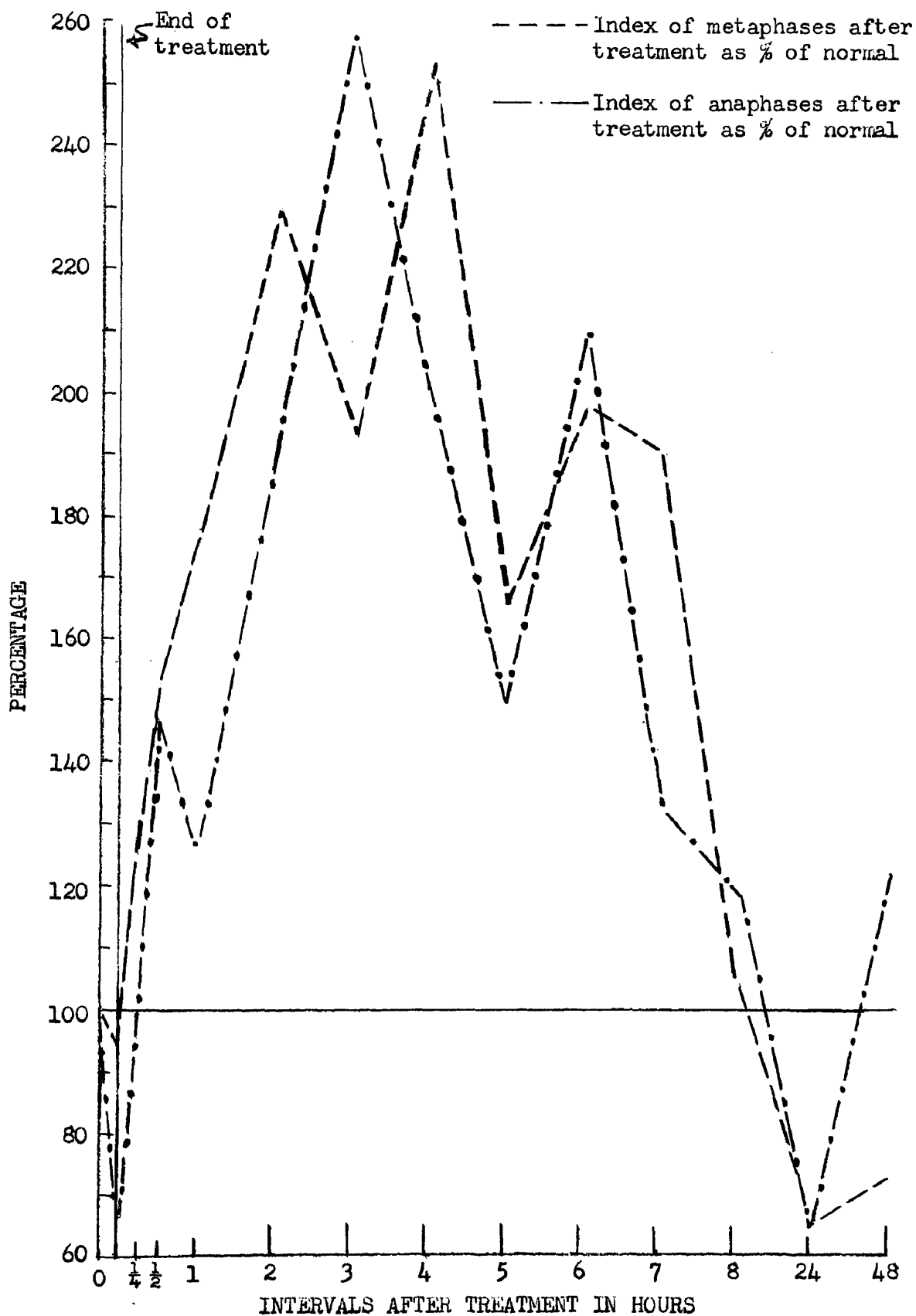
The indices for metaphase and anaphase after treatment showed an unusually rapid increase over the normal for up to four hours. Another high index occurred for both again at six hours (Text Figure 4).



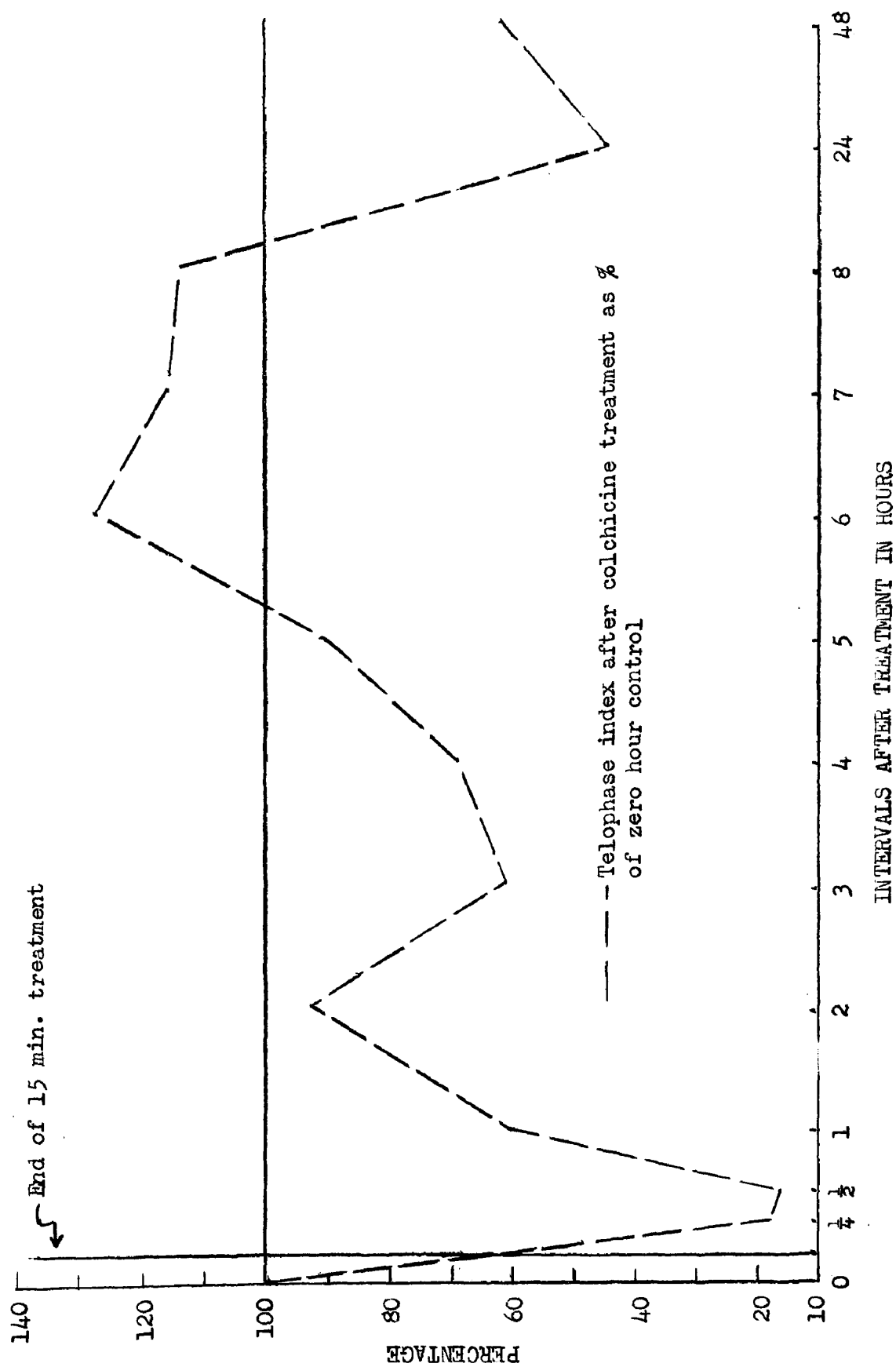
Text figure 2. Changes in Mitotic Index after Colchicine Treatment Compared to a Control.



Text figure 3. Changes in Prophase and Mitotic Indices after Colchicine Treatment.



Text figure 4. Changes in Metaphase and Anaphase Indices after Colchicine Treatment



Text figure 5. Changes in the Telophase Index after Colchicine Treatment.

The first increase of metaphase and anaphase seem to be related to the original increase in the mitotic index after treatment. The second increase at six hours appears to be related to the increase also found in the prophase as well as the mitotic index at that time.

The indices for telophase showed clearly that the stage virtually disappeared at fifteen and thirty minutes of recovery, in negative correlation to the indices established for metaphase and anaphase (Text Figure 5). The highest index for telophase occurred at six hours, at the same time as the peak for the mitotic index and the prophase index and the secondary peak for metaphase and anaphase occurred.

The stalling effect which has been attributed to colchicine by many workers is evident from the piling up of metaphases and anaphases and the disappearance of telophases. In every case all indices declined sharply after seven hours and were substantially lower than zero hour controls at 24 and 48 hours.

Summary: By use of mitotic indices and indices for the various stages in mitosis it was determined that colchicine increases the mitotic index, stimulates the entry of cells into division, causes an accumulation of metaphases and anaphases and prevents them from going into telophase at the normal rate.

Time-Effect Relationships

When the data after treatment and during recovery were compared with zero hour controls it became evident that the degree and kind of aberration appeared to be related to the time of recovery after the

colchicine treatment. The data, summarized in Appendix Table II to VII, could be conveniently considered as falling into the following four time-effect categories:

1. immediate effects, after fifteen minutes of treatment,
2. effects at 15 minutes to 4 hours of recovery,
3. effects at 5 to 8 hours of recovery, and
4. effects at 24 and 48 hours of recovery.

Immediate effects. No visible effects were noted upon prophase. However, many of the cells in postprophase stages of division showed some immediate effect. There was a tendency for prometaphase chromosomes to remain in the tangled clump typical of normal mitosis and to pass into metaphase and postmetaphase in situ. Chromosomes morphologically at metaphase showed an absence of normal metaphase arrangement. They ranged from loosely disorganized clumps to tightly balled configurations. No evidence of scattering of metaphase chromosomes was found nor were any metaphases, that were already organized at the time of treatment, blocked in place. Apparently cleavage of metaphase chromosomes was able to take place. Several types of postmetaphase abnormalities were also immediately seen. Well organized anaphases were stalled or arrested in their movement so that the anaphase and later telophase nuclei remained in close proximity to one another; when telophase occurred there was a failure of cytokinesis and binucleate interphase cells and cells with bridged nuclei were formed. Another anaphase disturbance was noted when very early anaphases appeared to have been stalled before they had come under the influence of orderly anaphase movement. They appeared quite

haphazard and formed into tetraploid telophases. Other anaphases were tightly balled and clumped (often resembling a ball of yarn) and appeared to have been derived from metaphases which had remained in the prometaphase association. In addition to telophases with bridged and arrested nuclei unipolar telophase configurations were found. They arose from all mitoses in which anaphase movement sufficient to separate the chromatids failed. They were obviously polyploid.

Summary: The immediate effects of colchicine increased the duration of the prometaphase clump so that many of the metaphase and anaphase associations were produced in situ. They reverted into unipolar telomorphs and became polyploid interphases. Early anaphases were arrested and also formed into polyploid nuclei. A stalling in later anaphase movement and the failure of cytokinesis produced binucleate cells in which the nuclei were sometimes bridged.

Effects at fifteen minutes to four hours of recovery. This period can for all practical purposes be considered as one of extension in time and intensification of effect of most of the deviations already noted at the end of the treatment. The effects upon the postprophase figures are represented graphically in Text Figures 6 to 8. No effects upon prophase were observed. The frequency of the balled type of prometaphase reached its peak at the end of 15 minutes of recovery and thereafter gradually declined. Over 50% of all prometaphases seen in the first four hours of recovery were rather tightly balled. At the same time almost all of the metaphases were either clumped or tightly balled at the center of the

cell. The remainder showed a tendency toward a looser arrangement, being slightly scattered in the vicinity of the cell center. The only normal metaphases observed occurred at two hours of recovery. They were beginning to show signs of over-contraction but, unlike the others, formed into what appeared like very normal metaphase plates. At the close of the period, at four hours of recovery, some highly scattered metaphase chromosomes made their initial appearance. Like the c-pairs of Levan, they were highly contracted and held together at the kinetochores only. Postmetaphases were represented by clumped and balled akinetic anaphases. They gave rise to an almost identical proportion of unipolar telomorphs. An interesting observation made at both 15 and 30 minutes of recovery was that four of the six slides scored for those time intervals showed no telophases of any kind (see Text Figure 5). The same slides showed numerous binucleate cells in interphase as well as others with atypically close nuclei and nuclei connected by one or more bridges. At one hour of recovery all telophases were unipolar telomorphs. At the same time there was a reappearance of slightly scattered anaphases similar to those seen at the end of the treatment. They were seen with increasing frequency up to four hours when their peak of frequency was reached. At three and four hours highly scattered and contracted anaphases were noted. The first multipolar telomorphs were seen at two hours and they increased in frequency throughout the remainder of the period. Micro-nucleate interphases, microcytes, and large, apparently polyploid, cells were commonly seen at four hours.

Summary: The period of recovery from fifteen minutes to four

hours was characterized by a continuation and intensification of the effects initiated during treatment, and the appearance of scattered metaphases and anaphases. Multipolar telomorphs were commonly seen.

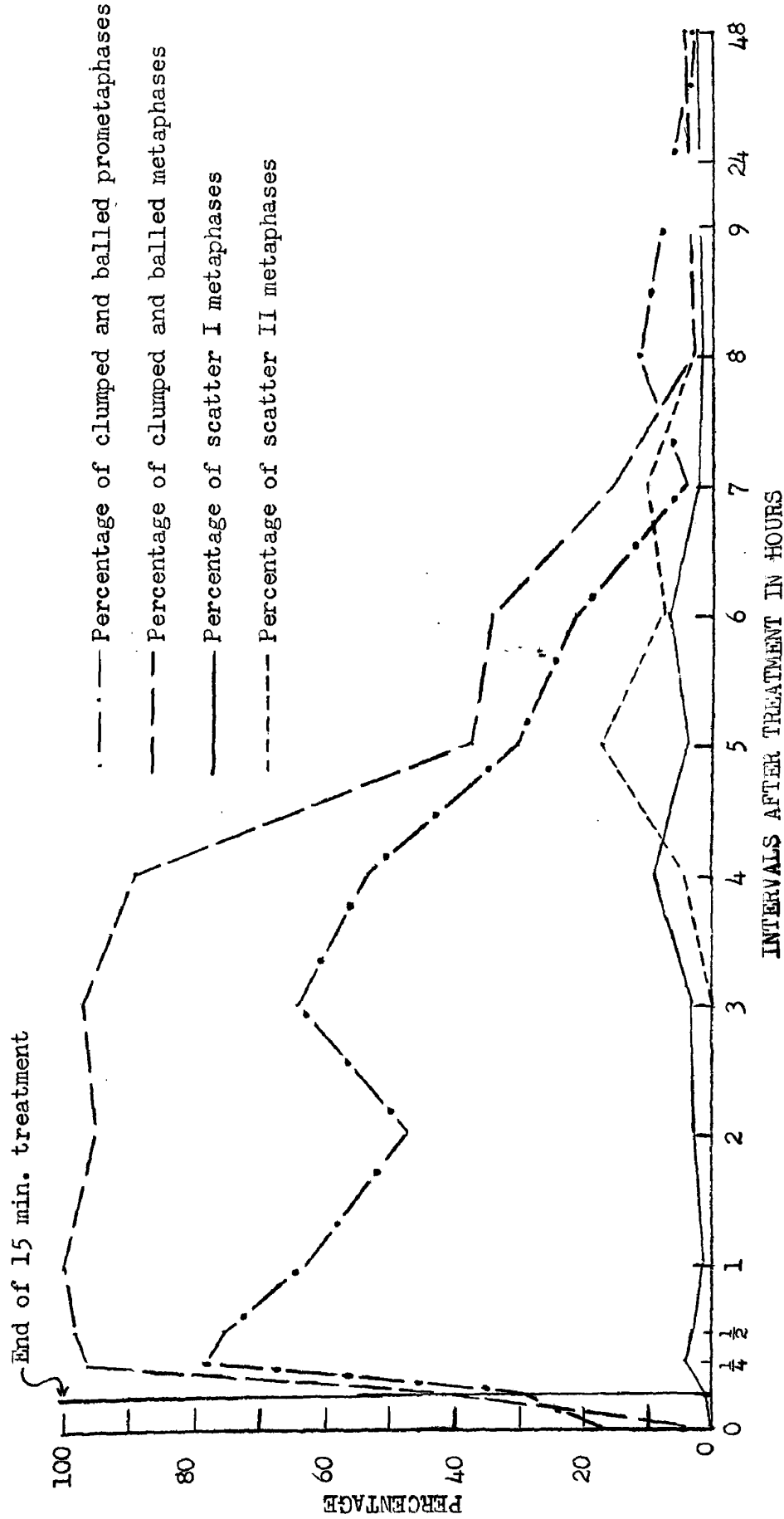
Effects at five to eight hours of recovery. This period was observed to be one that was conspicuously marked by a rather high incidence of scattered metaphases and anaphases and multipolar telomorphic activity. The last two hours saw the return of normal bipolar mitoses. The incidence of balled prometaphases and balled and clumped metaphases fell to approximately normal levels. In both stages the decrease at five hours was sharply evident from the four hour samples. At the same time, the highly scattered metaphases reached their peak at 5 hours of recovery. They were, likewise, almost entirely eliminated at eight hours.

The same general trend was also observed for postmetaphases. Both the clumped and balled anaphases had disappeared at seven hours. The scattered anaphases, like the corresponding metaphases, were most common at five hours. They were no longer found at eight hours. Telophases were represented by a decreasing number of unipolar telomorphs, which, like the clumped anaphases, disappeared at seven hours. Multipolar telomorphs were at the height of their frequency at five and six hours; they fell off sharply by eight hours. A large number of multinucleate cells resulted from the multipolar telomorphs. In addition to haphazard multipolarity, some evidence for true multipolar spindle activity was seen. This form of erratic or unbalanced spindle behavior is well illustrated by figures 14, 15 and 16 in Plate II.

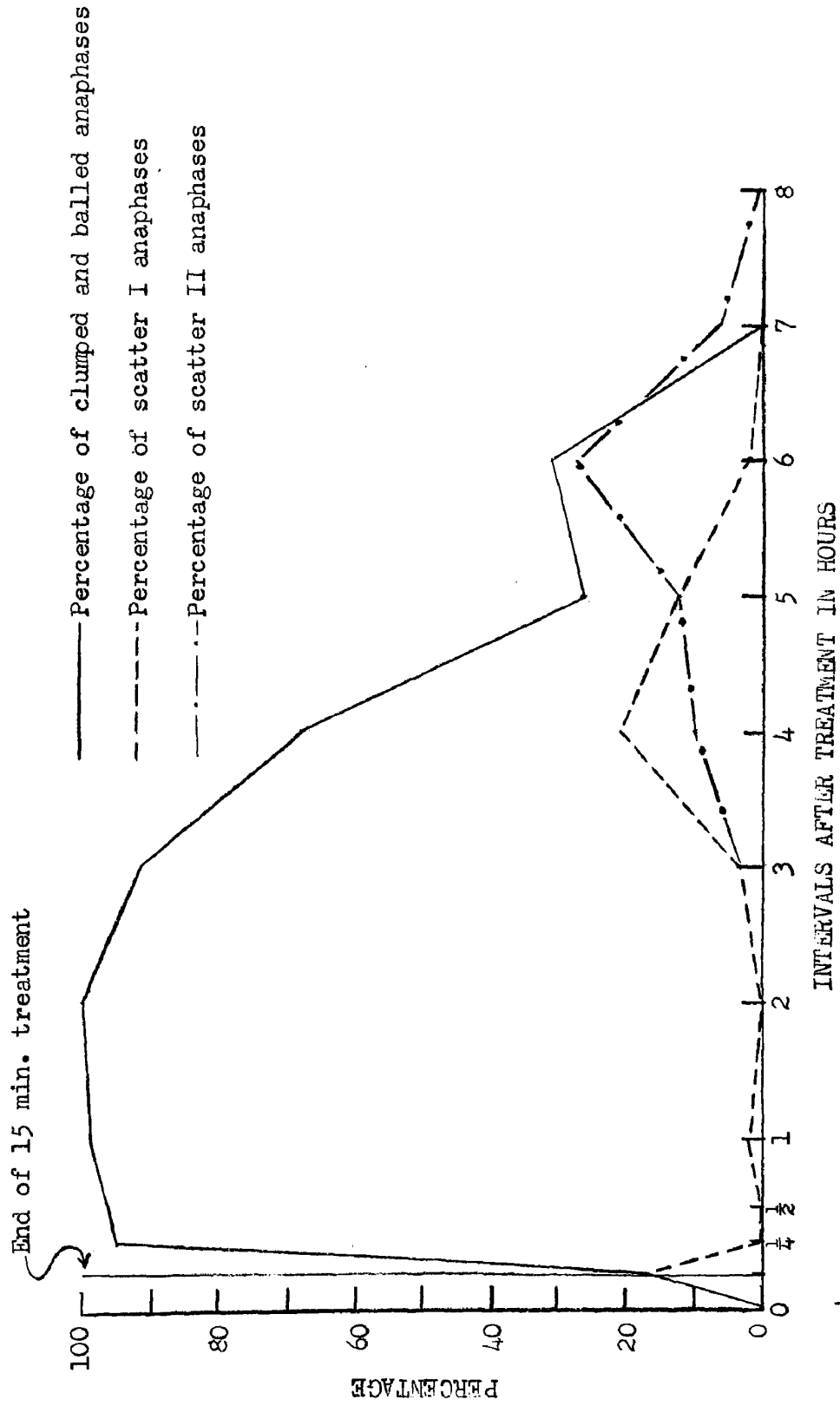
Except for the presence of an abnormal metaphase plate and anaphase movement, multipolar spindle activity is difficult to differentiate from akinetic multipolar activity since the end result is the same, namely, unbalanced micronuclei.

Summary: The five to eight hours stages of recovery saw a rapid decrease of the effects first seen at the end of treatment and persistent through the first four hours. There was an increase in the number of scattered metaphases, anaphases and multipolar telomorphs. There was a gradual return to normal mitosis and by eight hours the frequency of aberrations was at near normal levels.

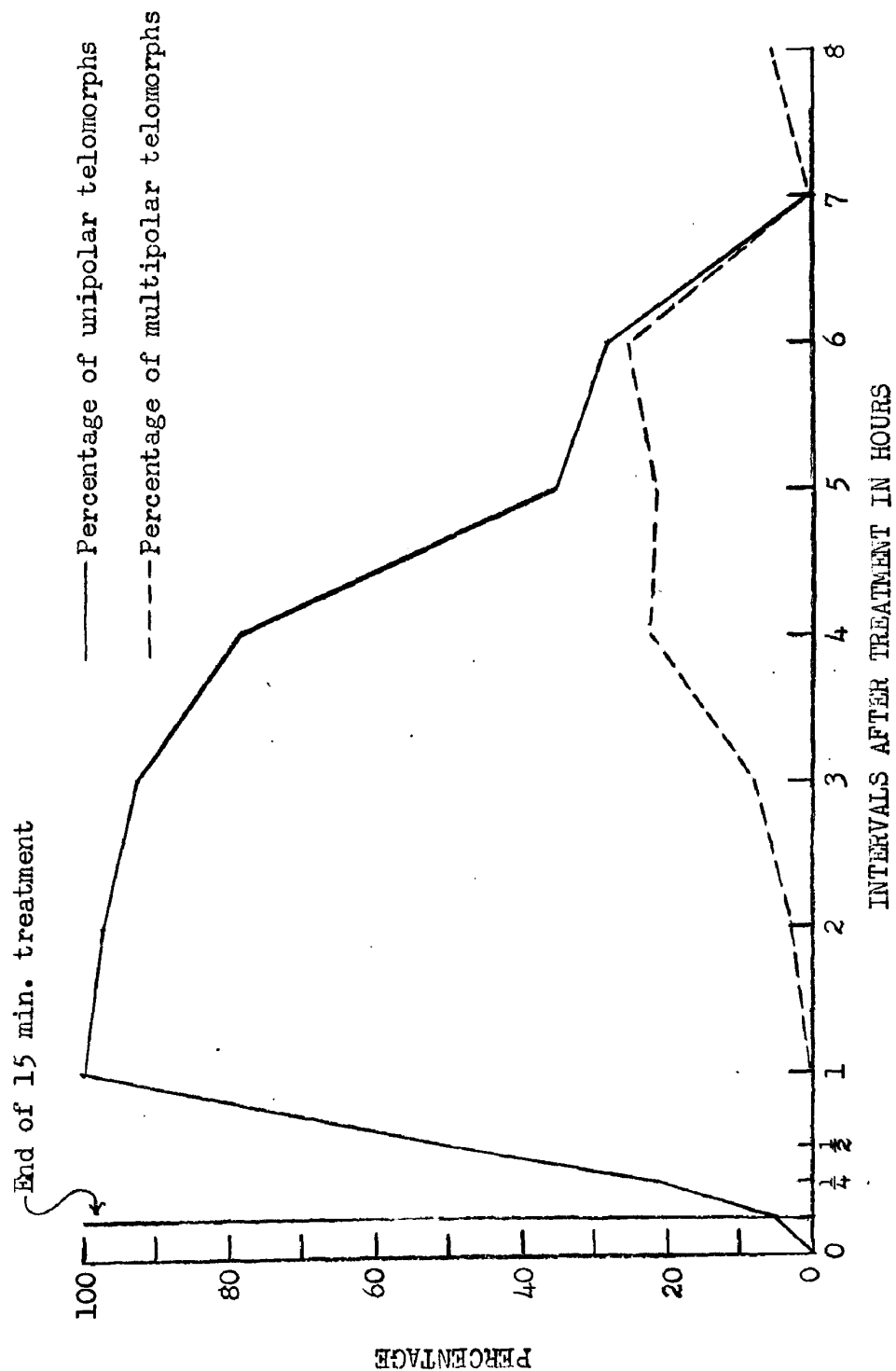
Effects at twenty-four and forty-eight hours of recovery. The mitotic frequency after one and two days of recovery was significantly lower than in the controls. This difference was particularly evident at metaphase (Appendix Table I). No particular deviations from controls were found in the division figures except in the presence of a few slightly scattered metaphases. Polyploid cells were seen, but only somewhat less than 8% of the dividing cells were polyploid. Actually, 7.5% at twenty-four hours and 8% at forty-eight hours made up the proportion of polyploid cells in division. It was ascertained in some cases that the polyploidy was not strictly euploid. The number of chromosomes was often observed to be somewhere between the diploid fourteen and the tetraploid twenty-eight. Many large polyploid interphases were found as well as numerous multinucleate cells and microcytes of varying nuclear content and size. Occasionally binucleate cells and cells with bridged, dumbbell-shaped, lobed and otherwise irregular nuclei were also observed. No evidence of any



Text figure 6. Variations in the Proportions of Abnormalities in Prometaphase and Metaphase.



Text figure 7. Variations in the Proportions of Abnormalities in Anaphase.



Text figure 8. Variations in the Proportions of Abnormalities in Telophase.

mitotic activity was observed in any of the binucleate or micro-nucleate cells nor in any of the microcytes. No cell was ever seen in division in which there was definitely established to be less than the normal complement of chromosomes.

Summary: By twenty-four and forty-eight hours normal mitotic conditions prevailed. Many interphases showed abnormalities that had originated during their mitotic cycle under the effect of colchicine. Binucleate, polyploid and multinucleate cells and microcytes were in evidence.

Normal Mitosis and Colchicine Effects on Allium

It was stated earlier that onions were used only to establish the length of treatment required to induce a quick and uniform colchicine effect upon mitosis. A variety of reasons, especially the lack of mitotic uniformity between individual bulbs, made cytological work with Allium difficult. Therefore, after Bowen (1953) showed how well Pisum seedlings could be used in the present type of work, quantitative studies with Allium were discontinued. However, a number of experiments with fairly satisfactory results were conducted and the observations made on them served to confirm the results obtained from Pisum.

The normal mitotic cycle in Allium appears to be about the same as in Pisum except for one striking difference. The prometaphase chromosomes do not enter into such a tightly clumped configuration as they do in Pisum. Instead they tend to form into a rather loose, "brush-heap", type of arrangement from which metaphase orientation takes place.

After a fifteen minute treatment with colchicine the same general results as seen in Pisum were observed: arrested anaphases, bridged and unusually close telophase nuclei, and the tendency of the metaphase chromosomes to remain in the haphazard prometaphase heap and to pass into anaphase and telophase in place. Unipolar telomorphs were produced as an immediate result of the treatment. Binucleate interphases and interphases with bridged nuclei were also soon observed. At thirty minutes to one hour after treatment slightly scattered metaphases in which the chromosomes were approximately normal length were seen. At one and two hours after treatment there was a sharp decline in the number of telophases. From three to six hours numerous highly scattered metaphases and anaphases and many multinucleate telomorphs were seen. Few unipolar telomorphs were noted after three hours. At six and seven hours normal mitosis was observed in considerable quantity and by eight hours normal mitotic conditions prevailed. Checks made at twenty-four and forty-eight hours revealed very little mitotic activity, many polyploid cells, countless micronucleate cells and equally numerous microcytes. See Plate III for illustrations representing the kinds of abnormalities found in Allium.

In summary, the colchicine effect upon Allium was about the same as it was on Pisum. It differed essentially in that prometaphases were not so tightly clumped and therefore relatively fewer tightly organized postmetaphase abnormalities were found.

DISCUSSION

Colchicine and Polyploidy

The time-honored concept of the cytological effect of colchicine has been that its action is due to a specific effect, in any of several possible ways, upon the spindle. The end result after an inactivation of the function and formation of the spindle has generally been agreed to be the production of polyploid cells. Spindle impairment can occur by the effect of colchicine on either the achromatic spindle or, the kinetochores or both. When both components of the spindle are impaired, the chromatids resulting from the cleavage of the metaphase chromosomes fail to separate into two equivalent nuclei and pass instead into a telophase stage in which the nucleus is tetraploid. The presence of abnormal telophases and interphases, other than polyploid ones, has been regarded as a sign of partial spindle activity either during colchicine treatment or in the early stages of recovery. Multinucleate cells and microcytes, which commonly accompany polyploidy in tissues subjected to the action of colchicine, have been explained to have arisen from the presence of spindle activity that was only partially organized.

The general conclusions of earlier workers are, in part, substantiated by the present investigations. The immediate effect of colchicine is the inactivation of the spindle mechanism so that prometaphase chromosomes remain passively in the clump typical of that

stage and pass, in situ, into metaphase and postmetaphase. Thereby, tetraploid nuclei are formed. In cells which had a functional spindle at the beginning of treatment the spindle is destroyed and anaphase movement is stalled. Chromosomes organized at a metaphase plate undergo cleavage and a mutual repulsion of kinetochores but are reconstituted into a single, though diffuse, tetraploid nucleus. Later anaphases are stalled, cytokinesis does not occur and a binucleate cell is formed. If the anaphase nuclei are still connected to one another by longer chromatid arms at the time spindle function is impaired the nuclei remain connected to one another and form bridged, dumbbell-shaped and otherwise irregular telophase and interphase nuclei. Bhaduri (1939), Cornman (1942) and Hindmarsh (1952) have also arrived at the conclusion that colchicine impairs the function of spindles already formed at the time of treatment. However, Bhaduri and Hindmarsh felt that the stalled anaphases later tended to fuse to each other. Shimamura (1939) also reported seeing fusing nuclei. From the present results it seems highly improbable that fusing takes place.

The results also show that during the first four hours after the end of treatment the tendency for chromosomes to remain associated in the prometaphase clump was prolonged and intensified. There is, furthermore, a tendency for the chromosomes to remain blocked at morphological metaphase. As a result of the accumulation of metaphases the mitotic index is seen to go up. The prophase index, meanwhile, responds indifferently to the mitotic index, indicating that there is no corresponding increase in the absolute number of

cells entering division. Likewise, there is a reduced number of nuclei entering into telophase. The relationship of the metaphase index to the mitotic index and the prophase and telophase indices shows rather convincingly that there is a real accumulation of metaphases. It was shown by Guttman (1952) that in Allium the mean duration of metaphase was quadrupled during colchicine treatment. It is quite evident that colchicine has a similar effect on metaphase in Pisum. The condition is apparently related to a delay in kinetochore cleavage caused by a paralyzing action upon the kinetochore by the drug. The inactivation of the kinetochore might also be related to the tendency of the chromosomes to remain in the prometaphase clump.

In evaluating the importance of blocked metaphases it is important to note that the accumulation occurs while the metaphases are almost wholly represented by balled and clumped associations. No scattered c-pairs are present until the end of four hours. Of equal importance is the fact that, although metaphase is blocked, the lag between metaphase and anaphase is eliminated at four hours and furthermore that the anaphases are analogous in chromatid association to the metaphases. Finally, although there is a lag in telophase formation, the telophases that occur during the period are largely of the unipolar type that arise from analogous earlier stages. After four hours the metaphase index shows a sharp decline which is directly related to a corresponding decrease in the mitotic and anaphase indices. Contrarily there is rise in the telophase index indicating that the accumulated metaphases are passing into interphase.

Further evidence for the blocking effect of colchicine is provided by a comparison of the present results with the mean durations of the stages of mitosis in Pisum as established by Brown (1951). According to Brown's determinations the durations at 25° C. were: interphase 17.0 hours; prophase 1.3 hours (78.0 minutes); metaphase 0.24 hours (14.4 minutes); anaphase 0.07 hours (4.2 minutes); telophase 0.22 hours (13.2 minutes). The almost complete absence of telophases at fifteen and thirty minutes of recovery after a fifteen minute treatment shows that the blocking of metaphase is almost 100% effective at those times. Although not convincingly shown by the present results there is an indication that the duration of metaphase and anaphase has almost been tripled. Normally the duration from the beginning of metaphase to the beginning of telophase is about nineteen minutes. After treatment the peak for clumped metaphases occurs forty-five minutes before the peak of unipolar telomorphs.

The mean duration of the mitotic cycle from the beginning of prophase to the end of telophase was shown by Brown to be somewhat less than two hours (109.8 minutes). Even if the duration of the mitotic cycle were doubled by colchicine, as suggested for Allium by Guttman, practically all cells which were in prophase at the beginning of treatment should have passed through mitosis by the end of four hours.

In summary, it appears that there is a stalling of metaphases for about four hours after treatment and that the stall occurs while the chromosomes are associated in the clumped configuration which is normally found in prometaphase. These blocked and clumped metaphases finally pass into anaphase and telophase in situ and polyploid

interphases are formed.

Colchicine and Scattered Mitosis

As was mentioned earlier in the discussion there was little evidence during the first four hours of highly scattered metaphase chromosomes. From five to six hours, however, scattered configurations became very common in both metaphase and anaphase and there was simultaneously an increase in the proportion of multipolar telomorphs. Scattered c-pairs have often been considered indicative of the c-mitotic (*i.e.*, the polyploidizing) effect of colchicine and other substances. This conclusion is unwarranted from the present results for several reasons: First, it was shown that the prophase and postprophase chromosomes present at the beginning of treatment were the source of polyploid nuclei. Secondly, it appears that the appearance of highly scattered metaphases occurs when the proportion of multipolar telomorphic activity is seen to be increasing.

From these observations it was obvious that the origin of scattering must be related to an effect by colchicine upon the interphase nuclei. The logical point at which the effect is probably initiated appears to be at the "antephase", which has been described by Bullough (1952, p. 145) as,

. . . undoubtedly the most sensitive phase in the entire course of a division, although there is evidently a second sensitive point in the metaphase. Almost all the known mitotic inhibitors act primarily on one or the other of these phases, though some act equally well on both.

The effect of colchicine upon antephase is also shown in another manner. From four to six hours after treatment there was an increase

in the absolute number of cells entering into mitosis. This increase was attributed to a stimulatory effect by colchicine on the rate of mitosis. The cells entering into division during the stimulated period are not all aberrant; there is also an increasing trend toward normal mitosis. From the mixed population of normal and abnormal configurations it appears that perhaps the latest antephasis give rise to scattered mitosis and the earlier antephasis are stimulated to enter division sooner than they would normally.

In summary, scattered mitosis and the increase in the number of cells entering into division are caused by the action of colchicine upon the antephasis and are therefore preprophase effects.

The Relationship of Colchicine Effects to the Mitotic Mechanism

The immediate suppression by colchicine of the spindle is an effect that has often been described. It has been explained in many ways, the consensus of opinion being that colchicine somehow changes the submicroscopic structure of the achromatic components in such a way that the formation of spindles ceases and the function of spindles already formed is impaired. Thus, after kinetochore cleavage, the chromatids fail to undergo normal anaphase movement. The morphological development of the chromatids continues and polyploidy is the inevitable result.

The effect of colchicine upon the kinetochores of the chromosomes and upon the centers which seem to direct kinetochore behavior is not as well explained. From the results of the present studies it appears that colchicine exerts an equally important effect upon

these two, and that the effect results in a very different sequence of events than those arising from the failure of the achromatic sphere.

The tendency for metaphase chromosomes to remain in the prometaphase clump is probably due to a paralysis of the kinetochores, as well as to the absence of a spindle. The delay in kinetochore cleavage, which accounts for the accumulation of metaphases, is another result of an effect by colchicine upon the kinetochores.

The presence of scattered metaphases probably comes about as a result of the inactivation of the kinetochores during the antephase. By paralyzing the kinetochores even before prophase begins colchicine prevents the chromosomes from participating in the normal prophase to prometaphase movement cycle. Thus, the contraction of the chromosomes to morphological metaphase occurs more or less about the periphery of the nucleus. Over-contraction occurs as long as there is a corresponding delay in the separation of the chromatids. The degree of scattering of the metaphase chromosomes probably depends upon a variety of factors. There may be mutual repulsion of the highly contracted chromosomes much as in diakinesis. The uncoiling and contraction of the chromosomes while they are under the influence of neither kinetochore nor centers would also tend to propel them at random within the cell, especially after the confining nuclear membrane has disappeared. Finally, if the suggestion earlier made that tensions due to differential contraction between segments of a chromosome arm has any validity, the forces involved may also tend to pull the chromosomes in an effort to

neutralize the strain set up in the zone of tension. As a result the chromosomes would yield and move at random since the controlling influences of the kinetochores and the centers are absent. By postulating that scattered metaphases arise from the lack of prophase movement to prometaphase and with the presence of several non-kinetochore forces inherent in the chromosomes themselves there is no further need to attempt to derive them by explosive movements of clumped metaphase chromosomes.

The present results showed that highly scattered metaphases invariably led to the production of micronucleate telomorphs. After a long delay, cleavage of the kinetochores occurs and there appears to be a slight but distinguishable repulsion between homologous chromatids very similar to the repulsion that occurs in normal anaphase before organized movement becomes effective. After a momentary repulsion the kinetochores seem to exhibit an attraction for each other. Levan (1938) stated that the presence of such anaphases was due to a partial spindle recovery. Bowen (1953) reported finding similar anaphases during extended treatment when the presence of any kind of spindle would be highly unlikely. It seems to be more acceptable to consider these anaphases as "star anaphases" in the sense that the term was originally proposed (Wilson, Tsou and Hyypio 1952).

The significance of the mutual attraction of two or more kinetochores after chromatid formation is that it illustrates another clue to the nature of the kinetochore. After a short period of mutual repulsion between sister chromatids (which seems to be displayed already by the overcontracted scattered metaphase

chromosomes) the kinetochores are attracted to each other during anaphase. This fact suggests that the organization of a normal anaphase is, in part, due to a similar kinetochore attraction which is held in check by the controlling influence of the achromatic figure.

In summary, colchicine destroys the achromatic component of the spindle, paralyzes the kinetochores and centers so that they have no control over the movement of prophase chromosomes. The development of morphological prophase is not hindered and the forces inherent in the chromosomes themselves account for the scattered distribution of the metaphase chromosomes. Colchicine does not destroy the natural affinity of kinetochores for one another. After a short period of mutual repulsion between sister chromatids the kinetochores of two or more chromatids show a mutual attraction for each other.

Concluding Remarks

The present studies have produced some enlightening information on the cytological effects of colchicine. The technique employed was chiefly responsible for the type of information that was obtained. Short treatments are not a new idea; they have been used with considerable success by Levan (1938), Bhaduri (1939), Cornman (1942) and recently by Hindmarsh (1952). These four workers have contributed more by their short-time studies to the knowledge of the colchicine effect and its relationship to the mechanism of mitosis than all the others who have worked with the drug. The

pitfall of most workers has been that they overlooked the early effects by waiting until the appearance of scattered metaphase chromosomes before making their observations (Appendix A). Naturally, they found polyploidy associated with all the other effects that colchicine is capable of causing and came to the most logical conclusion: that colchicine inactivated the spindle mechanism producing scattered chromosome configurations and when the scattered chromosomes clumped or the anaphase and telophase nuclei fused polyploid interphases were formed.

The present results have shown that colchicine is a much more complex antimitotic agent than has been generally supposed. It is c-mitotic to the extent that it suppresses spindle formation and function in cells already in division at the time of treatment. It is also a preprophase poison because it prevents the movement of prophase chromosomes to their normal prometaphase position.

Because of its preprophase effect colchicine is not an efficient polyploidizing agent. The ideal agent would be one which prevented only anaphase movement but allowed the prophase chromosomes to congregate at the prometaphase position where they would pass into all the later phases in situ. It appears that when colchicine is being used as a polyploidizing agent the best results would be obtained by relatively short treatments. Thus, a maximum number of polyploid cells would be produced by a minimum amount of effect upon preprophase.

SUMMARY

1. The Pisum test was used to determine the qualitative and quantitative effects of colchicine on mitosis. Root tips of Pisum sativum were exposed to a solution of 1000 ppm colchicine for fifteen minutes after which they were washed and returned to recover in nutrient solution. Cytological observations were made at zero hour, at the end of treatment, at fifteen and thirty minutes, and at one hour and hourly intervals thereafter until normal mitosis had returned. Twenty-four and forty-eight hour samples were also observed.
2. Mitotic indices were determined for normal and colchicine affected mitosis. It was found that colchicine increased the duration of metaphase during the first four hours after treatment and stimulated the frequency of mitosis by increasing the number of prophases entering division at five and six hours after treatment.
3. The immediate effects of colchicine stalled chromosome movement at the prometaphase clump and resulted in the formation of unipolar telomorphs which became polyploid interphase nuclei. Early anaphases were arrested and also formed into polyploid nuclei. Stalling of later anaphases and the failure of cytokinesis resulted in the formation of binucleate cells in which the nuclei were often bridged.
4. The period of recovery from fifteen minutes to four hours was

characterized by a continuation and intensification of the tendency of the chromosomes to remain in the prometaphase clump.

There was also an appearance of scattered metaphases, anaphases and multipolar telomorphs.

5. The five to eight hour stages of recovery saw a rapid decrease in the effects that had persisted since the beginning of treatment. There was an increase in the number of scattered figures and multipolar telomorphs. A gradual return to normal mitosis took place and by eight hours after treatment the frequency of abnormalities was at near normal levels.
6. At twenty-four and forty-eight hours normal mitotic conditions prevailed. Many interphases showed abnormalities which had originated during their formation under the influence of colchicine. Binucleate, polyploid, and multinucleate cells and microcytes were seen.
7. Polyploidy originates when the chromosome movement cycle is blocked at the prometaphase clump by the lack of an achromatic spindle and the inactivation of the kinetochores.
8. Scattered mitosis and the increase in the number of cells entering division at five and six hours after treatment are caused by an action upon antephase and are, therefore, preprophase effects.
9. Colchicine inactivates the kinetochores and centers so that they have no control over the movement of prophase chromosomes. The morphological development of prophase is not hindered and the forces inherent in the chromosomes themselves account for the scattered distribution of the metaphase figures. The delay in

cleavage of the metaphase chromosomes is due to the inactivation of their kinetochores. Colchicine does not destroy the natural affinity of the kinetochores at anaphase for each other. After a short period of mutual repulsion between sister chromatids the kinetochores of two or more show a mutual attraction for one another.

10. Qualitative observations made on Allium served to confirm the results obtained with Pisum.
11. The complex cytological effects of colchicine make it an inefficient polyploidizing agent.
12. The normal mitotic cycle in Pisum is described in terms of the movement of chromosomes during cell division.

PLATE I

Mitotic Stages from Untreated Pisum sativum

- Fig. 1 Interphases
- Fig. 2 Early prophases
- Fig. 3 Midprophase
- Fig. 4 Late prophase
- Fig. 5 Late prophase, prometaphase and early prophase
- Fig. 6-7 Early prometaphases
- Fig. 8 Prometaphase
- Fig. 9 Prometaphase with lagging chromosome
- Fig. 10 Prometaphase and early anaphase
- Fig. 11 Early metaphase and prometaphase
- Fig. 12 Early and late prophase; early metaphase
- Fig. 13-14 Metaphase
- Fig. 15 Early prophase and anaphase
- Fig. 16 Telophase with two lagging chromatids

Each division of the scale represents ten microns

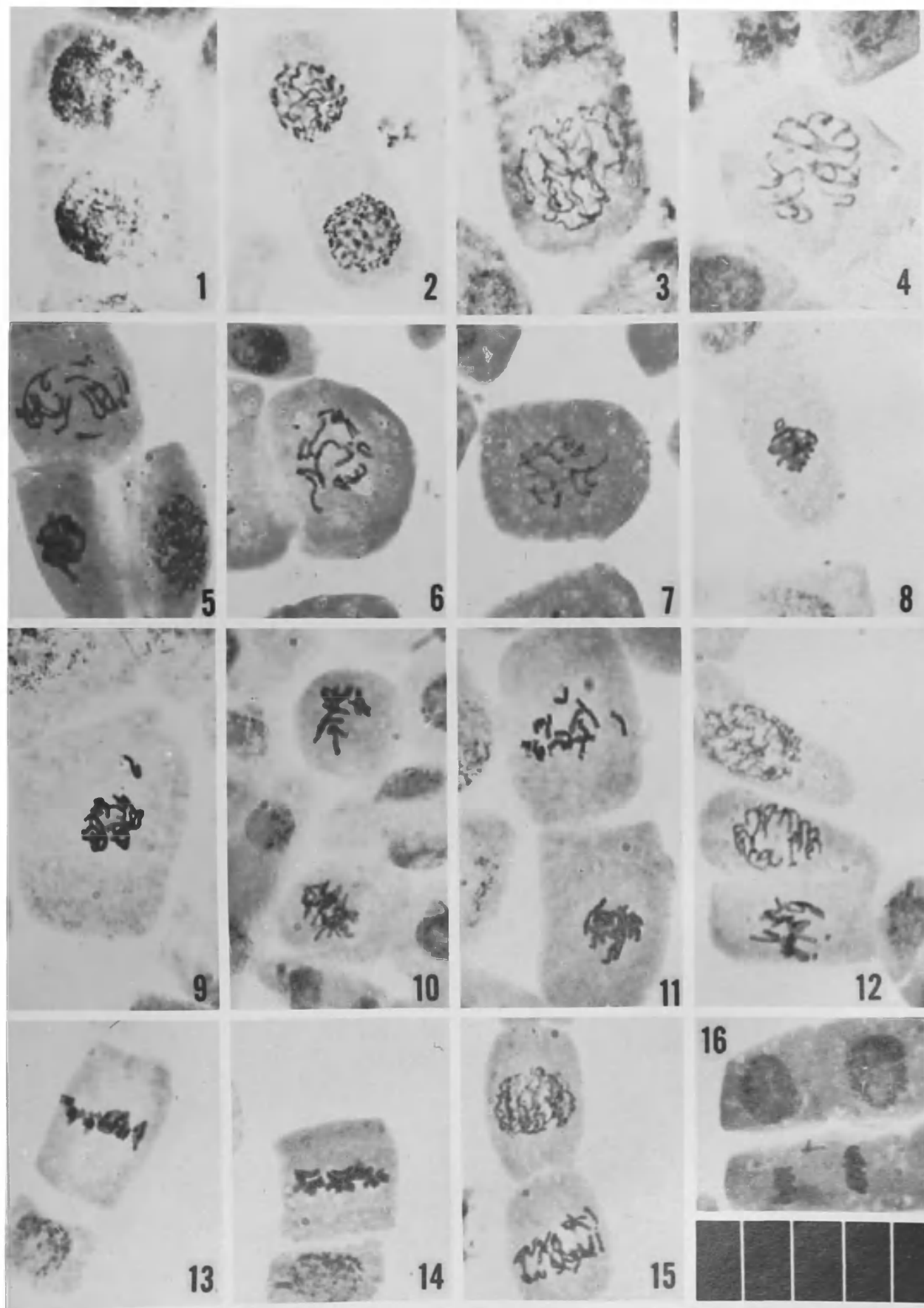


PLATE I

PLATE II

Effects of Colchicine on Pisum sativum

Mitoses from material treated with 1000 ppm colchicine solution
for fifteen minutes

- Fig. 1 Ball anaphase and ball metaphase at four hours recovery
- Fig. 2 Unipolar telomorph at one hour recovery
- Fig. 3 Arrested anaphase at thirty minutes recovery
- Fig. 4 Binucleate interphase at four hours recovery
- Fig. 5 Slightly scattered metaphase at six hours recovery
- Fig. 6 Slightly scattered metaphase at three hours recovery
- Fig. 7 Slightly scattered anaphase at fifteen minutes recovery
- Fig. 8 Slightly scattered metaphase and analogous anaphase at eight hours recovery
- Fig. 9 Severely scattered metaphase at eight hours recovery
- Fig. 10-11 Severely scattered anaphases at eight hours recovery
- Fig. 12 Multinucleate telomorph at eight hours recovery
- Fig. 13 Clumped metaphase showing attractions between kinetochores and telomeres, thirty minutes recovery
- Fig. 14 Metaphase with abnormal plate at six hours recovery
- Fig. 15 Multipolar anaphase showing true spindle activity at six hours recovery
- Fig. 16 Multinucleate interphase at six hours recovery

Each division of the scale represents ten microns

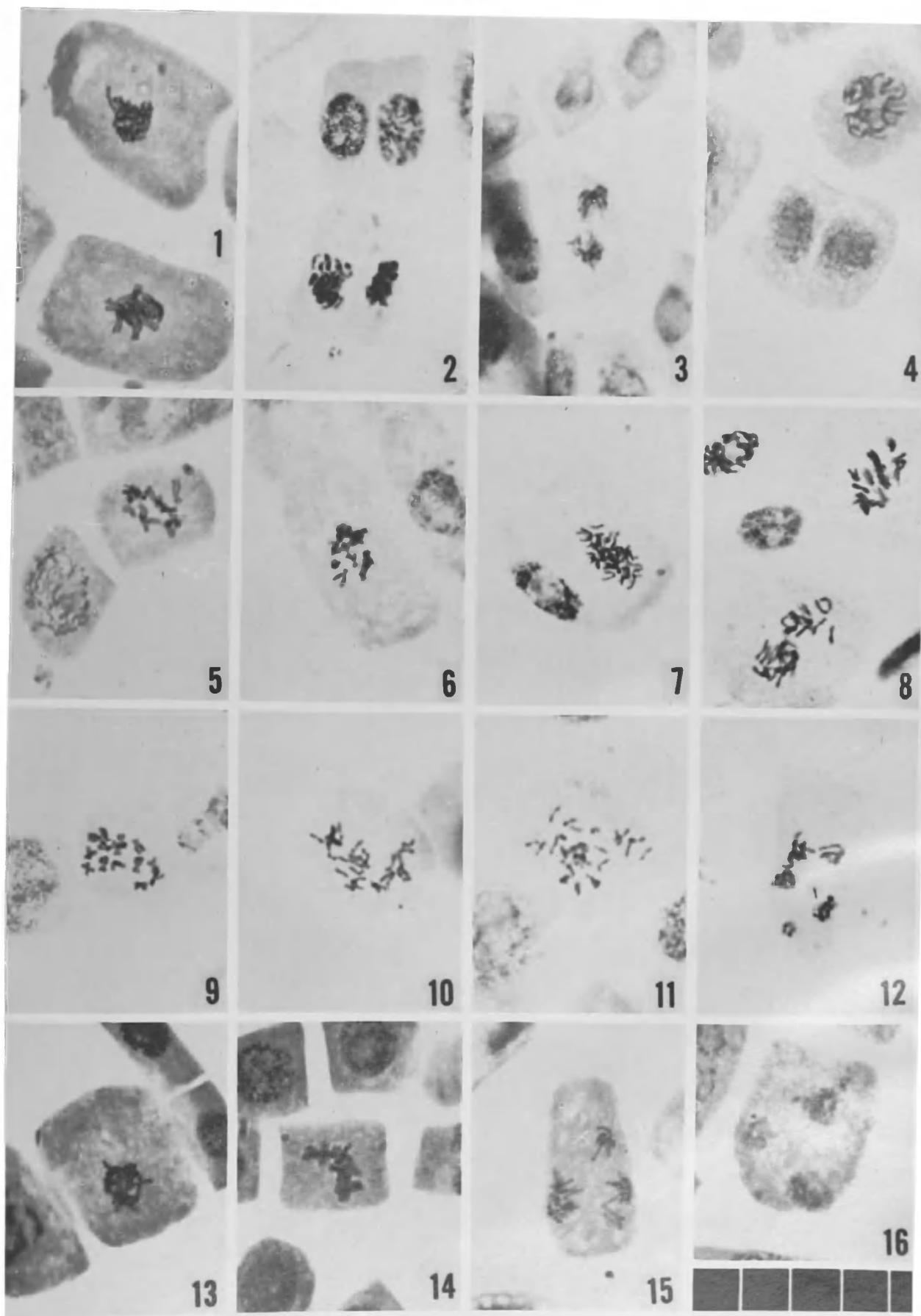


PLATE II

PLATE III

Effects of Colchicine on Allium cepa

Mitoses from material treated with 1000 ppm colchicine solution
for fifteen minutes

- Fig. 1 Arrested anaphase at fifteen minutes recovery
- Fig. 2 Early telophase with bridge at fifteen minutes recovery
- Fig. 3 Late telophase with bridge at fifteen minutes recovery
- Fig. 4 Normal prometaphase at fifteen minutes recovery
- Fig. 5 Ball prometaphase at fifteen minutes recovery
- Fig. 6 Unorganized metaphase at one hour recovery
- Fig. 7 Clumped anaphase at forty-five minutes recovery
- Fig. 8 Clumped "star" anaphase at end of treatment
- Fig. 9 Unipolar telomorph at fifteen minutes recovery
- Fig. 10 Polyploid interphase at twenty-four hours recovery
- Fig. 11-12 Scattered metaphases at three hours recovery
- Fig. 13 Scattered anaphase at three hours
- Fig. 14 Anaphase showing attraction of kinetochores at four hours
 recovery
- Fig. 15 Multinucleate telomorph at four hours recovery
- Fig. 16 Multinucleate interphase; one microcyte partly cut off
 at twenty-four hours recovery

Each division of the scale represents ten microns

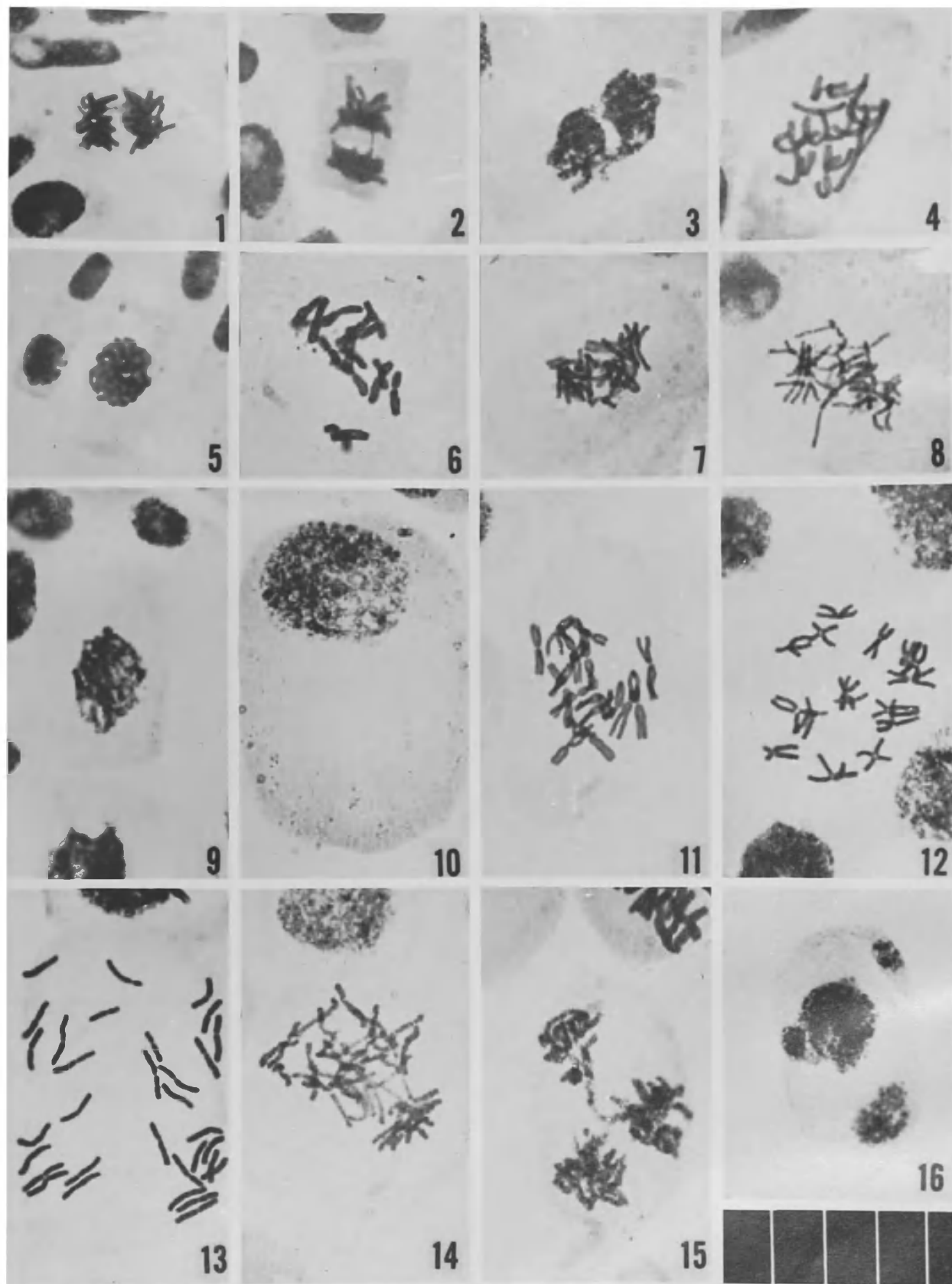


PLATE III

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APPENDIX A

A SUMMARY OF DURATIONS OF TREATMENT WITH COLCHICINE

- Nebel and Ruttle (1938) living stamen hairs of Tradescantia reflexa
Treatment: 1 to 24 hours
Observations: Unspecified, but 90 min. showed spindle failure
- Levan (1938) Allium cepa and A. fistulosum root tips
Treatment: 7, 15, 30 minutes, 1, 2, 24 and 72 hours
Observations: 0, 15, 30 minutes, 1, 3, 6, 12, 24, 48, and 72 hours after end of treatment
- Beams and King (1938) Triticum vulgare root tips
Treatment: 30 minutes up to 12 hours
Observations: immediately and at intervals not specified
- Shimamura (1939) Allium cepa root tips
Treatment: 2 hours followed by 1 hour of washing
Observations: after washing, at 1, 2, 3, 4, 12 and 24 hours
- Bhaduri (1939) Vicia faba root tips
Treatment: 5, 10 minutes, $\frac{1}{2}$, 1, 3, 6, 12, 24, 96 hours, 4 and 7 days
Observations: 3, 5, 10, 15 minutes, $\frac{1}{2}$, 1 hour and so forth up to 48 hours, 4, 7, and 15 days
- Cornman (1942) Colchicum byzantinum root tips
Treatment: $\frac{1}{2}$, 1, 2, 4, $8\frac{1}{2}$ hours
Observations: at end of treatments and after unspecified recovery intervals
- Hawkes (1942) Allium cepa seedling root tips
Treatment: 6, 12, 24 and 48 hours
Observations: 24 and 48 hours after treatment
- Berger and Witkus (1943) Allium cepa and Spinacia oleracea seedling root tips
Allium cepa
Treatment: 5 and 12 hours
Observations: immediately and at 5 and 24 hours
- Spinacia oleracea
Treatment: 1 and 5 hours followed by $\frac{1}{2}$ to 1 hour of washing
Observations: immediately, and at 5, 24, 32, 48, 53, 63, 66, 69 and 72 hours after treatment
- Hindmarsh (1952) Allium cepa root tips
Treatment: up to 24 hours
Observations: every 5 minutes for first hour, hourly thereafter up to 24 hours

Bowen (1953) Pisum sativum root tips

Treatment: 8 hours

Observations: hourly during treatment

APPENDIX B

APPENDIX TABLE I.

CYTOLOGICAL ANALYSIS OF ZERO HOUR CONTROLS AND COLCHICINE TREATED MATERIAL

	(A) Zero Hour Control	(B) Recovery; 15 min. to 4 hrs.	(C) Recovery from 5 to 8 hrs.	(D) Recovery at 24 and 48 hrs.
Number of Root Tips	25	18	12	8
Number of Division Figures/10,000 Cells	630 $\pm$ 32.5	732 $\pm$ 46.0*	783 $\pm$ 65.8*	396 $\pm$ 39.0**
Number of Divisions in Prophase	375 $\pm$ 21.0	394 $\pm$ 35.1	426 $\pm$ 54.0	213 $\pm$ 21.4
Number of Divisions in Prometaphase	51 $\pm$ 3.6	52 $\pm$ 7.0	53 $\pm$ 4.9	43 $\pm$ 5.2
Number of Divisions in Metaphase	95 $\pm$ 8.0	178 $\pm$ 15.5**	155 $\pm$ 23.3***	69 $\pm$ 7.2
Number of Divisions in Postmetaphase	109 $\pm$ 17.5	108 $\pm$ 13.6	148 $\pm$ 15.5	72 $\pm$ 10.6
Number of Divisions Abnormal	12 $\pm$ 2.5	329 $\pm$ 0.01	163 $\pm$ 64.6	5 $\pm$ 0.9

*indicates no significance; **means significant and *** probably significant.

APPENDIX TABLE II
MITOTIC INDICES FOR ZERO HOUR CONTROLS AND INTERVALS
AFTER COLCHICINE TREATMENT

<u>Time of Collection</u>	<u>Mitotic Index</u>
Zero hour	630
End of 15 min. treatment	623
15 min. recovery	630
30 " "	683
1 hr. "	710
2 " "	833
3 " "	703
4 " "	847
5 " "	690
6 " "	933
7 " "	867
8 " "	590
24 " "	362
48 " "	428

APPENDIX TABLE III
PROPHASE INDICES FOR ZERO HOUR CONTROL AND
INTERVALS AFTER COLCHICINE TREATMENT

<u>Time of Collection</u>	<u>Prophase Index</u>
Zero hour	375
End of 15 min. treatment	380
15 min. recovery	408
30 " "	401
1 hour "	403
2 " "	383
3 " "	310
4 " "	408
5 " "	349
6 " "	554
7 " "	485
8 " "	320
24 " "	362
48 " "	428

APPENDIX TABLE IV
SUMMARY OF CLUMPED PROMETAPHASES IN CONTROL AND
RECOVERY MATERIAL

<u>Time of Collection</u>	<u>Number</u>	<u>Percent</u>
Zero hour	8.4	17
End of treatment	23.3	29
15 min. recovery	24.6	79
30 " "	33.0	75
1 hr. "	23.0	62
2 " "	52.0	47
3 " "	30.0	64
4 " "	28.0	53
5 " "	14.3	30
6 " "	15.3	21
7 " "	3.3	4
8 " "	3.7	11
24 " "	0.8	2
48 " "	2.5	7

APPENDIX TABLE V
SUMMARY OF METAPHASE ABERRATIONS

Time	Normal		Clumps		Scatter I		Scatter II	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Zero hour control	92	96	3	4	0.0	0	0.0	0
End of treatment	44	50	44	50	0.0	0	0.0	0
15 min. recovery	0	0	122	96	2.7	4	0.0	0
30 " "	0	0	148	98	5.0	2	0.0	0
1 hr. "	0	0	164	99	3.3	1	0.0	0
2 " "	5	3	210	95	3.3	2	0.0	0
3 " "	0	0	179	97	5.3	3	0.0	0
4 " "	3	0.7	212	89	8.0	4	14.6	7
5 hr. recovery	41	26	85	54	5.3	3	27.0	17
6 " "	61	32	105	56	11.6	6	9.7	5
7 " "	46	72	22	12	3.3	2	24.0	13
8 " "	31	94	5	3	0.0	0	4.7	3
24 hr. recovery	65	94	2.25	4	1.25	2	0.0	0
48 " "	69	94	2.75	4	0.75	2	0.0	0

APPENDIX TABLE VI

SUMMARY OF ANAPHASE ABERRATIONS

Time	Normal		Clumps		Scatter I		Scatter II	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Zero hour control	49	100	0.0	0	0.0	0	0	0
*End of treatment	19	56	4.3	12	4.3	12	0	0
15 min. recovery	3	5	49.0	95	0.0	0	0	0
30 " "	2	4	69.0	96	0.0	0	0	0
1 hr. recovery	0	0	60.0	98	2.0	2	0	0
2 " "	0	0	94.0	100	0.0	0	0	0
3 " "	2	2	119.0	92	2.0	3	2.0	3
4 " "	0	0	81.0	68	11.3	21	5.7	10
5 hr. recovery	25	35	27.0	37	11.0	15	9.3	12
6 " "	23	22	48.0	47	10.3	10	21.0	20
7 " "	62	95	0.0	0	0.0	0	3.0	5
8 " "	58	100	0.0	0	0.0	0	0.0	0
24 hr. recovery	32	100	0.0	0	0.0	0	0.0	0
48 " "	60	100	0.0	0	0.0	0	0.0	0

*at 15 minute recovery the following category also occurred: Arrested, Abs. 6, 18%.

APPENDIX TABLE VII
SUMMARY OF TELOPHASE ABERRATIONS

Time	Normal		Arrested		Unipolar Telomorphs		Multipolar Telomorphs	
	Abs.	%	Abs.	%	Abs.	%	Abs.	%
Zero hour control	62.3	100	0	0	0.0	0	0.0	0
End of treatment	33.0	79	7	17	2.0	5	0.0	0
15 min. recovery	4.0	36	0	0	6.7	64	0.0	0
30 " "	0.0	0	4	40	6.3	60	0.0	0
1 hr. "	0.0	0	0	0	36.6	100	0.0	0
2 " "	0.0	0	0	0	54.0	97	2.7	3
3 " "	0.0	0	0	0	35.6	92	2.0	8
4 " "	0.0	0	0	0	34.4	78	8.0	22
5 hr. recovery	30.3	44	0	0	15.0	35	23.0	21
6 " "	30.0	38	0	0	19.3	24	30.0	38
7 " "	72.0	100	0	0	0.0	0	0.0	0
8 " "	71.0	95	0	0	0.0	0	4.7	5
24 hr. recovery	27.0	100	0	0	0.0	0	0.0	0
48 " "	38.0	100	0	0	0.0	0	0.0	0