THE MITOTIC EFFECT OF TECHNICAL LINDANE (γ-HEXACHLOROCYCLOHEXANE)

By

Te May Tsou Ching

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

Year 1954

Approved _____

ABSTRACT

The main object of this investigation was to study the abnormal cytological behavior induced by the insecticide, technical Lindane, which contains 25% wettable mixture of γ-hexachlorocyclohexane and an inert carrier; in order to explain some aspects of the mechanism of mitosis and some hazards encountered in practical application.

The root tips of onion bulbs and pea seedlings were treated in 0.1% suspension of the insecticide. The mitotic indices of these treated meristemic tissues remained essentially the same as "zero hour controls". The prophase frequencies gradually dropped, metaphase frequencies significantly increased, whereas post-metaphase frequencies remained unchanged in continuous treatments.

This investigation further suggested that there are at least two more or less independent components involved in spindle function: 1. a dipolar cytoplasmic orientation possibly characteristic of the cell at any stage, and 2. a nuclear component inherent in and directed by the chromosome or the kinetochore thereof. The insecticide is capable of inhibiting both components, thus inducing so-called "c-mitosis".

The immediate effects of the insecticide upon mitotic stages are contraction of chromosome, possibly breakdown of nuclear membrane, partial inhibition of "spindle function" and failure of cytokinesis. As treatment is prolonged, over contraction of chromosomes and complete destruction of the spindle mechanism occurs.

Inositol antagonism and radiomimetic effects of this insecticide have not been demonstrated in this investigation, thus further research is desirable. While Lindane is theoretically a good polyploidizing agent, there are a number of practical difficulties which reduce its potentiality below that of colchicine.

The final products of atypical mitosis induced by the insecticide are polyploid cells and aneuploid microcytes. The former are giant cells, but possess a much slower growth rate than that of normal ones. The latter usually tend to be less viable. Therefore the treated meristemic tissues are much reduced as far as number of cells and rate of growth are concerned.

The insecticide also causes "c-tumour" in the enlargement zone of treated root-tips. Most workers believe that this effect is independent of "c-mitosis" and probably is due to disturbance of hormone polarity at the cellular level. This disturbance causes abnormal growth, such as swelled shoot and root apex, twisted parts, lack of root hairs, distorted leaves, etc. The precise, correlated normal physiological balance of some sensitive plants is definitely affected, when they are grown in soil containing the insecticide either as soil treatment or residue of spary.

BIBLIOGRAPHY

Boswell, V. R. 1952. Residues, Soils, and Plants. Insects. 1952 Year Book of U.S.D.A.:284-297.

D'Amato, F. 1949. Sull'impiego Del Gammesano Come Agente Poliploidizzante. (Use of gammexane as a polyploidizing agent) Caryologia 1(2):209-222.

Kostoff, D. 1949. Atypical Growth, Abnormal Mitosis, Polyploicy and Chromosomal Fragmentation Induced by Hexachlorocyclohexane. Nature 162:845-846.

Nybom, N. and B. Knutsson, 1947. Investigation on c-mitosis in Allium Cepa. Hereditas 33:220-243.

Östergren, G. and A. Levan, 1943. The Connection Between c-mitotic Activity and Water Solubility in Some Monocyclic Compounds. Hereditas 29:381-443.

Scholes, M. E. 1953. The Effect of Hexachlorocyclohexane on Mitosis in Roots of Onion and Strawberry (Fragaria Vesca). Jour. Hort. Sci. 28(1):49-68.

Wilson, G. B., M. E. Hawthorne, and T. M. Tsou, 1951. Spontaneous and Induced Variations in Mitosis. Jour. Hered. 42:183-189.

_____, T. M. Tsou and P. Hyypio, 1952. Variations in Mitosis. II. The Interrelation of Some Basic Deviations. Jour. Hered. 43:211-215.

THE MITOTIC EFFECT OF TECHNICAL LINDANE (γ-HEXACHLOROCYCLOHEXANE)

By

Te May Tsou Ching

A DISSERTATION

Submitted to the School of Graduate Studies of Michigan
State College of Agriculture and Applied Science
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1954

Te May Tsou Ching

Candidate for the Degree of

Doctor of Philosophy

Final Examinations: May 18, 1954, 10-12 A.M. Botany Seminar Room

Dissertation: The Mitotic Effect of Technical Lindane (γ-hexachlorocyclohexane)

Outline of Studies:

Major Subject: Botany (Cytology)

Minor Subject: Zoology

Biographical Items:

Born: January 9, 1923, Soochow, China.

Undergraduate Studies: National Central University China, 1940-44

Graduate Studies: Michigan State College, 1949-54

Experience: Teaching Assistant - National Central University, 1944-47

Graduate Assistant - Michigan State College, 1951-54

Member of: Genetic Society of America

Sem. Bot.

Society of the Sigma Xi

Xi Sigma Pi Fraternity

ACKNOWLEDGMENTS

The writer wishes to give her sincere gratitude to her colleagues in the cytology group at Michigan State College. Their encouragement and discussion have made this investigation gay and interesting.

To the leader of the group, Dr. G. B. Wilson, the writer is especially indebted for his advice pertaining to the study and for his assistance in the preparation of the manuscript. To Mr. Philip Coleman, thanks are extended for his invaluable photographic work.

Appreciation is extended to the All College Research Committee for defraying many of the research expenses, as well as to the Agricultural Experimental Station of Michigan State College for a Graduate Research Assistantship which the writer held during her final year of graduate work.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
MATERIALS AND METHODS	5
I. The Insecticide	5
II. Experimental Procedure	6
III. Cytological Examination	8
OBSERVATIONS	10
I. Variation in Mitotic Effects With Duration	10
II. Aberrations	27
III. Comparison of Results	33
IV. The Abnormal Mitosis Induced by Technical Lindane	36
DISCUSSION	41
I. Mitotic Aspect	41
II. Chromosomal Fragmentation	44
III. Polyploidizing Effect	45
IV. Inositol Antagonism	46
V. Difference Between Lindane and Colchicine	47
VI. Explanation of Phyto-responses	50
SUMMARY	56
BIBLIOGRAPHY	58
PLATES	63
APPENDIX	68

INTRODUCTION

Numerous reports have been written concerning the phytotoxicity of the insecticide, technical Lindane or technical benzene hexachloride, since the insecticidal value of the chemical was verified by Slade (1945). Sensitivity to the insecticide differs in different species, even the same varieties of plants react differently at different ages, locations or by different handling methods (Boswell, 1952; Ashby, 1950). Thus the phytotoxicity of any insecticide is a representation of the balanced effect of all factors involved in field work; such as genetic potentiality of the plant in question; the soil; the climate; and the method of treatment, etc..On the whole, the phytotoxicity of this insecticide may be summarized as follows (Boswell, 1952; Cullinan, 1949; Ashby, 1950; Greenwood, 1949; Stitt and Evanson, 1949; and Stoker, 1948):

1. Seeds sown in soil, which contains the insecticide either as soil treatment or as residue from previous spray were injured by the insecticide and showed reduced germination rate. Sometimes germination was not affected but emergence from the soil was.

2. When the insecticide was present in the soil in appreciable amounts, plants showed a definitely reduced rate of growth, reduced total growth and decreased yield (as of seed or fruit).

3. If a spray method was used, the young leaves were easily scorched or distorted by the insecticide. Young vegetable seedlings and orchard trees were often injured by spray of high concentration.

4. The plant grown in soil containing the insecticide usually had a

poorly developed root system; the roots were numerous, short, stubby, thickened, and virtually without root hairs. Thus root vegetable crops were definitely damaged by technical Lindane.

5. The edible crops were often off-flavored by the presence of technical Lindane in soil or as a spray. The mild flavored vegetables such as peas, beans, lettuce and potatoes expecially were tainted, but altered taste was not obvious in strong flavored onion or radishes.

If technical Lindane is used in a soil treatment or spray for above-ground parts, it eventually accumilates in the soil. Since it is insoluble in water, it remains quite stable in the soil and little or no decomposition occurs. Owing to the volatility of the chemical present in the insecticide and to its absorbtion by organisms in soil, the insecticide decreases in amount. Some workers reported that every year the accumulated amount decreased about 10 percent, if no additional application was made (West and Campbell, 1950).

The pure chemical has been used for theoretical study by Östergran and Levan (1945). They stated that it caused colchicine mitosis or c-mitosis, and that the c-mitotic property is related to water solubility and the relationship is an inverse one. In addition, there is a positive correlation between c-mitotic activity and lipid solubility.

Nybom and Knutsson(1947) confirmed the above statement and further proved that there is a negative correlation between the melting point of various isomers and their c-mitotic activity. They also started investigation of the insecticide "666" which causes essentially the same mitotic

effects as benzene hexachloride.

Kostoff (1948 and 1949) reported that atypical growth, abnormal mitosis, polyploidy and chromosomal fragmentation were induced by hexachlorocyclohexane. He applied commercial powder to seedlings of twelve species of angiosperms; Zea mays, Triticum vulgare, T. monococcum, T. compactum, Secale cereale, Setaria italica, Panicum miliaceum, Helianthus annus, Crepis capillaris, Vicia faba, V. sativa and Brassica nigra. Suppressed growth and thickened shoot and root apexes as well as cytological aberrations resulted. Furthermore, he pointed out that the chemical is a cheap polyploidizing agent. Reo and Kundu (1949) came to the same conclusion after they treated root tips of Corchorus capsularis with gammexane.

More intensive work has been done by D'Amato (1949). By immersing root tips or shoot tips of seedlings of twelve species of angiosperms in dilute ethanol solution of pure gammexane, polyploid cells were obtained. However, germination of seeds in supersaturated solution did not induce polyploidy in either root or stem tips.

Practical application of the insecticide as well as a cytological study of the effects were carried out by Scholes (1955) on onion seedlings with the insecticide. She stated that the frequency of nuclear division was unaffected, but low doses sometimes led to exclusion of nuclear fragments in subsequent cell division while higher doses led to polyploidy. Strawberry runners rooted in soil treated with the insecticide showed similar cytological derangements.

Owing to the interesting cytological effects of the chemical and

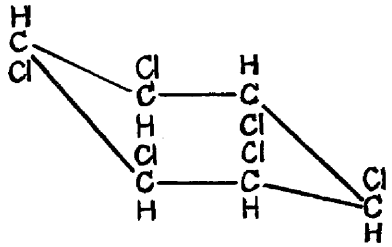
also the physiological disturbances caused by practical application, the insecticide provides a challenge to botanists. Cells are universally known as basic units of organism and any deviation of cellular behavior may cause physiological as well as genetical changes. Thus this insecticide has been selected for research at the cytological level, in order to explain some encountered hazards and if it is possible, to throw some light on one of the basic biological problems, i.e., the mechanism of mitosis.

MATERIALS AND METHODS

I. The Insecticide

The molecular formula of hexachlorocyclohexane or benzene hexachloride is $C_6H_6Cl_6$, theoretically it may have eighteen isomers, but only five have been syntnesized and the names alpha, beta, gamma, delta and epsilon have been given according to their sequence of discovery. The insecticidal property of the chemical was discovered in France in 1941. In the following year, the Labouratory of Imperial Chemical Industries in England verified the fact. Slade (1945) indicated that the gamma isomer is most effective as far as the insecteradicating property is concerned. Since then the compound has been used in Europe and America in various forms and under different commercial names, such as Gammexane, 666, Agrocide 7, Agtocide 3, Hexadow, Hexachlorane, "Lindane" etc..

The structural formula of γ-hexachlorocyclohexane is



The pure chemical is a volatile crystal with a melting point of 112.5° C, insoluble in water and soluble in organic solvents especially in acetone and ethanol. In a weak alkaline solution, it splits off three moles of hydrogen chloride to form a mixture of trichlorobenzenes.

(Shepard, 1951). The insecticidal property of the gamma isomer of this compound may be due to interference with the inositol ($C_6H_6(OH)_6$) metabolism of insects as suggested by Kirkwood and Phillips (1946). Since inositol is a member of the vitamin B complex and also is a common metabolite in animals, and in addition, suppressed the inhibitory effect of γ-hexachlorocyclohexane on growth of yeast and fungi, the suggestion is not an unreasonable one.

The name Lindane is applied to those grades of γ-hexachlorocyclohexane which have a purity of 99 percent or better. The insecticide "Lindane" used in this investigation was obtained through the courtesy of the Dow Chemical Company, Midland, Michigan. It consisted of 25 percent wettable mixture of the chemical and an inert carrier and is commonly used as a general insecticide on plants and animals. Owing to its insolubility in water, the concentration of test solutions was rather difficult to ascertain. Some primary experiments showed that there is no striking cytological difference between 0.01 % and 1.0 % suspensions. On the other hand the filtrate of those suspensions did not show full c-mitotic effect on meristemic cells within a reasonable time. Since only water is used for field work, a water-suspension of 0.1% by weight was selected for tests.

II. Experimental Procedure

The root tips developed from bulbs of the common commercial yellow variety of Allium cepa L., and from young seedlings of Pisum sativum var. Alaska provided the meristemic tissues for the study of the mitotic effects.

The onion bulbs were obtained from a single crop produced by Mr. Emil Pontack of Elsie, Michigan. The pea seeds were furnished by the Ferry-Morse Seed Company, and reported to be fresh stock from a disease-free strain of relative genetic uniformity.

The onion bulbs were rooted in aerated distilled water until the majority of roots were two centimeters in length; four roots from each bulb were excised and designated as "zero hour controls". Then the rooted bulbs were transferred to treatment suspension or aerated distilled water which was used as control.

The pea seedlings were germinated in moist paper towel about two and one-half days. The roots of selected young seedlings, about two to three centimeters long, were inserted in half strength modified Hoagland's nutrient solution (Huskins and Steinitz, 1948) by means of a wax coated metal grid placed on a beaker. Constant aeration and agitation was furnished by bubbling air through capillary openings in the ends of glass tubes inserted into the solution through the grid. The air was obtained from the laboratory service outlets but was first rendered free of dust and oil particles by being passed through a filter consisting essentially of eighty mesh charcoal. After six to twelve hours of nutrient solution treatment, four root tips were collected as "zero hour controls" for each run; the rest of the seedlings were transferred into the test suspension containing quarter strength modified Hoagland nutrient and 0.1 % of "Lindane" by weight. A control run treated only with the nutrient solution was also carried out simultaneously with each test.

Collections were made at intervals up to one hour for short time treatments of onion, up to twenty-four hours for onion and pea continuous treatments. For recovery runs, twenty-four hours were also used. All the experiments were carried out under laboratory conditions, with a room temperature of about 24° C and an average relative humidity of 50 percent.

Excised root tips were fixed in three to one absolute alcohol-glacial acetic acid mixture for 10 minutes at 60° C for pea, and 15 minutes for onion. Hydrolysis in one normal hydrochloric acid for nine minutes for pea, 12 minutes for onion at 60° C was followed by staining by the Feulgen technique. Squash preparations were then dehydrated over night in 95 % ethanol containing a small amount of fast green as a counterstain, and made permanent with Diaphane.

III. Cytological Examination

All examination of slides was done with a 90X oil immersion objective and 12.5X oculars. Critical illumination of the slide was provided by a ribbon filament lamp with a type B green filter between the lamp and the microscope mirror. The phase microscope also was used for checking the presence of nuclear membrane, spindle and cell plate.

Qualitative information concerning mitotic effects was obtained by random checking right after the temporary slides were made. Careful notes were taken on apparent frequency of division figures, types of abnormalities and finally evidences of poisoning effect in both dividing cells and resting nuclei.

Quantitative data were procured by a rigid system. Each slide was examined in horizontal strips in order to obtain a mitotic index and a mitotic formula. The former was based on 1,000 meristemic cells, the latter was calculated upon 100 dividing cells.

Data taken in this investigation are summerized in Appendix tables 1 to 6, and form the basis for all quantitative and statistical analysis of the mitotic effects.

OBSERVATIONS

I. Variation in Mitotic Effects With Duration of Treatment

Any spontaneous or induced variation in normal mitotic activities of a meristemic tissue may be considered as mitotic effects of a certain disturbance. The effects may be classified mainly as changes in mitotic index, mitotic formula and mitotic behavior. The so-called mitotic index is the frequency of mitotic figures per hundred merist-emic cells. The mitotic formula is an expression which is composed of percent distribution of various stages of mitosis. And finally, the mitotic behavior is usually described by deviation from normal stages or phases which are characteristic of the normal mitotic sequence.

1. Onion:

The results obtained from treatments for 15, 30, 45 and 60 minutes showed that the mitotic indices decreased then steadily increased (Fig.2); whereas the control root-tips excised within one hour had an index similar to that of the zero hour controls (Fig. 1). As for long time continuous treatments, the mitotic indices showed a slight drop at the half-hour point followed by a steady increase up to 2 hours;there the index reached a maximum which was maintained through 7 hours treatment, after which it dropped abruptly to normal (Fig. 4). The results of the recovery after one hour treatment were rather erratic, but an ascending tendency was

obvious (Fig. 5). The long time continuous controls were somewhat irregular; the average index, however, was close to that of zero hour controls (Fig. 3).

The mitotic formula of controls remained within the range of the zero hour controls; in other words, the mitotic formula at any time was more or less constant (Figs. 6 and 8). In continuous treatment, the prophase percentage gradually decreased and remained significantly lower than that of controls. The metaphase percentage on the other hand increased rather rapidly and remained definitely higher than that of controls (Figs. 7 and 9). In the materials which were recovered after treatment of one hour, the mitotic formula reached maximum deviation at one hour after the treatment, then gradually approached normal. However, the effect still remained, i. e., the prophase percentages were lower than that of normal, metaphase percentages were higher than that of normal, and postmetaphase remained within normal range (Fig. 10).

The mitotic behavior of treated onion root-tips was definitely different from that of controls. In untreated or control root-tips there were never more than 20 percent of the total of any mitotic stage (Figs. 1 and 3), but metaphase abnomalities quickly jumped to 100 percent within one hour's treatment and remained so provided the drug was not removed. If the drug was removed after one hour, the effect still remained but to a milder degree, and consequently some normal figures gradually appeared as "recovery" proceeded. Postmetaphase reacted similarly but somewhat later. Prophase responded quite rapidly showing some aberration within

one hour's treatment. However, most prophases were normal shortly after the roots were removed from treatment (Figs. 2, 4 and 5).

If one takes the mitotic indices into consideration, the mitotic formula may not be a good means for comparison. Thus it is necessary to put all data on the same basis and analyze them statistically in order to obtain a reasonably complete picture. The frequencies of prophase per 10,000 meristemic cells from 9-14 hours treatment and 1-4 hours recovery after treatment of one hour showed that they were statistically lower than that of normal, otherwise there were no differences from those of normal. The frequencies of metaphase were significantly higher than normal both in treatments and recoveries. As for post-metaphase, only 9-13 hours recovery material showed definitely higher frequencies than normal. The divisional rates were significantly higher than controls both after $\frac{1}{2}$-4 hours treatments and 9-13 hours recoveries at 5 percent level (Table 1).

2. Pea:

A definite drop of mitotic indices in the first six hours was obtained in continuous control pea root-tips followed by an increase at eight hours (Fig. 11). Continuous treatment of pea produced only apparently normal fluctuation of index(Fig. 12). The statistics showed no significant difference in mitotic index between continuous treatments and zero hour controls. The mitotic indices however during the first four hours of continuous control appeared to be decreasing but no difference was found after 5-8

hours (Table 2).

The percent distribution of various mitotic stages in continuous controls were rather close to that of the zero hour controls, but further statistical analysis revealed that the frequencies of prophase in 1-4 hours were significantly lower than that of the zero hour. The frequencies of other stages were not different from the control (Fig. 13, Table 2). In continuous treatments, the frequencies of prophase showed a slight uptrend at one hour then gradually decreased till 6 hours and then increased again. The frequencies of metaphase, on the contrary, went down first then up steadily till 6 hours and dropped again. The frequency changes of post-metaphase were rather insignificant diagramatically and statistically. As for prophase and metaphase, the decrease and increase were statistically significant after 5-8 hours treatment.

The mitotic behavior of continuous controls was very regular, and the abnormality of any stage at any time seldom exceeded ten percent of the total percent of the stage. Prophases had the fewest aberrations and metaphases the highest (Fig. 11). In the case of continuous treatment, the prophases, comparatively speaking, were most inert, while metaphases changed rapidly to 100 percent aberrant within four hours treatment. Post-metaphases also reached maximum aberrations at four hours, but more gradually. In comparison to continuous controls, even prophase showed almost double the abnormalities at any stage of treatment (Fig. 12).

TABLE I

COMPARISON OF CONTROL, CONTINUOUS TREATMENT AND RECOVERY RUNS OF ONION

Time (hr.)	No. of slide	No. of pro-phase per 10,000 cells	No. of meta-phase per 10,000 cells	No. of post-metaphase per 10,000 cells	No. of divi-ding figures /10,000cells
Control	16	261 ± 18.6	69 ± 4.7	116 ± 10.3	446 ± 28.7
Treatment					
½-4	5	270 ± 17.6	218 ± 44.6**	126 ± 21.5	614 ± 55.3*
5-8	4	181 ± 49.2	232 ± 27.9**	137 ± 5.1	550 ± 78.7
9-14	4	140 ± 26.3	142 ± 6.2**	126 ± 32.8	405 ± 38.6
Recovery					
1-4	4	198 ± 10.5**	138 ± 31.7*	151 ± 24.9	488 ± 43.8
5-8	4	287 ± 30.9	134 ± 16.0**	112 ± 21.1	533 ± 67.6
9-13	4	250 ± 36.4	182 ± 30.8**	153 ± 9.7*	585 ± 39.7*

* significant at 5% level

** significant at 1% level

TABLE II

COMPARISON OF ZERO HOUR CONTROL, CONTINUOUS CONTROL AND CONTINUOUS TREATMENT OF PEA

Time (hr.)	No. of slide	No. of pro-phase per 10,000 cells	No. of meta-phase per 10,000 cells	No. of post-metaphase per 10,000 cells	No. of dividing figures /10,000cells
Zero Hour Control	31	388 ± 18.5	123 ± 6.3	131 ± 6.3	631 ± 25.9
Continuous treatment:					
1-4	14	371 ± 37.3	154 ± 2.2	118 ± 33.8	643 ± 59.3
5-8	8	299 ± 37.1*	207 ± 18.8**	104 ± 21.3	606 ± 60.5
Continuous control:					
1-4	8	333 ± 17.1*	110 ± 9.6	120 ± 13.1	563 ± 22.3*
5-8	4	376 ± 69.6	124 ± 16.7	120 ± 9.4	618 ± 84.9

* significant at 5% level

** significant at 1% level

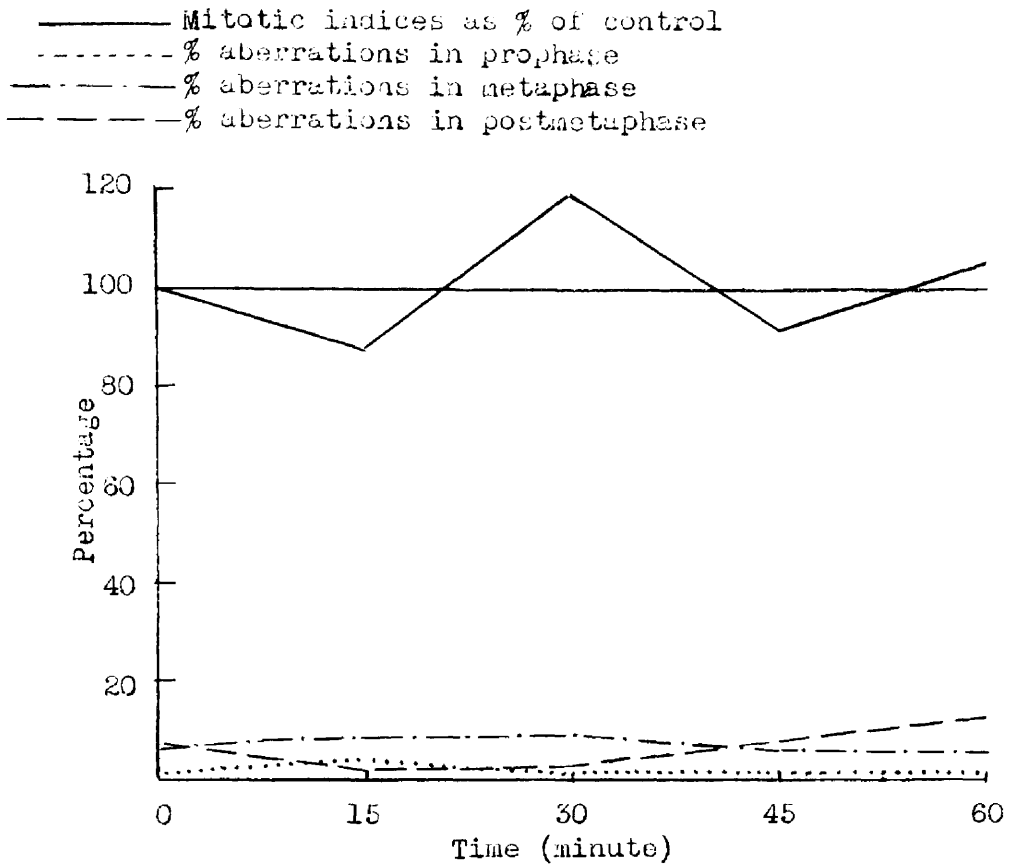


Figure 1. Quarterly Variation of Mitotic Indices and Aberrations in Various Stages in Control Runs of Onion

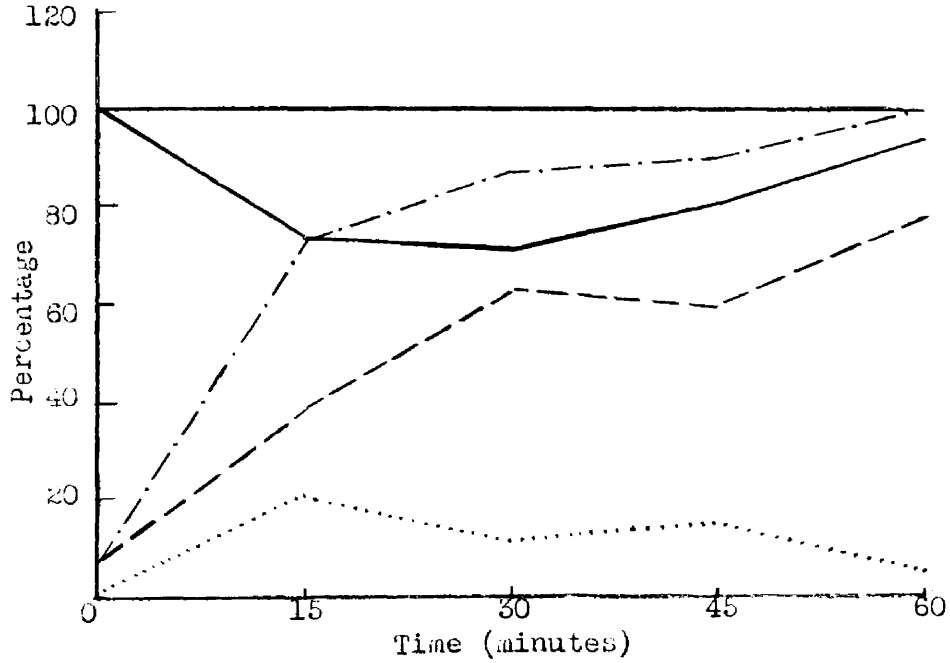


Figure 2. Quarterly Variation of Mitotic Indices and Aberrations in Various Stages in Treated Runs of Onion

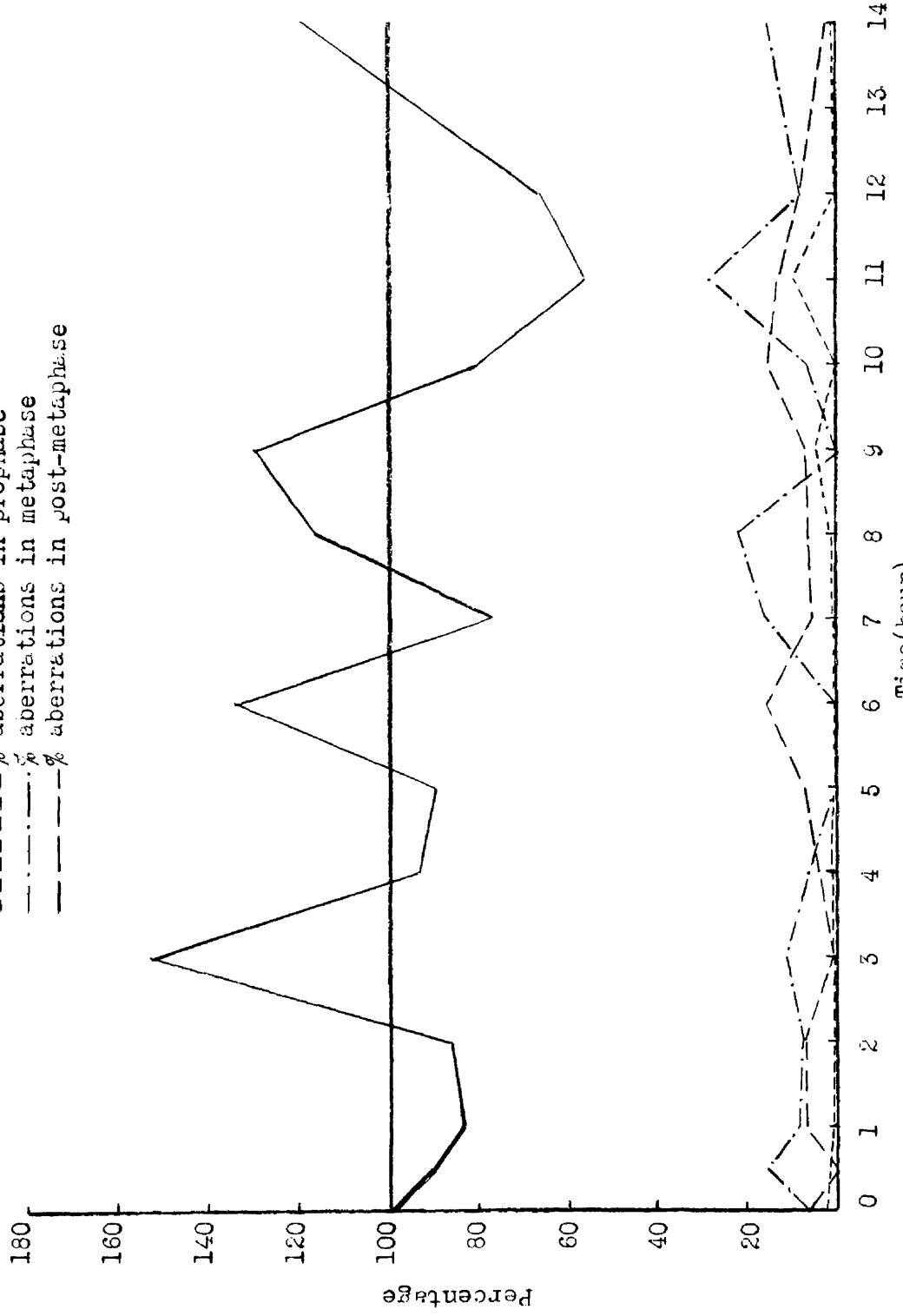


Figure 3. Hourly Variation of Mitotic Indices and Aberrations in Various Stages in Continuous Control of Onion

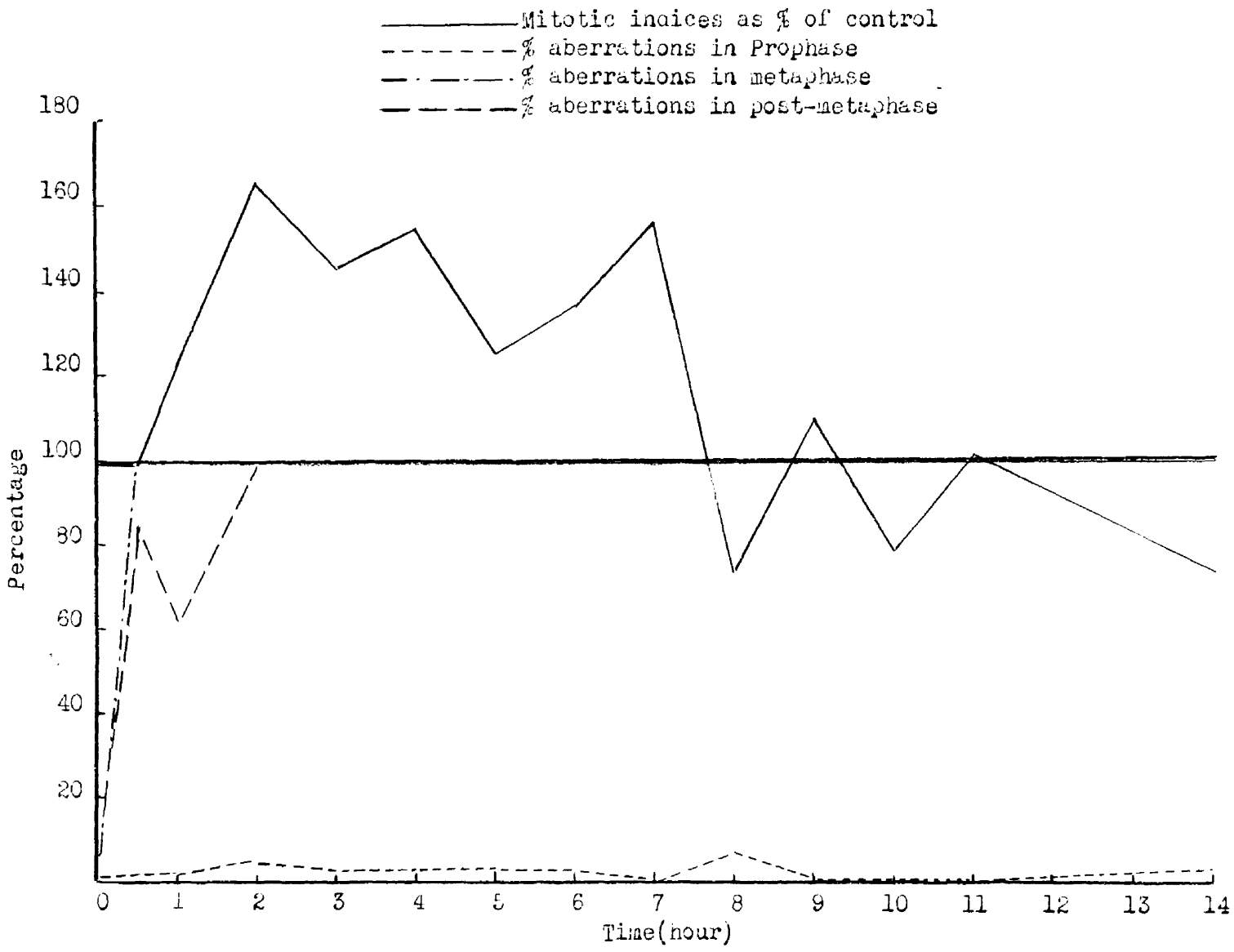


Figure 4. Hourly Variations of Mitotic Indices and Aberrations in Various Stages in Continuous Treatment of Onion

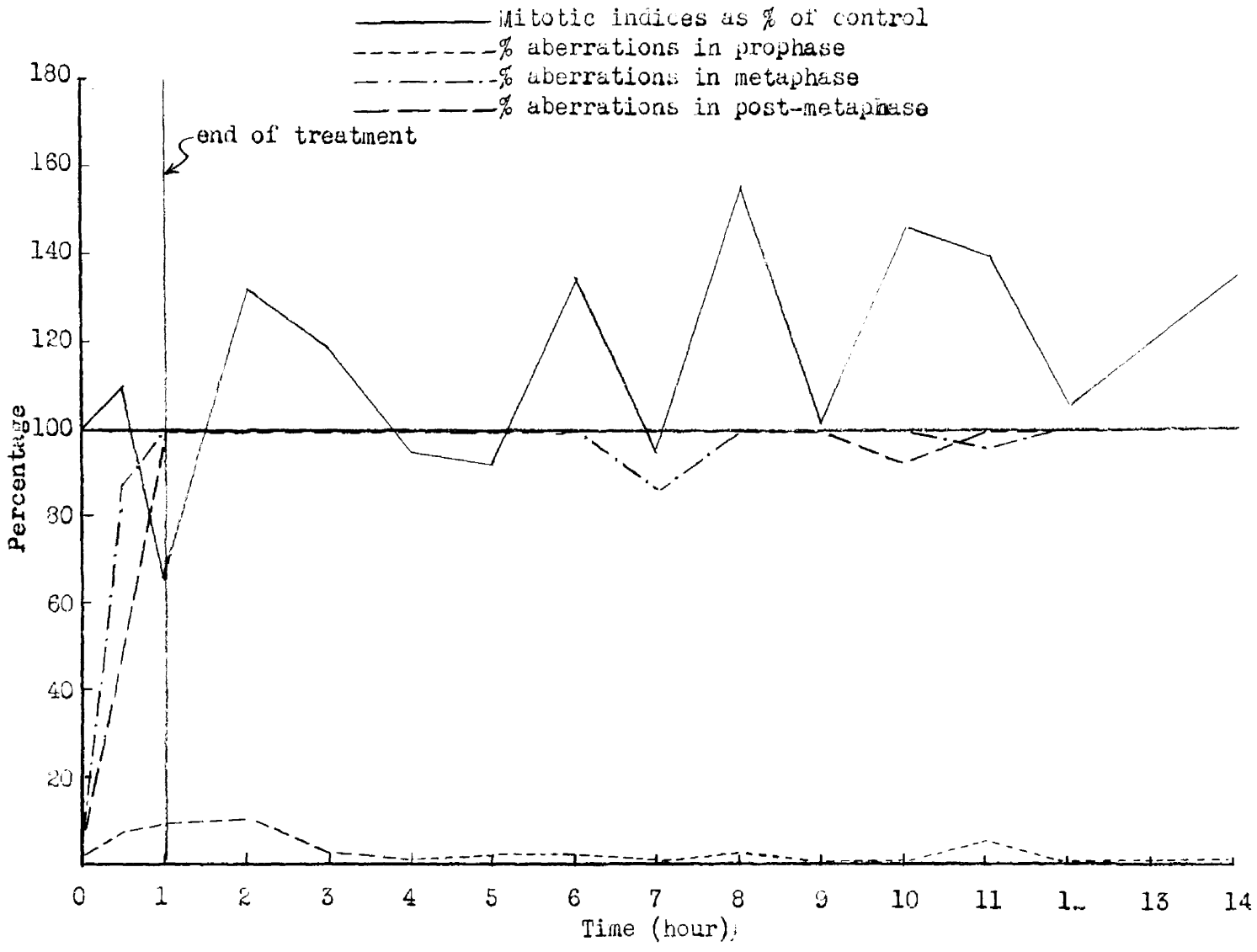
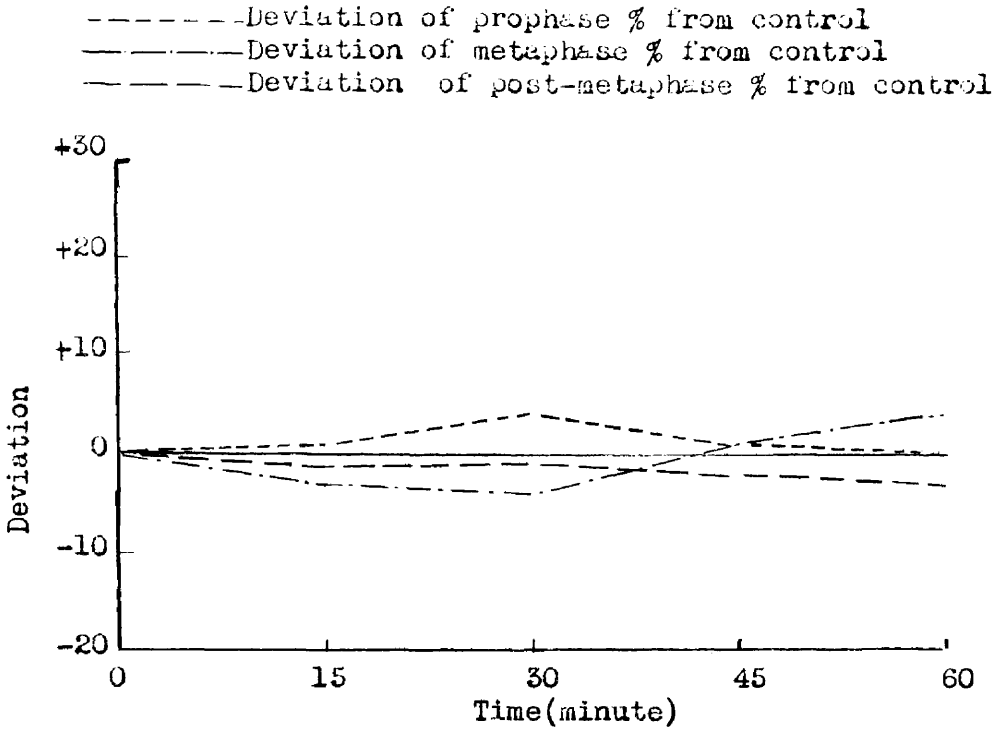


Figure 5. Hourly Variation of Mitotic Indices and Aberrations in Various Stages in Recovery Runs of Onion



Fifure 6. Quarterly Variation of Mitotic Formulae in Control Runs of Onion

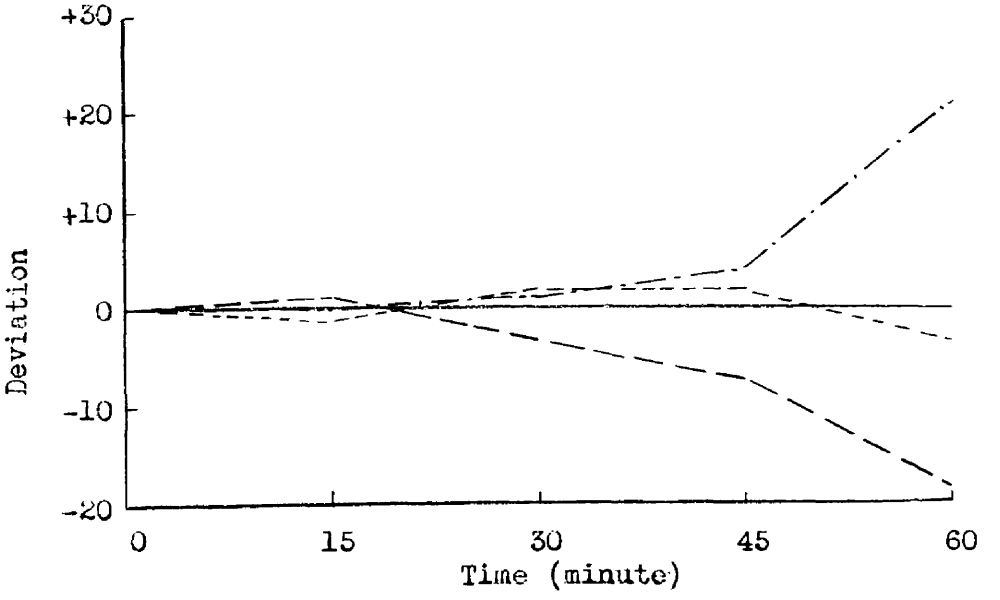


Figure 7. Quarterly Variation of Mitotic Formulae in Treated Runs of Onion

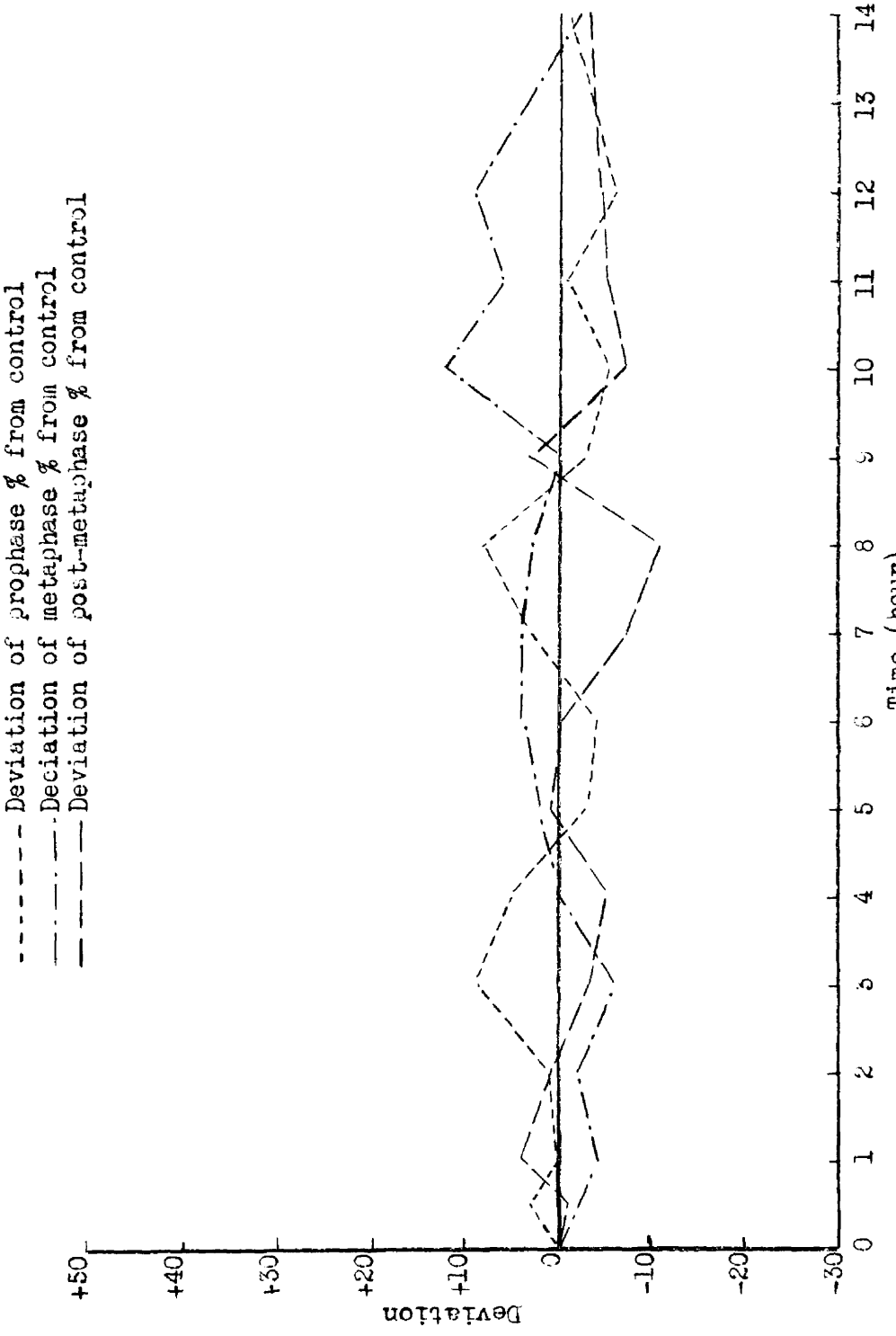


Figure 9. Hourly Variation of Mitotic Formulae in Continuous Control of Onion

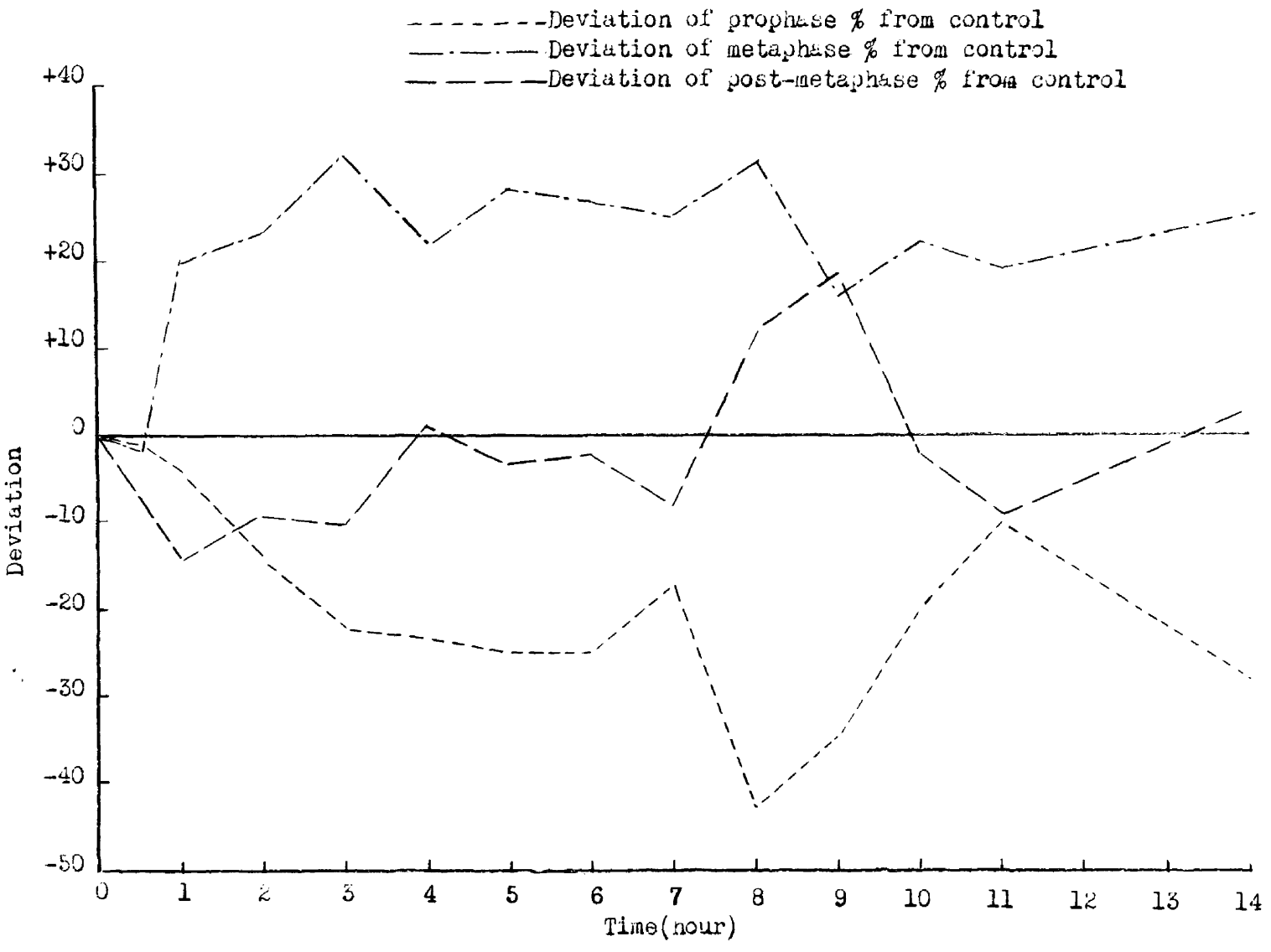


Figure 9. Hourly Variation of Mitotic Formulae in Continuous Treatments of Onion

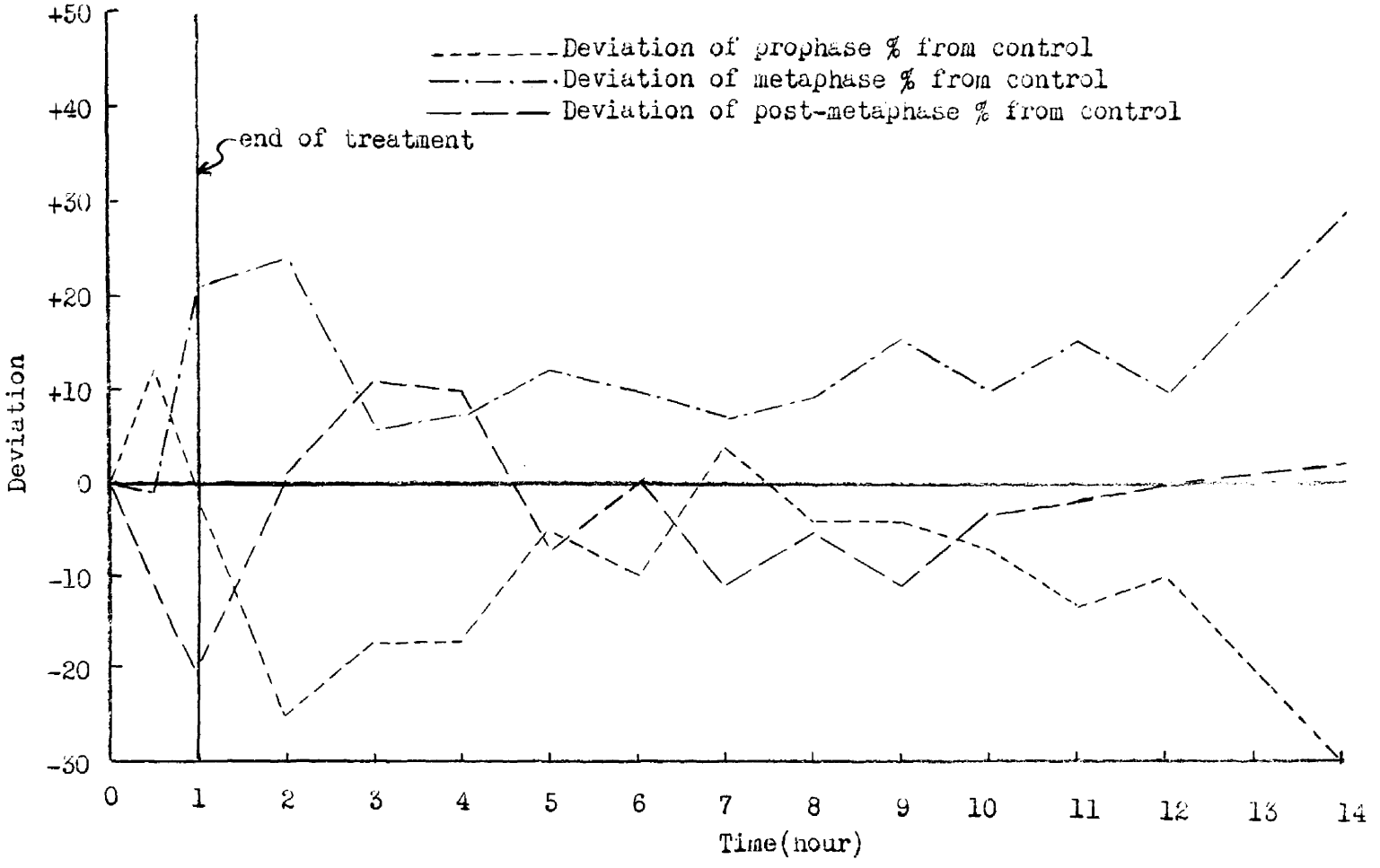


Figure 10. Hourly Variation of Mitotic Formulae in Recovery Runs of Onion

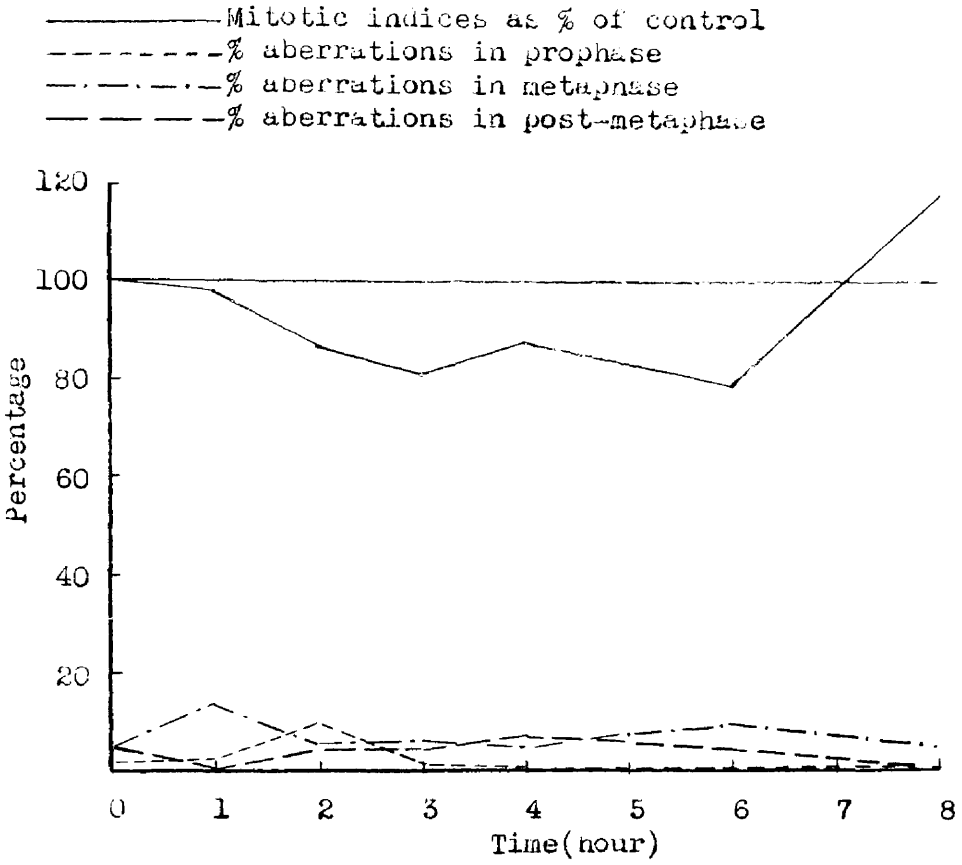


Figure 11. Hourly Variation of Mitotic Indices and Aberrations in Various Stages in Continuous Control of Pea

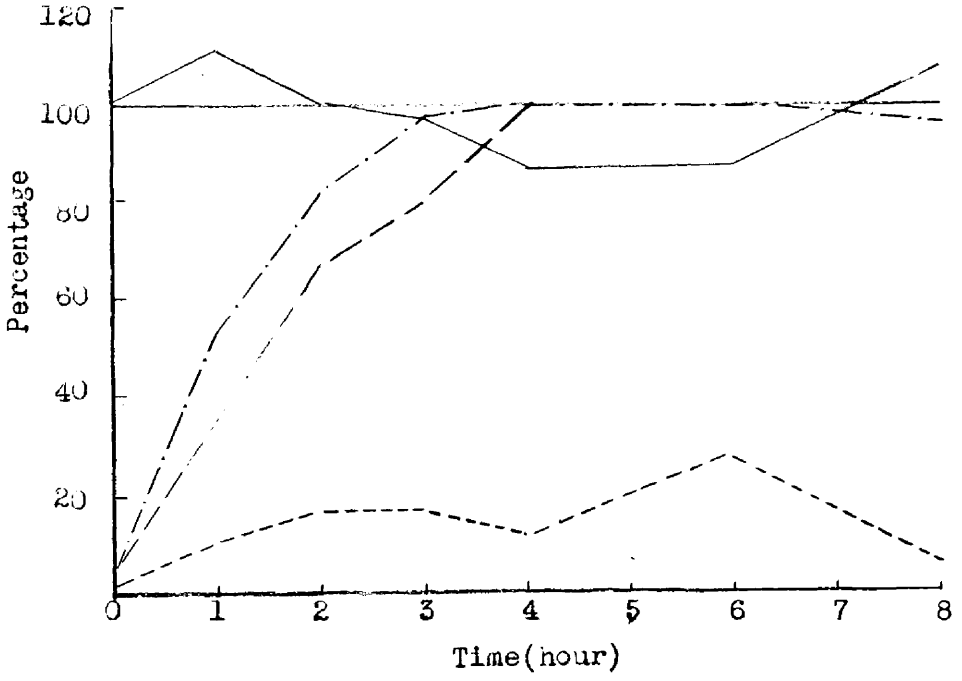


Figure 12. Hourly Variation of Mitotic Indices and Aberrations in Various Stages in Continuous Treatment of Pea

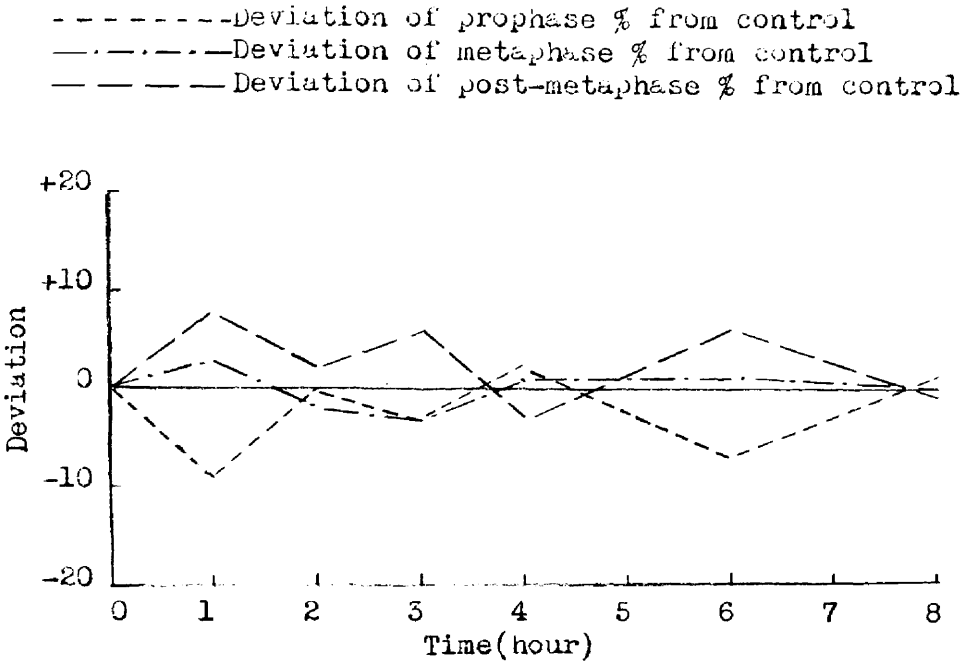


Figure 13. Hourly Variation of Mitotic Formulae in Continuous Control of Pea

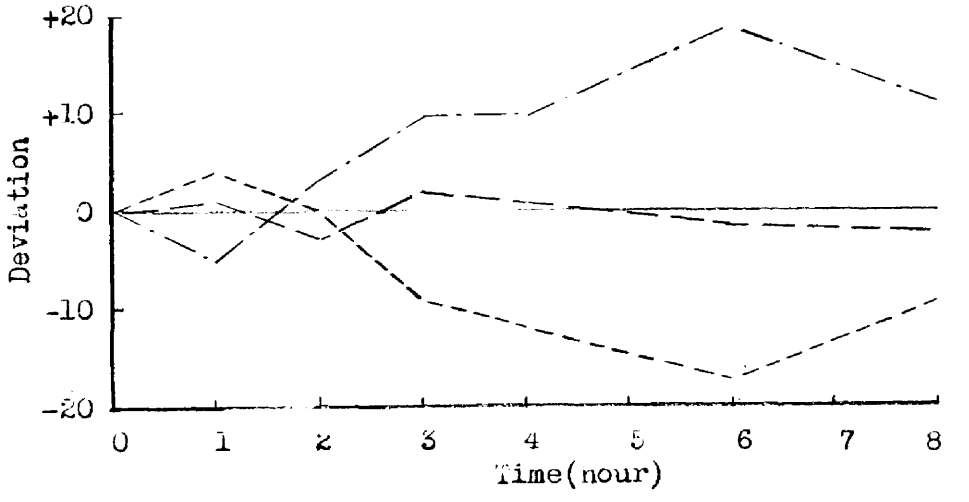


Figure 14. Hourly Variation of Mitotic Formulae in Continuous Treatment of Pea

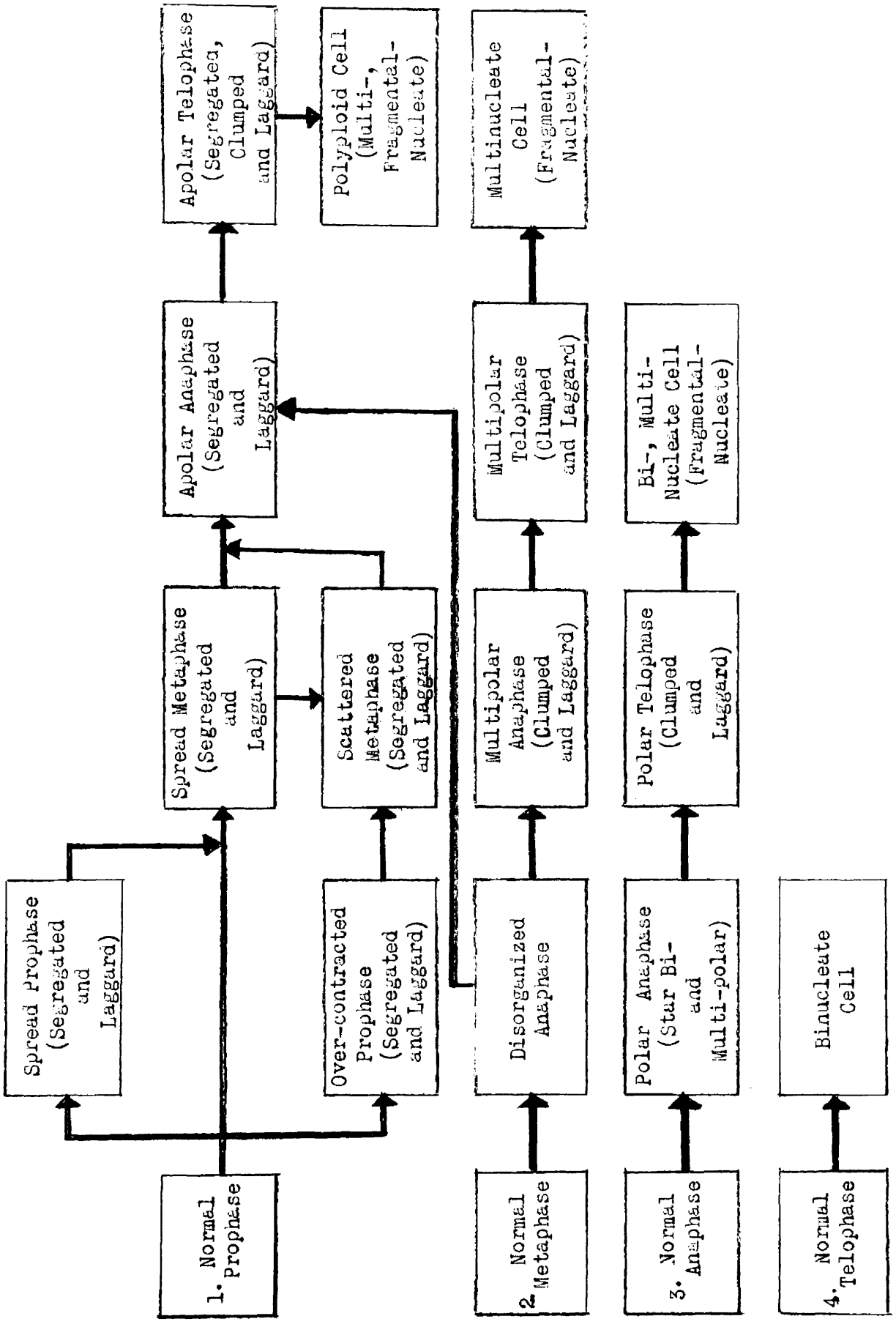


Figure 15. Ontogeny of Mitotic Deviations Induced by Lindane

II. Aberrations

For the purpose of comparison, normal mitosis for both onion and pea is shown in Plate I, Figs. 13, 14, 15, 16 and Plate IV. In general the normal sequence has been intensively studied and well established. The description can be obtained in any cytological or genetic text book. However, it is worthwhile to stress one often overlooked point here, that is, after the breaking down of the nuclear membrane at the end of prophase, the chromosomes clump in center of the cell and form a more or less solid ball with the nucleolus or nucleoli included. The chromosome ball may or may not go through a brief pause, then the chromosomes in the tangled mass start to line up on the equatorial plate. This untangling action and line-up movement may appear to be rather chaotic before all the chromosomes are arranged on the metaphase plate. This interphase stage is designated as prometaphase. It is rather infrequently seen in normal onion root-tips, and this may be attributed to its relatively short duration. However, there is no doubt that this series of events is normal for onion though not as clear cut as in peas in which 3-9 percent of the dividing cells in normal root-tips may be classified as prometaphase.

The entire process of mitosis involves continuous change, thus interstages and "awkward" figures can not be avoided in a sudden stop by fixation. Thus the abnormalities or aberrations are scored in a relative sense. Criteria used for classification of aberrations are degree of chromosomal contraction, arrangement or relationship of chromosomes or chromatids, direction of chromosome or chromatid movement or degree of spindle

inhibition, and finally reversion of chromosomes or chromatids. All the criteria are self-explanatory, except the degree of chromosomal contraction which needs some further explanation. The normal size is the size which can be found in the corresponding stage of untreated material. Chromosomes are referred to as overcontracted when they are much shorter and denser than usual for the stage concerned. For instance, in onion material the over-contracted metaphase chromosomes were only approximately one third of their normal length.

The classification and occurrence of aberrations may be described as follows.

1. Prophase:

There are three kinds of detectable aberrations in prophases; namely, spread, segregated, and overcontracted. The spread prophases are composed of contracted prophase chromosomes lying randomly inside a nuclear membrane which is more expanded than normally, thus they give a spread appearance (Plate I, Fig. 1; Plate II, Fig. 1, Plate V, Fig. 2). Segregated prophases, often referred to as "reductional groupings", are aggregated into two or more groups. The nuclear membrane may or may not be visible depending upon the developmental stage of the cell (Plate I, Fig. 1; Plate II, Fig. 1). Over contracted prophases are cells with over-contracted chromosomes arranged along the nuclear membrane or in a definite sphere (Plate I, Fig. 4).

The spread condition is a stage in the normal sequence of mitosis, but its duration is short and its appearance seldom exceeds five percent

in a root-tip. Treatment raised its incidence greatly both in onion and pea. This condition was common in shorter time of treatment and has never been found later than two hours recovery after one hour treatment of onion (Table 5). The segregated prophase has been observed in onion controls but not frequently. In this investigation, it occured rather sporadically both in treatment and recovery, the highest point being reached after 24 hours treatment. So far the overcontracted cases have not been observed in controls, and were rare in treatment and have only been found after 45 minutes treatment of onion and eight hour treatment of pea. None of these prophase abnormalities showed any very definite trend.

2. Metaphase:

All the metaphase aberrations may be classified into seven categories: (1) The least abnormal one is the disorganized metaphase which consists of more or less normal sized chromosomes but without definite orientation or disturbed orientation. Occasionally this type of abnormality appeared in control materials, but was common within one hour treatment of onion, though sporadic in recovered materials after one hour treatment of onion and continuous treatment of pea.

(2) Spread metaphase: This is characterized by contracted chromosomes in random arrangement. (Plate I, Figure 5). The highest frequency of this kind of abnormality was reached at one hour treatment in continuous treatment of onion and two hour for pea, then gradually tapered off as treatment was prolonged. There were two peaks in recovery runs of onion,

i.e. at one hour treatment and eight hour recovery after treatment. This variation was uncommon in controls but did occur.

(3) Scattered metaphase: This deviation is characterized by over-contracted chromosomes, or so-called diplo-chromosomes, which are scattered all over the cell (Plate I, Fig. 6; Plate V, Figure 3). This is the classic c-metaphase and has not been observed in control tissue. The frequencies of the scattered metaphase increased with time of treatment in both continuous treatments of onion and pea. In recovered materials of one hour treatment of onion, the peak was reached at two hours after treatment and gradually tapered off. At eleven hours the scattered metaphases appeared again but were much less frequent (Table 5).

(4) Segregated metaphase: Separations into groups may occur in nuclei in which the chromosome organization is otherwise normal, spread or scattered. The chromosomes may be normal, contracted or overcontracted depending upon the degree of the effect (Plate I, Fig. 2, Plate II, Fig. 2). This abnormal condition appeared sporadically both in treatment and recovery (Tables 3, 4, 5 and 6). Segregation of normal metaphase occasionally occured in onion and pea controls.

(5) The clumped condition: The chromosomes may be normal, contracted or overcontracted, and they are arranged in such a way that stickiness and clumping of chromosomes are obvious (Plate V, Fig. 1). This type of aberration was very common in continuous treatment of pea (Table 6), infrequent in continuous treatment of onion (Table 3 and 4), and rather inconspicuous in recovery runs (Table 5). None has been found in control materials.

(6) The ball type metaphase: In this type of aberration, the chromosomes band together to form a solid ball, in which only a few [illegible] mosomes can be observed. Although the occurrence of this type in an earlier stage of mitosis is known as typical normal prometaphase, it does not usually persist. It was also not especially persistent after "Lindane" treatment as it is after colchicine treatment (Bowen 1953, Hyypio unpublished).

(7) The telomorphic metaphase: The chromosomes of this type revert to telophase appearance in metaphase condition. Such a condition has never been found in control materials of either onion or pea, but is fairly common in colchicine treated animal tissue. Such reversion was not common in most cases but did appear in one onion run after one hour recovery following one hour's treatment (Table 5).

3. Post-metaphase:

All the post-metaphase aberrations so far observed may be put into five classes: (1) The mildest effect on post-metaphase is shown by disorganized figures, in which the normal arrangement of chromatids are somewhat distorted, although their shape and size are not different from control ones. This abnormality occasionally can be observed in untreated root-tips. This is a common aberration early in treatment of both pea and onion (Tables 3, 4, 5 and 6).

(2) The polar post-metaphase: The typical chromatics of this type of aberration are more or less normal in size and shape. Their arrangement

may be star-bipolar or multipolar, the former appears as two equal sized "stars" located more or less in the center of the cell (Plate II, Fig. 4, 5 and 6), the latter is composed of three or more unequal "stars" scattered about the middle of the cell (Plate II, Fig. 7 and 8). Similar figures have been found in controls but with very low frequency. In this investigation, the highest frequency was reached after one hour of continuous treatment of onion (Table 3 and 4) and two hours for pea after which it became gradually rare (Table 6). These star-anaphases and telophases were commonest in onion in the early recovery stages following one hour's treatment (Table 5).

(3) The apolar post-metaphase: This is the most common effect of continuous treatment and the frequency increased with time (Tables 4 and 6). In recoveries, no definite trend of any sort was apparent except that it appeared quite frequently (Table 5). The so-called apolar arrangement is a condition in which the overcontracted, bar-like chromatids do not show any direction of movement and lie loosely or clumped in one group at random in the cell. (Plate I, Fig. 7; Plate V, Fig. 4). If the chromatids show any sign of reductional grouping, then it may be subclassified into the following group.

(4) The apolar segregated post-metaphase (Plate I, Fig. 3): This was found mostly during continuous treatment of pea; this type of deviation showed a more or less increasing trend with time (Table 6).

(5) The clumped post-metaphase: This is not a clear cut multipolar or apolar segregated post-metaphase, but is characterized by stickiness

of chromatids and groupings. This type of abnormality showed no definite trend and was rare in treated pea roots in all runs (Tables 4, 5 and 6).

The last mentioned three types of aberrations have never been found in control materials, and are more characteristic of colchicine treated materials.

4. Resting stage:

Aberrant resting stages may be classified in five groups; namely, polyploid uninucleate, binucleate, multinucleate, fragmental nucleate, and pycnotic. The uninucleate cells have been found after two hours treatment in both onion and pea (Tables 4 and 6). Binucleate cells occured as early as 15 minutes in treated onion materials, but gradually decreased in number in longer treatments. Multinucleate cells were more frequently seen in both treated and recovered materials. Some workers used sticky, lobed, dumbbell, ring, etc., to describe the appearance. As a whole, the fragmental-nuclei resemble that of leukocytes in blood. Only in long time treatment (about 24 hours) or treatment of higher concentration (more than 1%), pycnotic nuclei have been observed; occasionally, some patches of cells appeared pycnotic in less affected and even untreated materials, this may not be considered as a result of treatment but rather as of unknown nature.

III. Comparison of Results

In summarizing the results obtained from this investigation, several remarks seem worth stressing both for theoretical and practical reasons.

1. Comparison of Onion and Pea:

Generally speaking, the pea seedlings provide better material for experimental cytology of the type reported herein. It is simply because the mitotic indices are higher and mitotic aberrations lower in untreated pea than that in onion (Tables 1 and 2). In this the writer agrees with Bowen (1953). The standard errors of the means of mitotic indices for pea and onion are almost identical (pea ±.259, onion ±.287, first row of Table 1 and 2). So individual variability is about the same. The fluctuation of the mitotic formula is similar in both cases (first row of Tables 1 and 2). The frequencies of metaphase in onion controls are more stable, and the frequencies of post-metaphase in untreated pea, on the other hand, are less variable.

As for reaction to treatment (Tables 3, 4 and 6), the onion meristemic cells are affected more quickly and uniformly, while the pea root-tips showed maximum abnormality at three hours later during the treatment. D'Amoto (1949) reported that onion is more sensitive to γ-hexachlorocyclohexane than pea, however the nutrient solution used in experiment may lessen the effect to a certain degree. The kinds of aberrations are similar in both materials, except that clumped metaphase is much more frequent in pea than in onion. Presumably the clumped metaphase is derived from clumped prometaphase; since the tightly packed type of prometaphase is more frequent in control pea, it is not unexpected that there would be more clumped metaphase in treated pea. In addition, the size of the meristemic cells of pea are far smaller than that of onion,

some less spread or scattered figures may be easily lumped into clumped condition. The clumped post-metaphase is a rather common effect in onion but not in pea, this may be explained by the fact that onion is more sensitive than pea to this particular insecticide.

2. Comparison of Control, Treated and Recovered Materials:

The control materials are rather normal and can be used as standard for comparison.

There are three definite types of aberrations in treated material (Tables 3, 4 and 6) i.e., spread prophase, scattered metaphase and apolar post-metaphase. The first type of abnormality does not have any trend, but the last two kinds definitely increase with time. Other aberrations described in the second part of this chapter may be considered as either intermediate between control and treated cells, such as disorganized figures, or as sub-groups of the above mentioned three distinct aberrations, e. g., apolar segregated anaphases. Some segregated polyploid figures observed at 24 hours treatment both in onion and pea may not be considered as sub-group of any simple aberration, since their origin is not due to chance alone but lie in binucleate or multinucleate cells which are formed in treatment of the previous mitotic cycle.

In recovered materials after one hour treatment (Table 5), no definite trend of aberrations can be observed. Spread metaphase and polar post-metaphase are rather common abnormalities. Probably, spread metaphase originates from relatively normal prophase. Polar post-metaphase indicates

partial inhibition of spindle activity, and also may be a sign of recovery after complete destruction of spindle function (Levan 1938). Since "Lindane" is insoluble in water, recovery is relatively difficult; and the characteristic effects, scattered metaphase and apolar post-metaphase still continue to appear sporadically presumably because of residual traces of the chemical in the tissue.

IV. The Abnormal Mitosis Induced by Technical Lindane

Depending upon the mitotic stage of the cell at the time the technical Lindane became effective upon it, there are four possible ways to complete the mitosis. The first one is that the complete mitotic cycle proceeds under the influence of the chemical, the last three will be partially affected by the chemical. The procedures are illustrated in Figure 15. The parenthetical names are possible subgroups.

In any treated root-tips, these four types of abnormal mitosis can be observed simultaneously; however, the first type will be predominent in longer treatments.

TABLE 3

PERCENT DISTRIBUTION OF ABERRATIONS IN MITOTIC STAGES

IN SHORT TIME TREATMENT OF ONION

Time (min.)	Prophase					Metaphase							Post-metaphase					
	Total %	% of Total				Total %	% of Total						Total %	% of Total				
		Normal	Spread	Segregated	Over-contracted		Normal	Disorganized	Spread	Scattered	Segregated	Clumped		Normal	Disorganized	Star-bipolar	Multipolar	Apolar
15	57	78	17	5	—	15	27	13	47	7	6	—	28	60	14	14	4	8
30	60	88	10	2	—	16	13	19	50	6	—	12	24	35	13	33	11	8
45	60	84	12	2	2	20	10	15	55	15	5	—	20	30	—	35	20	15
60	55	94	6	—	—	36	—	16	69	12	—	3	9	22	—	—	45	33

TABLE 4

PERCENT DISTRIBUTION OF ABERRATIONS IN MITOTIC STAGES

IN CONTINUOUS TREATMENT OF ONION

Time (hr.)	Prophase				Metaphase								Post-metaphase						
	Total %	% of Total			Total %	% of Total							Total %	% of Total					
		Normal	Spread	Segregated		Normal	Disorganized	Spread	Segregated	Scattered	Clumped	Ball		Normal	Disoeganized	Polar	Apolar	Apolar-seg.	Clumped
½	57	98	2	—	13	—	23	77	—	—	—	—	30	40	10	—	13	—	37
1	54	98	2	—	35	—	—	85	6	6	3	—	11	28	—	18	18	—	36
2	44	96	2	2	38	—	—	77	5	18	—	—	18	—	—	9	57	17	17
3	36	97	3	—	47	—	—	81	4	15	—	—	17	—	—	6	77	—	17
4	35	97	3	—	37	—	—	73	—	27	—	—	28	—	—	7	79	7	7
5	33	97	—	3	43	—	—	63	2	33	2	—	24	—	—	—	88	8	4
6	33	100	—	—	42	—	—	60	—	40	—	—	25	—	—	—	76	16	8
7	41	98	—	2	40	—	—	70	—	30	—	—	19	—	—	—	90	—	10
8	15	93	—	7	46	—	—	26	4	67	3	—	39	—	—	—	68	6	26
9	23	100	—	—	31	—	—	52	—	45	3	—	46	—	—	—	76	7	17
10	38	100	—	—	37	—	—	42	9	46	3	—	25	—	—	—	88	12	—
11	48	100	—	—	34	—	—	23	—	77	—	—	18	—	—	—	100	—	—
14	30	97	3	—	40	—	—	25	3	72	—	—	30	—	—	—	83	17	—
24	17	41	35	24	57	—	—	—	—	88	—	12	26	—	—	—	77	23	—

TABLE 5

PERCENT DISTRIBUTION OF ABERRATIONS IN MITOTIC STAGES

RECOVERY RUN OF ONION

Time (hr.)	Prophase				Metaphase						Post-metaphase						
	Total %	% of Total			Total %	% of Total					Total %	% of Total					
		Normal	Spread	Segregated		Normal	Disorganized	Spread	Scattered	Segregated	Telomorphic		Normal	Disorganized	Polar	Apolar	Apolar-seg.	Clumped
1	33	91	9	—	39	—	—	61	33	3	3	28	—	—	17	70	10	3
2	41	95	5	—	21	—	—	48	52	—	—	38	—	—	24	55	3	18
3	41	100	—	—	22	—	18	55	22	5	—	37	—	—	45	49	6	—
4	53	98	—	2	27	—	7	51	35	7	—	20	—	—	20	30	5	45
5	48	98	—	2	25	—	—	80	12	8	—	27	—	—	26	30	3	41
6	62	100	—	—	22	10	—	85	5	—	—	16	—	—	31	25	—	44
7	54	98	—	2	24	—	8	72	16	4	—	22	—	—	82	18	—	—
8	54	100	—	—	30	—	—	100	—	—	—	16	—	—	50	37	—	13
9	51	100	—	—	25	4	4	88	—	4	—	24	8	—	34	12	—	46
10	45	96	—	4	30	—	3	94	—	3	—	25	—	—	56	44	—	—
11	48	100	—	—	25	—	4	88	4	4	—	27	—	—	15	55	—	30
13	27	100	—	—	43	3	3	73	21	—	—	30	6	—	13	51	—	30
23	49	100	—	—	26	8	—	78	14	—	—	25	16	—	16	40	—	28

TABLE 6

PERCENT DISTRIBUTION OF ABERRATIONS IN MITOTIC STAGES

CONTINUOUS TREATMENT OF PEA

Time (hr.)	Prophase						Metaphase								Post-metaphase					
	Total	% of Total				Total %	% of Total							Total %	% of Total					
		Normal	Spread	Segregated	Over-contracted		Normal	Disorganized	Spread	Scattered	Segregated	Clumped	Ball		Normal	Disorganized	Polar	Apolar	Apolar-seg.	
1	66	88	12	—	—	15	53	7	20	7	—	13	—	19	68	11	11	5	5	
2	62	84	16	—	—	23	13	4	52	9	13	9	—	15	34	7	13	13	33	
3	51	84	16	—	—	30	3	3	36	33	3	22	—	19	26	—	5	37	32	
4	50	88	12	—	—	31	—	—	32	29	—	29	—	19	—	—	5	68	27	
6	45	76	14	—	—	39	—	2	31	18	26	20	3	16	—	—	6	44	50	
8	53	94	4	—	2	31	—	3	32	32	7	23	3	16	—	—	6	63	31	
24	42	81	2	17	—	40	—	—	5	60	23	12	—	18	—	—	—	50	50	

DISCUSSION

I. Mitotic Aspect

One immediate effect of this insecticide on mitotic meristemic cells is the complete suppression of cytokinesis. So long as treatment is continued no cell will divide regardless of nuclear organization. Even in recovered materials after treatment of one hour, no cell plate has been noted before 48 hours. That might be attributed to the fact that (1) the chemical is practically insoluble in water, so does not readily leach out within relatively short times, and (2) it takes part in cellular metabolic process and is utilized or translocated in the tissue. A binucleate cell is capable of undergoing a second mitosis which is normal if treatment is stopped. In the case of continuous treatment, each nucleus will travel any path illustrated in Figure 15, thus numerous complications result, Figures 1 - 6 in Plate III, Figures 5, 6, 7 in Plate V, demonstrate some of these.

Polar anaphases originate from normal early anaphase or metaphase as illustrated in Figure 15. Multipolar anaphases probably arise from partial breakdown of the spindle mechanism. "Star" bipolar anaphase is difficult to explain by spindle malfunction. In the short time treatment of onion, some intermediate stages between normal early anaphase and "star" bipolar or multipolar anaphase have been observed (Figures 4, 5 and 7 Plate II). The configuration and relatively abundant occurence of these cells led the writer to believe that there are definite attractions

among anaphase kinetochores after the "spindle mechanism" has been inhibited partially by any chemical or physical means. The spatial relationship is rather a determining factor in grouping, since "segregational groupings" are more or less random.

Gauldon and Carlson (1951) stated that:

> Observations on living neuroblasts reveal that stars (metaphases) can be formed in two ways: First, they may appear during either partial or complete destruction of a fully formed spindle body by strong concentration of colchicine. Second, enough spindle material will be organized at prometaphase to form a focal point of a star. Thus we see why Barber and Callan observed stars most frequently during the first hours of exposure to a relative strong solution, and why Levan and Peter observed them most frequently during recovery when the intracellular concentration of the alkaloid was being diluted.

They also sensed the attraction of metaphase kinetochores which may be present in partial inhibition of spindle either on the way to total breakdown or recovery.

The above mentioned facts further illustrated our notion (Wilson Hawthorne and Tsou,1951) that the spindle action in metaphase and anaphase can not be adequately defined in terms of a single force, but that at least two more or less independent forces are involved: First, the forces between kinetochores and so-called spindle "poles"; and second, the affinity among kinetochores. The kinetochore attraction is probably partly responsible for metaphase "line-up" and anaphase migration in normal cell division. Laggard chromosomes or chromatids could be considered as too far separated to be attracted, or are in equilibrium

with two or more attractive forces initiated from adjecent groups; a third possibility is that the kinetochore of laggards is partially inactivated by the chemicals (Daniel and Wilson, in press).

The cleavage of the kinetochore seems to be a unique process regardless of degree and kind of effect. Sometimes it may delayed as in the case of acti-dione and colchicine (Hawthorne and Wilson,1952; Bowen,1953). Omission of this process is rather rare in treated plant tissue, thus Levan (1954) claimed that the difference between colchicine effect in plants and animals is that there is usually reversion from metaphase in the latter.

The scattered metaphase is likely the result of complete inhibition of both "kinetochore-poles" and "kinetochore-kinetochore" forces, so that the "diplo-chromosomes" or "c-pairs" remain in the arrangement of prophase or, in most instances, may "explode" a little under the influence of a diakinetic like force. Segregated figures of this scattered type showed no definite orientation, so that it may be assumed that segregation is partly due to chance, and partly due to attraction of kinetochores. Apolar postmetaphase is just the continuation of this type of abnormal metaphase.

Spread prophase caused directly by chromosomal contraction appears to be a more or less immediate effect of technical Lindane on onion meristemic cells (Table 3, Fig. 1 in Plate II; Fig. 1 in Plate 1). Overcontraction of chromosomes seems to be a common effect of any chemical, from the simplest ethanol (Vaarama,1947) to heavy metal salts (Macfarlane, 1953), alkaloids (colchicine) nucleates (Powell, 1952) and antibiotics

(Wilson, 1950; Wilson and Bowen, 1951; Bowen and Wilson, 1954). It is unlikely that chromosome contraction can be explained by simple dehydration as Sigenaga (1949) proposed, since some effective concentrations were too low to cause a hypertonic condition. It, as in many other biological phenomena, is a result of complicated bio-physico-chemical interactions, involving surface actions on the matrix and degree of chromonema spiralization.

The relatively frequent appearance of segregated prophases in short time treatment of onion suggested that "segregational groupings" or "reductional groupings" can not be defined as splitting of the spindle into several parts, but is rather due to chromosome contraction plus relative expansion of the nuclear membrane and possibly inter-kinetochore forces. As for metaphase and anaphase, the segregated figures have been discussed above and it has been suggested that chromosomal autonomy and chance also play some important role in the initiation of these aberrations.

II. Chromosomal Fragmentation

Isolated chromatids and chromosomal fragments have been observed in angiosperm seedlings after direct treatment with the insecticidal powder (Kostoff, 1949). D'Amato (1950) treated onion root-tips with dilute ethanol solution of pure gammexane and found a frequency of chromosomal fragments in 150-160 anaphase figures obtained from three root-tips after three days recovery of approximately 22 percent.

The writer has analyzed more than thirty series of onion tests, with each series containing about thirty root-tips. The frequency of true

free chromosomal fragments was only one for each of six treated root-tips. On the other hand, four cases also have been found in untreated materials. Attached fragments were more frequently observed in both treated and untreated pea root-tips; this may be attributed to the entanglement of the four secondary constrictions of chromomosmes in pea.

Levan and Tjio (1948) found as high as sixty percent of phenol treated cells containing fragments. Spontaneous chromosome fragmentation has also been detected in Vicia faba (Levan and Lotfy, 1950). Moreover radiomimetic activity has been demonstrated for chlorine in meristemic cells of Pisum rootlets (Von Rosen, 1953). In view of conflicting results concerning the potential radiomimetic effects of Lindane, further investigation seem desirable.

III. Polyploidizing Effect

Nybom and Knutsson (1947) claimed that Lindane is a better polyploidizing agent than acenaphthene and only second to colchicine. The writer did several experiments on water soaked rye (Secale cereale L.), radish (Raphanus sativus L.) and pea seeds. The majority of meristemic cells became polyploid within 48 hours treatment with 0.01 to 0.5 percent suspension of the technical Lindane. Selected polyploid seedlings have been planted in green house or field, but, to date, only diploid parts have grown out. Soaking seeds directly in a suspension of the insecticide also was used as a means of inducing polyploidy, but none of the emergent seedlings grew to maturity.

With colchicine one may expect successful recovery of polyploidy once in several hundred treatments. With Lindane the percentage of success is obviously much less.

IV. Inositol Antagonism

According to Kirkwood and Phillips (1946), the growth inhibition of Saccaromyces cerevisiae by γ-hexachlorocyclohexane can be overcome by a sufficient amount of m-inositol. Buston, Jacobs and Goldstein (1946) reported that m-inositol is an antagonist of Lindane on Nematospora gossypii in some growth experiments. In addition, Chargaff, Stewart and Magasanik (1948) claimed that "...meso-inosital is able to inhibit the metaphase arrest and tumor formation induced in Allium cepa by colchicine or gammexane.".

On the contrary, Schopfer, Posternak and Boss (1947) did not succeed in demonstrating an antigammexane action of m-inositol in other species of Saccaromyces, and Eremothecium ashbyii, Candida spp., Phycomyces blakesleanus and Ustilago violacea. Failure of m-inositol to protect sea urchin eggs from the toxic action of δ-and γ-hexachlorocyclohexane has been also reported by Chaix and Lacroix (1948).

In between, D'Amato (1949) considered that m-inositol delayed the effect of gammexane on onion root-tipsin a similar manner as sugar solution, which merely changed cell permeablity.

These conflicting reports led the writer to re-investigate. Exact repetition of Chargaff's experiments failed to show any antagonism of

Lindane and m-inositol. As a matter of fact, m-inositol alone induced similar effects in onion dividing cells as those induced by Lindane. Further experiments, using equal parts of technical Lindane and m-inositol of comparable concentration on pea seedlings, gave the same result. The slides made from the first four hours treatment as well as after twenty-four hours treatment demonstrated perfect Lindane effect --"c-mitosis".

Woolley (1952) explained the antagonism of m-inositol and γ-hexachlorocyclohexane or Lindane in terms of solubility and configuration, i.e, the former enters the cell through the aqueous phase of the cytoplasmic membrane, the latter the lipid phase; since both chemicals have the same configuration, antagonism arises. According to Östergren and Levan (1943), c-mitotic activity is a kind of narcotic effect. Höber (1945) says:

> Typically, narcotics do not enter into a chemical reaction with cell components; they are chemically "indifferent". The reactions they enter into with cells are of physico-chemical rather than of chemical nature. They make contact with cells by secondary valences, changing the surface properties of exterior or interior cellular structures and microstructures, which become apparent as changes of dispersity, of hydration, of collodial aggregation, of dissolving power, of absorption affinity.

Therefore inositol antagonism may be found only in inositol-requiring organisms, but not in others.

V. Difference Between Lindane and Colchicine

Ever since Östergren and Levan (1943) used hexachlorocyclohexane to study c-mitotic activity, it has been recognized that this chemical

and colchicine belong to the same category as far as their mitotic effects, induction of polyploidy and c-tumour are concerned. Östergren (1945) stated that both chemicals cause narcotized mitosis and proposed a precipitation hypothesis of narcosis to explain the mechanism of c-mitosis. Levan (1951) classified the effect of both chemicals as reversible physiological reactions, but"...colchicine differs from the substances (include Lindane) of simple narcotic effect in so far as c-mitotic activity is extended far down into concentrations which are at a great distance from the saturation point."

Judging by the results that have been obtained by Bowen (1953) and Hyypio (unpublished), Lindane and colchicine differ in degree but not in kind of effect on mitotic activity. For instance, ball metaphase is a prominent aberration after colchicine treatment, but relatively rare in the case of Lindane. It may be attributed to the fact that colchicine immobolizes prometaphase chromosomes whereas Lindane affected chromosomes still are capable of moving out of the prometaphase mass; but due to inhibition of spindle function and kinetochore attraction, the arrangement of chromosomes is completely destroyed.

According to Powell (1951), various nucleates show effects at late prophase, with colchicine primary effects are at metaphase. The results obtained in this investigation suggest that Lindane exerts an initial effect at prophase which is revealed at late metaphases. This suggestion also explains why spread prophases and metaphase can persist in continuous treatments; and why Lindane affected chromosomes are capable

of moving out from prophase.

The quantitative disagreements are: First, Lindane is not as potent a mitotic stimulator (Table 1 and 2) as colchicine as Bowen concluded (1953). This point agrees with the result of Scholes (1953). Second, Lindane does not block metaphase to anaphase movement to such a degree or for so long a time as Hindmarsh (1951) showed in her colchicine treated material (Tables 3, 4 and 6 post-metaphase frequency decreased only within one hour, after that the percentages remained essentially the same). Third, the frequency of prophase is relatively reduced in Lindane treated materials (Tables 1, 2, 4 and 6). This may be attributed to shorter duration of prophases or to a relatively increasing number of metaphase-like figures which without chromosomal contraction and absence of nuclear membrane will be classified as prophase. This suggests that the nuclear membrane may break down in a rather shorter time than normally. As was suggested by Huston (1952) when she studied the cytological effect of the fungicide, Thiolutin, there is a correlation between "over-contraction" and increase in the relative number of metaphases.

All this may indicate another possibility that the total division cycle is somewhat lengthened as Guttman (1952) noted in colchicine treated material, but not in the same proportion to that of a normal one or of a colchicine affected one.

In 24 hour treated pea root-tip, octoploid cells were detected. According to Brown (1951), the mitotic cycle of pea root meristemic cells is about nineteen hours. Thus the cycle is definitely shortened

by the chemical. Bowen found the same effect in colchicine treated pea root-tips (1953). Whether the duration of mitosis or interphase is being shortened is difficult to determine from fixed material.

VI. Explanation of Phyto-responses

Growth has been separated traditionally into two rather distinct phases, cell division and cell enlargement. The former involves the mechanism of cell division and chemical reactions of protoplasm synthesis, the latter with growth of cell wall, possibly some bio-synthesis, and the physical process of hydration or vacuolation. The superficial cytological course of cell division and cell elongation have proved to be rather similar in roots and shoots, although they are different with respect to their physiological behavior.

The mechanism of cell division is still not well known. However, the insecticide affects the normal cell division as observed by the writer and many other workers. The products of the abnormal mitosis are polyploid, binucleate, and multinucleate cells and fragmental nuclei. In subsequent cell division, the tetraploid becomes octoploid in continuous and residue treatment. The binucleate and multinucleate cells, under influence of the chemical, may divide and form polyploid cells with higher or lower number of nuclei, depending upon the distance between nuclei. If the chemical is completely leached out, cell plates may form and aneuploid microcytes are formed. The fragmental nuclei react sometimes as polyploid cells other times as multinucleate, largely depending on the manner of

grouping. Levan and Östergren (1943) stated that an increase in the number of chromosomes in plants results in slower growth and lower growth energy. Aneuploid cells tend to be less viable, and Kostoff (1949) thought that they may become dead cells in tissues. Reduced cells or microcytes generally posses reduced viability.

As for protoplasm synthesis, Chao and Loomis (1947) stated that hormones were formed as a by-product of protein synthesis; while Baldovinos's (1953) results suggested that protein synthesis was dependent upon hormones in non-green tissue. Nevertheless, some correlation between growth hormones, protein synthesis and cell division seems to be present.

Burström (1951) stated that; "Cell elongation proceeds in two phases, the first phase involves an increasing elasticity of the wall, which has been explained on the basis of a loosening elasticity of the wall, which has been explained on the basis of a loosening of the joints between the micellae." Some protoplasm synthesis occurs at this stage in order to keep pace with enlargment of the cell. "The second phase is characterized by a hardening of the wall, as evidenced by a decreasing elasticity. During the second phase there is, further, a very rapid supply of nutrients to the cell so that the osmotic concentration does not decrease despite the rapid increase in cell volume." Increase in cell volume involves largely an absorption of water. "Elongation does not take place uniformly over the whole cell surface. Apical growth is a well known feature of many cells, and such differential growth is probably a wide spread phenomenon in ordinary tissues." The mechanism of cell elongation whether

it is a hormone problem or a question of morphologic and metabolic changes in the cell is rather beyond our present knowledge. However hormones are almost certainly involved.

Hence we may say that hormones are likely responsible for the direction of growth, and growth is the product of metabolism. Anything interferring with hormone activity or metabolic processes, will disturb the precise, correlated growth.

It is a well known fact that Lindane and colchicine induce swelling or c-tumour in the elongation zone of roots or stems of most plants. Östergren (1944) considered that c-mitosis and c-tumour effects are related but independent, and that the c-tumour effect is due to "...narcosis of the cell growth control." Havas (1949) further stated that colchicine induces "...deviation of the polarity of hormones' trans-location." It is probable that Lindane behaves in a similar way, and disturbs the distribution of hormones.

In the course of this investigation, another interesting physiological response has been noted. The onion root-tips after 72 hours treatment with 0.1% technical Lindane suspension showed a much reduced meristem below the c-tumour, and sometimes protoxylem strands extended to the promeristem or elongation zone. A similar result was reported by Macfarlane and Schmock (1948) in colchicine affected onion root-tips. They believed this is an irreversible cytoplasmic effect of poisoning, which is comparable to the effect of Phenylmercuric nitrate on onion meristemic cells. Phenylmercuric nitrate is a respiratory poison which probably attacks the -SH groups of succinic dehydrogenase, lactic and gluco

dehydrogenases, cytochrome oxidase and catalase. In conclusion, they proposed that the colchicine and colchicine-like reactions are possible responses to enzymatic poisoning.

All the metabolic processes involve mostly enzymatic activity, thus it is not improbable that prolonged treatment with colchicine or Lindane will inhibit metabolism and growth.

In field contitions, at planting season, the amount of residual "Lindane" in the soil and the kind of seeds probably are the determining factors for germination of seeds. When colchicine was used for seed treatment, the following results were observed. Low concentration stimulates germination. More concentrated solutions delay germination, and sometimes, even inhibit. Bond (Croker and Barton, 1953) suggested that a concentration which was low enough to stimulate germination is still effective enough to induce polploidy.

2, 4-dichlorophenoxy-acetic acid has been reported to cause c-mitosis and c-tumour in onion roots (Croker, 1953). It also prevents oxygen from penetrating into barley seeds and thus inhibits aerobic germination (Croker and Barton, 1953). Lindane differs from 2,4-D in two respects. First, Lindane is known to cause narcotic effects on the cell; 2,4-D, on the other hand, reacts chemically with cellular components. Second, 2,4-D is a definite hormone-mimetic substance. Since they induce similar cytological effect, there may be some correlation present.

Hocking (1950) found that a mixture of trichlorobenzenes prepared by the break down of the alpha isomer of hexachlorocyclohexane caused plant

deformation and ultimately inhibition of germination with relatively small doses.

In addition, Lindane dissociates a little in solution to give some chlorine ions. Chlorine is known to prevent germination of seeds (Croker and Barton, 1953). Even if the residue is not concentrated enough to inhibit germination, the growth of emerged young roots and shoots is definitely affected by physiological and cytological disturbances caused by the insecticide.

The writer carried out a germenation test on pea seeds by the paper towel method. The germination percentages after three days for control and 1,000 ppm suspension were 99 and 97 respectively. The average lengths of shoot and root were 1 and 3.7 inches respectively for control seedlings, 0.7 and 3.0 inches for treated seedlings. After ten days treatment they increased to 7.8 and 6.0 inches for control seedlings, and 4.75 and 3.5 inches for treated seedlings. The control seedlings were straight and with numerous side roots; on the other hand, the treated seedlings were twisted and with very few thickened side roots on stubby, brownish main roots.

Root hairs are projecting tubes of epidermal cells, and chiefly function as absorption organs for water and mineral nutrients from the soil. The production of root hairs is believed to be due to hormone action, this may be the reason why there are no root hairs on "Lindane" affected plants.

As for scorching of young leaves, the effect probably is partly due to physiological blocking of the carrier, and partly due to narcotic

influence of the chemical on the leaf tissue. The "off-flavored" effect is believed to be due to chlorinated impurities present in the chemical. Since the pure chemical has been used for preparation of the commercial insecticide, no off-flavor has been reported.

SUMMARY

The results obtained from this investigation could be summarized as follows:

1. The 0.1% suspension of Lindane did not stimulate mitosis in root meristemic tissues of onion bulbs and pea seedlings.

2. Under the influence of Lindane, the prophase frequencies in dividing cells decreased than that of untreated materials. The metaphase frequencies increased significantly, and the post-metaphase frequencies remained essentially the same. Therefore, the "piling-up" of metaphase was probably due to shortening of prophase.

3. The immediate mitotic effects caused by Lindane were contraction of chromosomes; possibly break down of nuclear membrane; inhibition of cytoplasmic "spindle" mechanism; and failure of cytokinesis. As treatment was prolonged, over-contraction of chromosomes, and inhibition of chromosomal attraction occured.

4. Inositol antagonism and radiomimetic effects of this insecticide have not been demonstrated in this investigation, thus further research is desirable. While Lindane is theoretically a good polyploidizing agent, there are a number of practical difficulties which reduce its potentiality below that of colchicine.

5. The final products of atypical mitosis induced by the insecticide were polyploidy cells and aneuploid microcytes. The former are gaint cells, but possess a much slower growth rate than that of normal ones. The

latter usually tend to be less viable. Therefore the treated meristemic tissues are much reduced as far as number of cells and rate of growth were concerned.

6. The insecticide also caused "c-tumour" in the enlargement zone of treated root-tips. Most workers believe that this effect is independent of "c-mitosis" and probably is due to disturbance of hormone polarity at the cellular level. This disturbance causes abnormal growth, such as swelled shoot and root apex, twisted parts, lack of root hairs, distorted leaves etc. The precise, correlated normal physiological balance of some sensitive plants is definitely affected, when they are grown in soil containing the insecticide either as soil treatment or residue of spary.

BIBLIOGRAPHY

Ashby, D. C., 1950, The Phytotoxic Effects of DDT, BHC, Panathion and Toxaphene on Tobacco. Ann Appl. Biol. 37:624-639

Baldovinos, G., 1953. Growth of the Root Tip. Growth and Differentiation in plants. W. E. Loomis, Editor. The Iowa State College Press, 1953.

Boswell, V. R., 1953. Residues, Soils and Plants. Insects. 1952 Year Book of U.S.D.A.:284-297.

Bowen, C.C., 1953. A Comparative Study of the Effects of Several Antimitotics. Unpublished Ph. D. Thesis, Michigan State College, 1953.

_____, and G. B. Wilson, 1954. A Comparison of the Effects of Several Antimitotic Agents. Jour. Hered. 45:3-9.

Brown, R. 1951, The Effects of Temperature on the Durations of the Different Stages of Cell Division in the Root Tip. Jour. Exp. Bot. 2:96-110.

Burström, H. 1951. Mechanism of Cell Elongation. Plant Growth Substances. F. Skoog, Editor. University of Wisconsin Press, 1951.

Buston, H. W., S. E. Jacobs, and A. Goldstein, 1946. Cause of Physiological Activity of Gammexane. Nature 158:22.

Chao, M. D. and W. E. Loomis, 1947. Temperature Coefficients of Cell Enlargement. Bot. Gaz. 109:225-231.

Chaiz, P. and L. Lacroix, 1948. Action des ζet δ-hexachlorocyclohexane sur l'oeng d'oursin avant et apres la fecondation. Bioch. et Bioph. Acta 2:86-90.

Chargaff, E., R.N. Stewart, B. Magasanik. Inhibition of Mitotic Poisoning by Meso-inosital. Science 108:556-558.

Crocker, W. and L. V. Barton, 1953. Physiology of Seeds. Chronica Botanica Company, Waltham, Mass., 1953.

Croker, B. H., 1953. Effects of 2,4-dichlorophenoxyacetic acid and 2,4, 5-trichlorophenoxyacetic acid on mitosis in Allium cepa. Bot. Gaz. 114(3):274-283.

Cullinan, F. P., 1949. Some New Insecticides - Their Effect on Plants and Soils. Jour. Econ. Ent. 42(2):387-391.

D'Amato, F., 1949. Sull'impiego Del Gammesano Come Agente Poliploidizzante (Use of gammexane as a polyploidizing agent). Caryologia 1(2):209-222.

_____, 1949. Early Influence of m-inositol and Sugars of Gammexane Induced c-mitosis. Caryologia 1(2):223-228.

_____, 1950. Notes on the Chromosome breaks induced by Pure Gammexane. Caryologia 2:361-364.

Daneil, A. and G. B. Wilson, 1954. The Antimitotic Effect of Endothal. (in press)

Frear, D. E. H., 1949. Chemistry of Insecticides, Fungicides and Herbicides. D. Van Nostrand Co., Inc., N. Y., 1949.

Gaulden, M. E. and J. G. Carlson 1951. Effects of Colchicine on the Grasshopper Neuroblast. Expmt. Cell Res. 2:416-433.

Greenwood, M. L. and J. M. Tice. Palatobility Tests on Potatoes Grown in Soil Treated with the Insecticides Benzene Hexachloride, Chlordane and Chlorinated Comphene. Jour. Ag. Res. 78(11):477-482.

Guttman, R., 1952. An Interpretation of Some Mitotic Irregularities Using Poison Distribution. Amer. J. of Bot. 39(8):528-534.

Havas, L. J., 1949. Hormone-mimetic and Growth Effects of Colchicine. Exp. Cell Res. Suppl. I:597-601.

Hawthorne, M. E., and G. B. Wilson, 1952. The Cytological Effects of the Antibiotic Acti-dione. Cytologia 17:71-85.

Himdmarsh, M. H., 1952. The Effect of Colchicine on the Spindle of Root Tip Cell. The Proc. of Linn. Soc. 77:300-306.

Höber, R., 1945. Physical Chemistry of Cells and Tissues. The Blakiston Co. Philadelphia, 1945.

Hocking, B., 1950. On the Effect of Crude Benzene Hexachloride on Cereal Seedlings. Sci. Ag. 30:183.

Huskins, C. L. and L. N. Steinitz, 1948. The Nucleus in Differentiation and Development. Jour. Hered. 39:66-77.

Huston, M. J., 1952. Cytological Effects of Certain Organic Chemicals. Unpublished M.S. Thesis. Michigan State College, 1952.

Kirkwood, S. and P. H. Phillips, 1946. The Antiinositol Effect of γ hexachlorocyclohexane. J. Biol. Chem. 163:251-254.

Kostoff, D., 1948. Cytogenetic Changes and Atypical Growth Induced by Benzene Hexachloride, Curr. Sci. 10:17.

_____, 1949. Atypical Growth, Abnormal Mitosis, Polyploidy and Chromosomal Fragmentation Induced by Hexachlorocyclohexane. Nature 162:845-846.

_____, 1949. Induction of Cytogenetic Changes and Atypical Growth by Hexachlorocyclohexane. Science 109:467-468.

Levan A., 1938. The Effect of Colchicine on Root Mitoses in Allium. Hereditas 24:471-486.

_____, and G. Östergren, 1943. The Mechanism of c-mitotic Action Observation on Naphthalene Series. Hereditas 29:381.

_____, and H. J. Tjio, 1948. Induction of Chromosome Fragmentation by Phenols. Hereditas 34:453-484.

_____, and T. Lotfy, 1950. Spontaneous Chromosome Fragmentation in Seedlings of Vicia Faba. Hereditas 36:471-482.

_____, A., 1952. Chemically Induced Chromosome Reactions in Allium Cepa and Vicia Faba. Cold Spring Harbor Symposium. Quant. Biol. 16:235-242.

_____, 1954. Colchicine Induced c-mitosis in Two Mouse Ascites Tumours. Hereditas 40:1-64.

Macfarlane, E.W.E. and N. G. Schmock, 1948. The Colchicine and Colchicine-Like Reaction as a Possible Response to Enzymic Poisoning. Science 108:712-713.

Nybom, N. and B. Knutsson, 1947. Investigation on c-mitosis in Allium Cepa. Hereditas 33:220-234.

Östergren, G. and A. Levan, 1943. The Connection Between c-mitotic Activity and Water Solubility in Some Monocyclic Compounds. Hereditas 29:381-443.

_____, 1950. Cytological Standards for the Quantitative Estimation of Spindle Disturbances. Hereditas 36:371-382.

_____, 1950. Consideration on Some Elementary Features of Mitosis. Hereditas 36:1-18.

Östergren, G. and A. Levan, 1951. Narcotized Mitosis and the Precipitation Hypothesis of Narcosis. Colloques internat. do la centre national de la recherche scientifique. 26:77-87.

Powell, S. S., 1951. Comparative Effects of Colchicine and Various Nucleic Acid Salts upon Somatic Mitosis. Unpublished M.S. thesis. Michigan State College, 1951.

Rao, N. S. and B. C. Kundu, 1949. Effect of Gammexane on the Root Tips of Corchorus Capsularis L. Science and culture 14(11):484.

Scholes, M.E., 1953. The Effect of Hexachlorocyclohexane on Mitosis in Roots of Onion and Strawberry (Fragaria Vesca). Jour. Hort. Sci. 28(1):49-68.

Schopfer, W. H., Posternak, T. and M.L.Boss, 1947. Le Gammexane (γ-hexachlorochohexane) est-il l'antivitamine du mesoinositol? Schweiz. Zeitschr. f. Pathol. und Bakteriol. 10:443-457.

Shepard, H. H., 1951. The Chemistry and Action of Insecticides. McGraw Hill Book Co., Inc. 1951.

Sigenaga, M., 1949. Experimental Studies of Abnormal Nuclear Cell Divisions. Cytologia 15:45-60.

Slade, R. E. 1945. A new British Insecticide, the Gamma Isomer of Benzene Hexochlorid. Chem. and Ind. 64:314.

Stitt, L. L. and J. Evanson, 1949. Phytotoxicity and Off-Quality of Vegetables Grown in Soil Treated with Insecticides. Jour. Econ. Ent. 425:615-617.

Stoker, R. I. 1948. The Phytotoxicity of DDT and Benzene Hexachloride. Ann. Appl. Biol. 35:110-123.

Vaarama, A., 1947. Experimental Studies on the Influence of DDT Insecticide Upon Plant Mitosis. Hereditas 33:191-219.

Von Rosen, G., 1953. Radiomime-tic Activity and the Periodical System of the Elements. Bot. Notiser 1953 (1):140-142.

West, T. F. and G. A. Campbell, 1950. DDT and Newer Persistent Insecticides. Chapman and Hall Co. London, 1950.

Wilson, G. B., M.E. Hawthorne, and T. M. Tsou, 1951. Spontaneous and Induced Variations in Mitosis. Jour. Hered. 42:183-189.

Wilson, G. B., T. M. Tsou and P. Hyypio, 1952. Variations in Mitosis. II. the Interrelation of Some Basic Deviations. Jour. Hered. 43:211-215.

Woolley, D. W., 1952. A Study of Antimetabolites. John Wiley and Son Inc., New York, 1952.

PLATE I

Control and Affected Mitotic Stages of Allium Cepa

Figures:

1. Spread segregated prophase with two fragments and two dicentric chromosomes taken after three hours treatment with 1,000 ppm suspension.

2. Segregated c-metaphase taken after nine hour treatment with 10 ppm suspension.

3. Segregated apolar anaphase taken after four hours treatment with 1,000 ppm suspension.

4. Over-contracted prophase taken after 24 hours treatment with 1,000 ppm suspension.

5. Spread metaphase taken after 30 minutes treatment with 1,000 ppm suspension.

6. Scattered metaphase taken after six hours treatment with 500 ppm suspension.

7. Apolar anaphase taken after five hours treatment with 500 ppm suspension.

8. Normal tetraploid metaphase taken after 48 hours recovery of 24 hours treatment with 150 ppm suspension.

9. Normal polyploid anaphase and resting stage after 48 hours recovery of 24 hours treatment with 150 ppm suspension.

10. Clumped metaphase with two fragments taken after 5 hours treatment with 1,000 ppm suspension.

11. Normal anaphase with two fragments taken from untreated material.

12. Micro-nucleus taken from untreated material.

13. Normal prophase taken from untreated material.

14. Normal metaphase taken from untreated material.

15. Normal early anaphase taken from untreated material.

16. Normal anaphase and telophase taken from untreated material.

Each division of the scale represents ten microns.

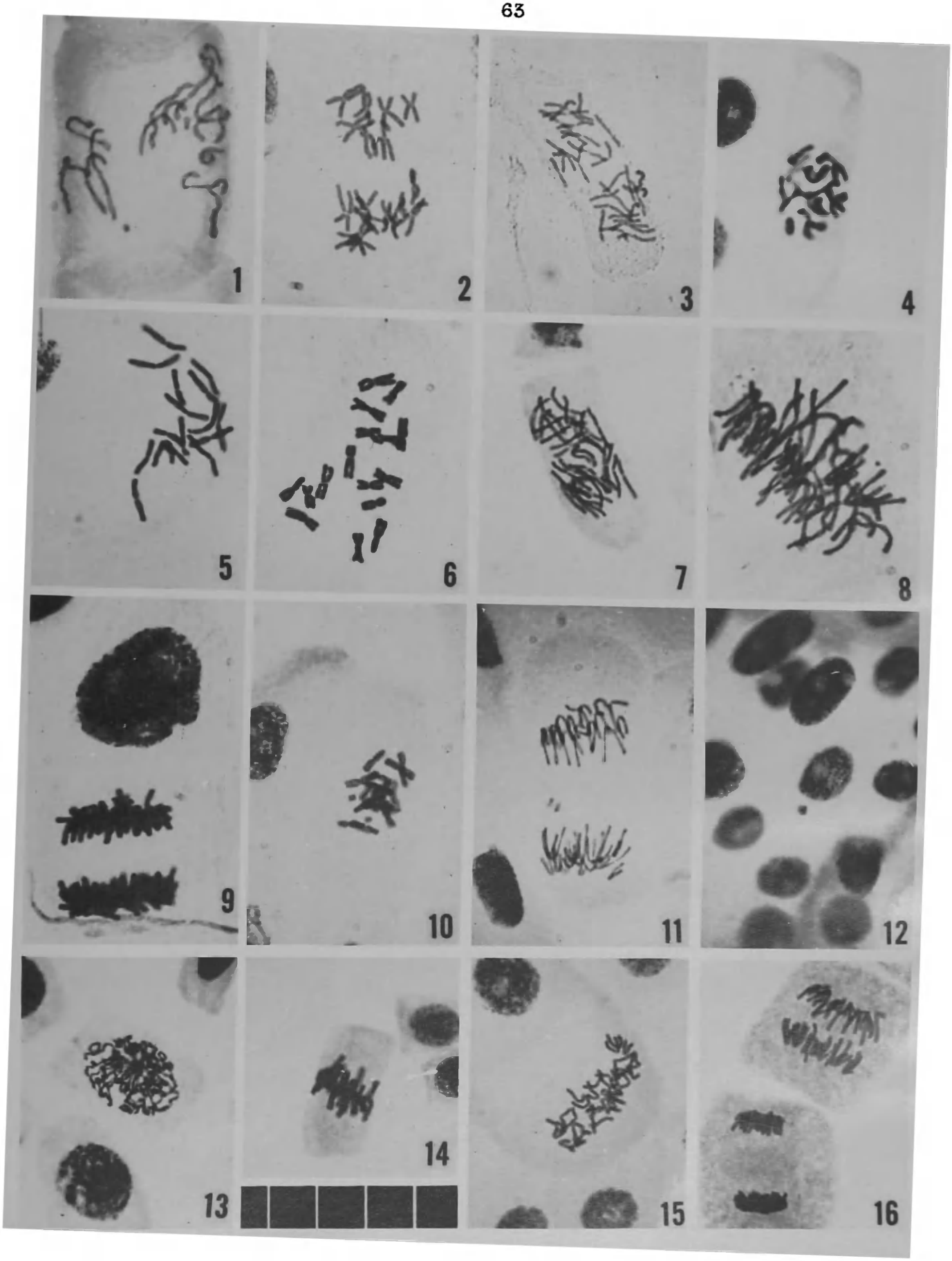


PLATE I

PLATE II

Affected Mitotic Stages of Allium Cepa Treated With 1,000 ppm Suspension

Figures:

1. Spread segregated prophase taken after 30 minutes treatment.
2. Segregated contracted metaphase taken after 30 minutes treatment.
3. Tetrapolar anaphase taken after 15 minutes treatment.
4. Early "star" bipolar anaphase taken after 30 minutes treatment.
5. "Star" bipolar anaphase taken after 15 minutes treatment.
6. "Star" bipolar anaphase taken after 30 minutes treatment.
7. Early multipolar anaphase taken after 15 minutes treatment.
8. Multipolar anaphase taken after 3 hours treatment.
9. Trinucleate cell taken after 24 hours recovery of 3 hours treatment.

Each division of the scale represents ten microns.

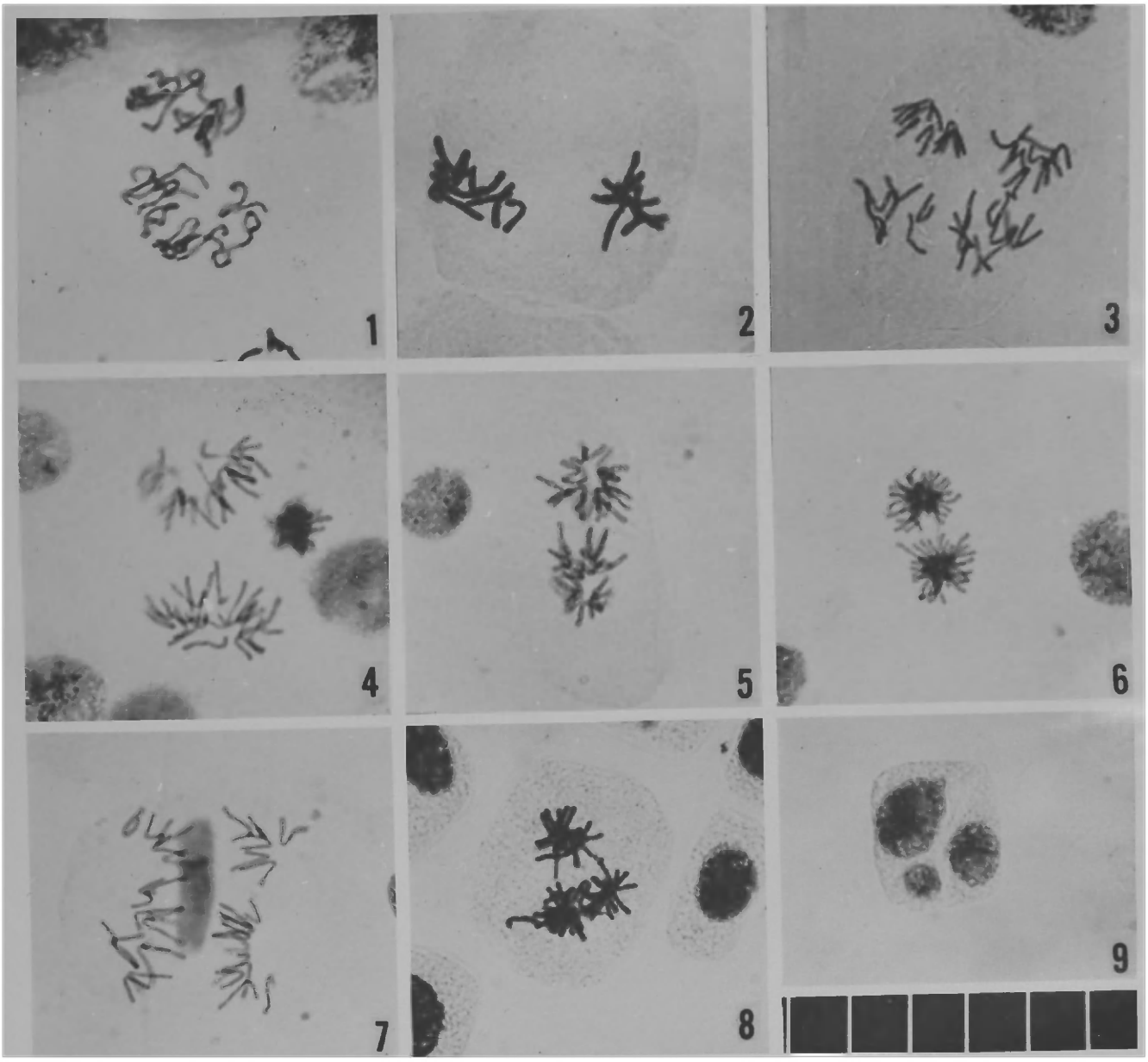


PLATE II

PLATE III

Mitoses of binucleate Cells of Allium Cepa Taken From 47 Hours Recovered Root-Tips After 1 Hour Treatment With 1,000 ppm Suspension

Figures:

1. Prophase
2. Prometaphase
3. Early anaphase
4. Anaphase
5. Telophase

Each division of the scale represents ten microns.

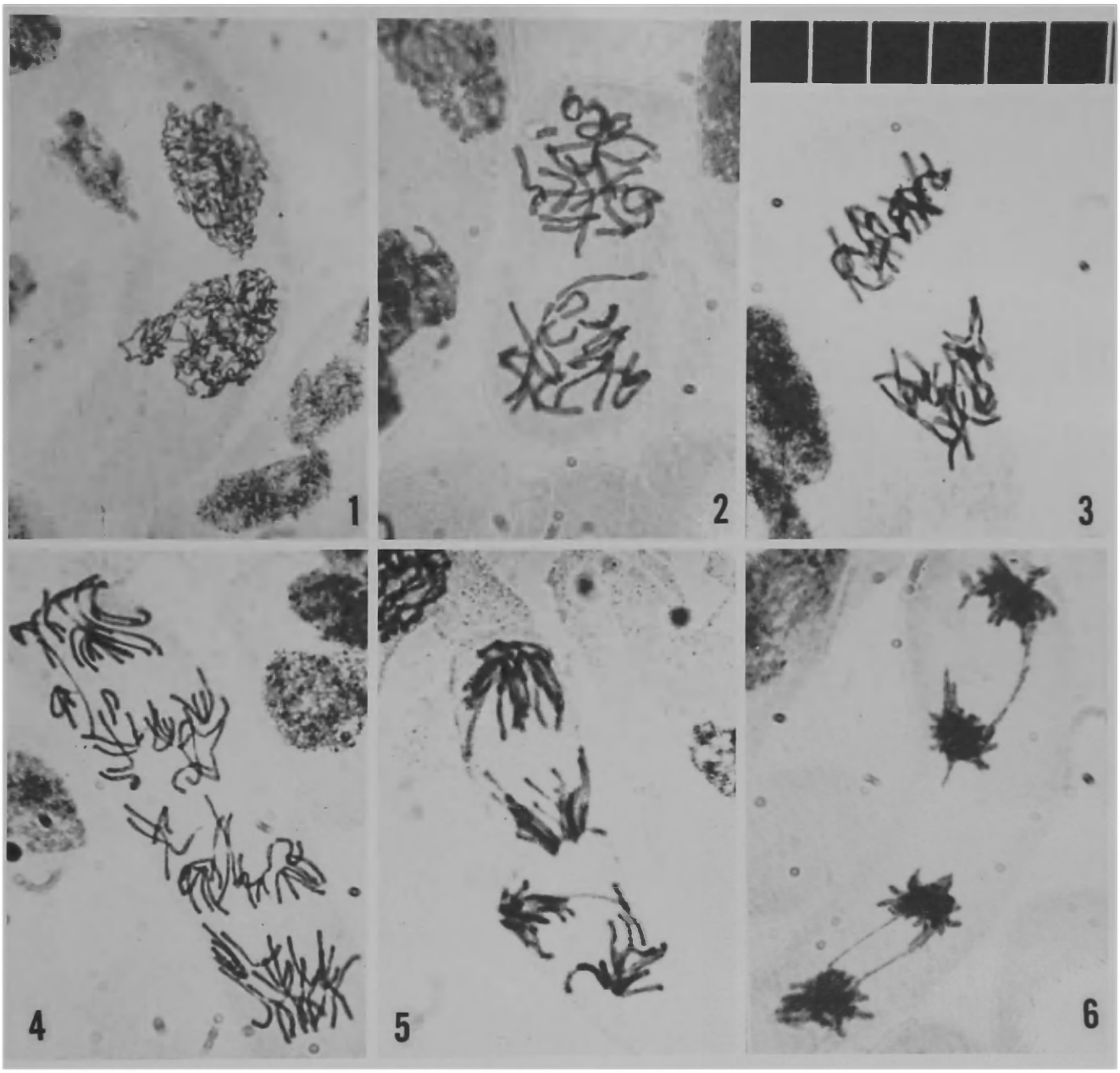


PLATE III

PLATE IV

Mitotic Stages From Untreated Pisum Sativum

Figures:

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

Each division of the scale represents ten microns.

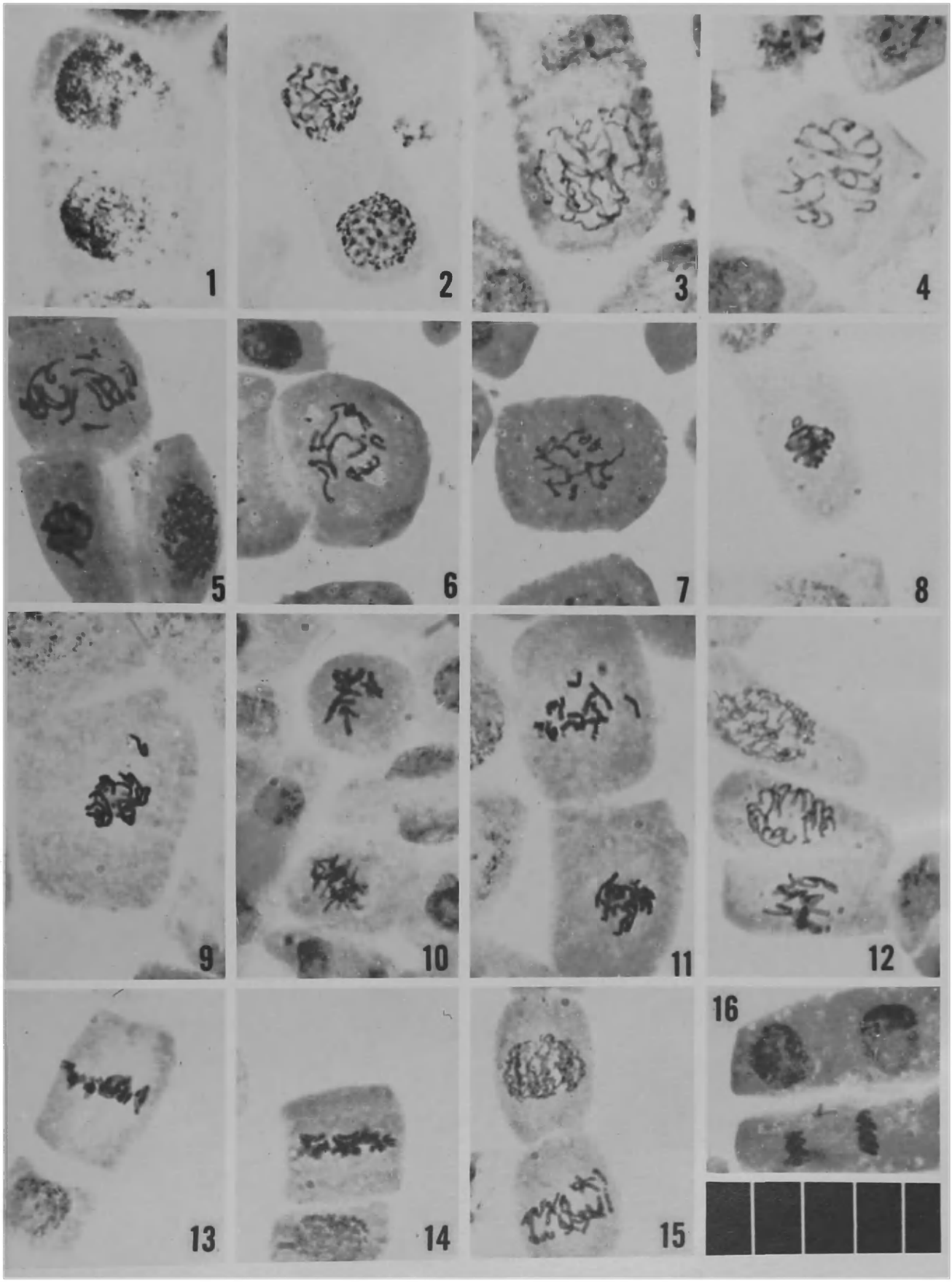


PLATE IV

PLATE V

Affected Mitotic Stages in Pisum Sativum Treated with 1,000 ppm Suspension

Figures:

1. Clumped metaphase and normal prometaphase taken after one hour treatment.
2. Spread and segregated prophase, clumped telophase taken after four hours treatment.
3. Scattered metaphase taken after four hours treatment.
4. Apolar anaphase taken after one hour treatment.
5. Binucleate scattered metaphase taken after 24 hours treatment.
6. Binucleate prophase with one fragmental nucleus taken after 24 hours treatment.
7. Apolar segregated polyploid anaphase taken after 24 hours treatment.
8. Nine nucleate cell taken after 24 hours treatment.
9. Polyploid apolar anaphase taken after 24 hours treatment.
10. Tetraploid scattered metaphase taken after 24 hours treatment.
11. Octoploid scattered metaphase taken after 24 hours treatment.
12. Tetraploid normal prophase and metaphase taken after six days recovery after eight hours treatment.

Each division of the scale represents ten microns.

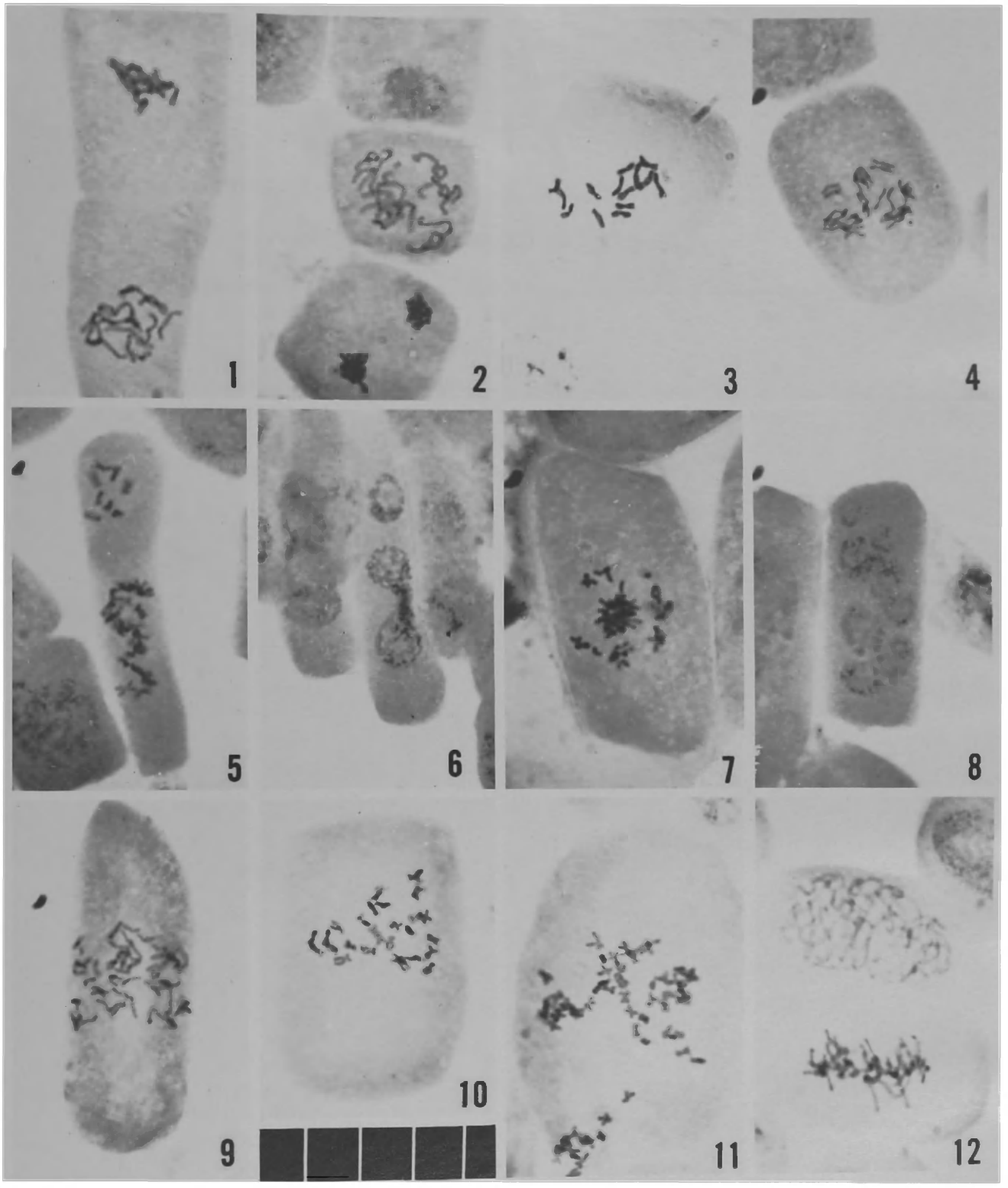


PLATE V

APPENDIX TABLE I

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES

IN SHORT TIME TREATMENT OF ALLIUM CEPA

		Time(min.)	0	15	30	45	60
		No. of slide	16	4	4	4	4
Prophase	Early	Normal	23	13	16	13	24
		Spread	—	1	1	1	—
		Segregated	—	1	—	—	—
	Mid	Normal	31	30	32	35	25
		Spread	1	8	5	6	2
		Segregated	—	3	1	1	—
		Overcontracted	—	—	—	1	—
	Late	Normal	3	1	5	3	3
		Spread	—	—	1	1	1
		Segregated	—	—	—	—	—
Metaphase		Normal	14	4	2	2	—
		Disorganized	—	2	3	3	2
		Spread	—	7	8	11	29
		Scattered	—	1	1	3	4
		Segregated	—	1	—	1	—
		Clumped	—	—	2	—	1
Anaphase		Normal	11	7	2	2	—
		Disorganized	2	4	3	—	—
		Star bipolar	—	2	5	1	—
		Multipolar	—	1	1	—	1
		Apolar	—	2	2	2	2
Telophase		Normal	14	10	6	6	2
		Star bipolar	—	2	3	4	—
		Multipolar	—	—	2	4	3
		Apolar	—	—	—	1	1
		Apolar+seg.	—	—	—	—	—
Total		Prophase	58	57	60	60	55
		Metaphase	15	15	16	20	36
		Anaphase	13	16	13	5	3
		Telophase	14	12	11	15	6
		Mitotic index	4.5	3.5	3.2	3.6	4.2
		Bi,multi-nucleate	—	—	—	—	—

APPENDIX TABLE II

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES IN CONTINUOUS CONTROL OF ALLIUM CEPA

	Time(hr.)	½	1	2	3	4	5	6	7	8	9	10	11	12	14	24
	No. of slide	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Prophase Early	Normal	23	21	27	25	28	28	26	23	28	25	16	22	24	27	23
	Spread	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Segregated	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Prophase Mid	Normal	35	34	30	40	30	25	23	35	35	27	27	22	24	29	31
	Spread	—	—	—	1	—	—	—	1	2	—	4	—	—	—	—
	Segregated	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—
	Overcontract	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Prophase Late	Normal	5	3	2	1	5	2	5	2	1	3	5	6	4	1	4
	Spread	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
	Segregated	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Metaphase	Normal	11	10	12	8	14	17	19	16	14	15	25	15	22	11	17
	Disorganized	2	—	—	—	1	—	—	2	4	—	—	—	—	—	—
	Spread	—	1	—	—	—	—	—	1	—	—	2	6	—	2	1
	Sp+seg.	—	—	1	1	—	—	—	—	—	—	—	—	—	—	—
	Scattered	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Sc.+seg.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Clumped	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Anaphase	Normal	9	13	11	6	14	13	13	5	6	12	9	11	11	11	10
	Disorganized	—	2	2	—	1	2	4	1	1	1	3	2	2	1	1
	Polar	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1
	Apolar	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—
	Apolar+seg.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Telophase	Normal	17	16	15	18	8	13	10	14	9	16	8	8	10	19	12
	Polar	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Apolar	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Apolar+seg.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total	Prophase	61	58	59	67	63	55	54	61	66	55	53	57	52	57	58
	Metaphase	13	11	13	9	15	17	19	19	18	15	27	21	24	13	18
	Anaphase	9	15	13	6	14	15	17	6	7	14	12	14	14	12	12
	Telophase	17	16	15	18	8	13	10	14	9	16	8	8	10	18	12
Mitotic index		4.0	3.8	3.9	6.8	4.2	4.0	6.0	3.4	5.2	5.8	3.6	2.5	2.8	5.3	4.6
Bi-,multi-nucleate		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

APPENDIX TABLE III

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES IN CONTINUOUS TREATMENT OF ALLIUM CEPA

	Time(hr.)	½	1	2	3	4	5	6	7	8	9	10	11	14	24
	No. of slide	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Prophase Early	Normal	14	22	12	8	9	8	10	12	2	9	8	19	11	1
	Spread	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Segregated	--	--	--	--	--	1	--	--	--	--	--	--	--	--
Prophase Mid	Normal	38	27	25	24	22	21	21	20	12	13	25	23	15	3
	Spread	--	--	1	1	1	--	--	--	--	--	--	--	1	--
	Segregated	--	--	1	--	--	--	--	--	1	--	--	--	--	--
	Overcontract	--	--	--	--	--	--	--	--	--	--	--	--	--	4
Prophase Late	Normal	4	4	5	3	3	3	2	9	--	1	5	6	3	3
	Spread	1	1	--	--	--	--	1	--	--	--	--	--	--	6
	Segregated	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Metaphase	Normal	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Disorganized	3	--	--	--	--	--	--	--	--	--	--	--	--	--
	Spread	10	30	29	38	27	27	25	28	12	16	16	8	10	--
	Sp.+seg.	--	2	2	2	--	1	--	--	2	--	3	--	1	--
	Scattered	--	2	7	7	10	14	17	12	31	14	17	26	29	50
	Sc.+seg.	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Clumped	--	1	--	--	--	1	--	--	1	1	1	--	--	--
	Ball	--	--	--	--	--	--	--	--	--	--	--	--	--	7
Anaphase	Normal	3	--	--	--	--	--	--	--	--	--	--	--	--	--
	Disorganized	3	--	--	--	--	--	--	--	--	--	--	--	--	--
	Polar	--	2	2	1	2	--	--	--	--	--	--	--	--	--
	A-polar	3	2	5	7	10	12	10	9	12	21	9	14	18	10
	A-polar+seg.	--	--	3	--	2	2	4	--	2	--	1	--	--	3
	Clumped	--	--	1	--	1	--	--	--	1	--	--	--	--	--
Telophase	Normal	9	3	--	--	--	--	--	--	--	--	--	--	--	--
	Polar	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Apolar	1	--	5	6	12	9	9	8	15	14	13	4	7	10
	Apolar+seg.	--	--	--	--	--	--	--	--	--	3	2	--	5	3
	Clumped	11	4	2	3	1	1	2	2	9	8	--	--	--	--
Total	Prophase	57	54	44	36	35	33	33	41	15	23	38	48	30	17
	Metaphase	13	35	38	47	37	43	42	40	46	31	37	34	40	57
	Anaphase	9	4	11	8	15	14	14	9	15	21	10	14	18	14
	Telophase	21	7	7	9	13	10	11	10	24	25	15	4	12	12
	Mitotic index	4.4	5.5	7.5	6.5	6.9	5.6	6.1	7.0	3.3	4.9	3.5	4.5	3.3	4.3
	Bi, multi-nucleate	--	--	--	+	+	+	+	+	++	+	+	+	+	++++

APPENDIX TABLE IV

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES IN

RECOVERY RUN OF ALLIUM CEPA

	Time(hr.)	½	1	1+1	1+2	1+3	1+4	1+5	1+6	1+7	1+8	1+9	1+10	1+11	1+13	1+23
	No.of slide	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Prophase Early	Normal	25	27	14	22	17	22	23	26	19	15	16	13	21	12	23
	Spread	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3
	Segregated	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Prophase Mid	Normal	39	23	15	16	22	27	20	31	32	34	32	28	26	10	21
	Spread	3	5	3	1	—	—	—	—	—	—	—	—	—	—	—
	Segregated	—	—	—	—	—	1	1	—	1	—	—	2	—	—	—
	Overcontract	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Prophase Late	Normal	2	2	1	2	2	3	4	5	2	5	3	2	1	5	2
	Spread	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Segregated	—	—	—	—	—	—	—	2	—	—	—	—	—	—	—
Metaphase	Normal	2	—	—	—	—	—	—	2	—	—	1	—	—	1	2
	Disorganized	—	—	—	—	4	2	—	—	2	—	1	1	1	1	—
	Spread	11	29	24	10	12	13	20	19	17	30	22	28	22	32	20
	Scattered	—	6	13	11	5	10	3	1	4	—	—	—	1	9	4
	Segregated	1	1	1	—	1	2	2	—	1	—	1	1	1	—	—
	Clumped	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Anaphase	Normal	1	—	—	—	-	—	—	—	—	—	1	—	—	1	2
	Disorganized	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Multipolar	2	1	5	9	12	4	7	5	5	8	8	9	4	4	4
	Apolar	—	2	14	12	10	5	8	4	4	6	2	9	10	16	10
	Apolar+seg.	1	—	3	1	2	1	1	—	—	—	—	—	—	—	—
Telophase	Normal	7	—	—	—	—	—	—	—	—	—	1	—	—	1	2
	Multipolar	2	3	—	—	5	—	—	—	13	—	—	5	—	—	—
	Apolar	—	1	5	9	8	1	—	—	—	—	1	2	5	2	—
	Apolar+seg.	—	—	1	7	—	9	11	7	—	2	11	—	8	6	7
Total	Prophase	70	57	33	41	41	53	48	62	54	54	51	45	48	27	49
	Metaphase	14	36	39	21	22	27	25	22	24	30	25	30	25	43	26
	Anaphase	7	3	22	22	24	10	16	9	9	14	11	18	14	21	16
	Telophase	9	4	6	16	13	10	11	7	13	2	13	7	13	9	9
Mitotic index		4.9	2.9	5.9	5.3	4.2	4.1	6.0	4.2	6.9	4.2	6.5	6.2	4.7	6.0	4.3
Bi-,multi-nucleate		—	+	+	+++	++	4+	4+	5+	5+	6+	6+	6+	4+	+++	4+

APPENDIX TABLE V

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES IN CONTINUOUS CONTROL OF *PISUM SATIVUM*

	Time(hr,)	0	1	2	3	4	6	8	24
	No. of slide	31	2	2	2	2	2	2	2
Prophase early	Normal	29	29	29	26	25	23	40	28
	Spread	-	-	-	-	-	-	-	-
	Segregated	-	-	-	-	-	-	-	-
Prophase mid	Normal	27	16	24	25	24	24	20	20
	Spread	1	-	-	-	-	-	-	-
	Segregated	-	-	-	-	-	-	-	--
	Over-contract-		-	-	-	-	-	-	--
Prophase Late	Normal	5	5	3	7	5	8	3	10
	Spread	-	1	6	1	-	-	-	-
	Segregated	-	-	-	-	-	-	-	-
Metaphase	Normal	19	20	17	16	20	19	19	17
	Disorganized	1	1	-	-	-	1	-	1
	Spread	-	2	1	1	1	1	1	-
	Sp.+ seg.	-	-	-	-	-	-	-	-
	Scattered	-	-	-	-	-	-	-	-
	Sc.+ seg.	-	-	-	-	-	-	-	--
	Clumped	-	-	-	-	-	-	-	-
	Ball	-	-	-	-	-	-	-	-
Anaphase	Normal	9	9	6	7	5	8	6	8
	Disorganized	1	-	1	1	1	1	-	-
	Polar	-	-	-	-	-	-	-	-
	Apolar	-	-	-	-	-	-	-	1
	Apolar+seg.	-	-	-	-	-	-	-	-
Telo-phase	Normal	8	17	13	16	9	15	11	14
	Polar	-	-	-	-	-	-	-	-
	Apolar	-	-	-	-	-	-	-	-
	Apolar+seg.	-	-	-	-	-	-	-	-
Total	Prophase	62	51	62	59	64	55	63	59
	Metaphase	20	23	18	17	21	21	20	18
	Anaphase	10	9	7	8	6	9	6	9
	Telophase	8	17	13	16	9	15	11	14
Mitotic index		6.3	6.2	5.5	5.1	5.5	5.0	7.4	4.1
Bi-,multi-nucleate		-	-	-	-	-	-	-	-

APPENDIX TABLE VI

PERCENTAGE DISTRIBUTION OF MITOTIC STAGES IN CONTINUOUS TREATMENT OF PISUM SATIVUM

		Time(hr.)	1	2	3	4	6	8	24
		No. of slide	4	4	4	4	4	4	4
Prophase	Early	Normal	20	22	16	18	15	24	13
		Spread	--	--	--	--	--	--	--
		Segregated	--	--	--	--	--	--	3
	Mid	Normal	35	28	27	26	21	23	18
		Spread	6	9	6	3	6	1	1
		Segregated	--	--	--	--	--	--	3
		Overcontracted	--	--	--	--	--	1	--
	Late	Normal	4	2	2	3	3	3	3
		Spread	1	1	--	--	--	1	--
		Segregated	--	--	--	--	--	--	1
Metaphase		Normal	8	3	1	--	--	--	--
		Disorganized	1	1	1	--	1	1	--
		Spread	3	12	11	10	12	10	2
		Sp.+seg.	--	3	1	--	9	2	--
		Scattered	1	2	10	9	7	10	24
		Sc.+seg	--	--	--	--	1	--	9
		Clumped	2	2	6	11	8	7	5
		Ball	--	--	--	--	1	1	--
Anaphase		Normal	5	2	1	--	--	--	--
		Disorganized	2	1	--	--	--	--	--
		Polar	1	1	1	1	1	1	--
		Apolar	1	2	5	6	3	6	7
		Apolar+seg.	1	3	3	3	5	3	5
Telophase		Normal	8	3	4	--	--	--	--
		Polar	1	1	--	--	--	--	--
		Apolar	--	--	2	7	4	4	2
		Apolar+seg.	--	2	3	2	3	2	4
Total		Prophase	66	62	51	50	45	53	42
		Metaphase	15	23	30	31	39	31	40
		Anaphase	10	9	10	10	9	10	12
		Telophase	9	6	9	9	7	6	6
Mitotic index			7.0	6.3	6.1	5.5	5.5	6.8	10.6
Bi-,multi-nucleate			+	+	+	++	+++	++++	++++++