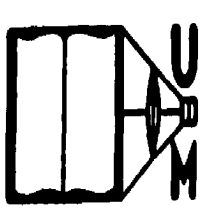
TITLE THE CYTOLOGICAL EFFECTS OF THE ANTIBIOTIC ACTI-DIONE

AUTHOR MARY ELIZABETH HAWTHORNE

UNIVERSITY MICH. STATE COLL. DATE 1951

DEGREE Ph.D. PUBLICATION NO. 3077



This is to certify that the

thesis entitled

The Cytological Effects of the Antibiotic Acti-dione

presented by

Mary Elizabeth Hawthorne

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany (Cytology)

G.B.Wilson
Major professor

Date 17 Aug.1951.

O-169

THE CYTOLOGICAL EFFECTS OF THE ANTIBIOTIC ACTI-DIONE

By

Mary Elizabeth Hawthorne

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

1951

FRONTISPIECE

FIGURE

A. Prophase "reductional" grouping from untreated material.

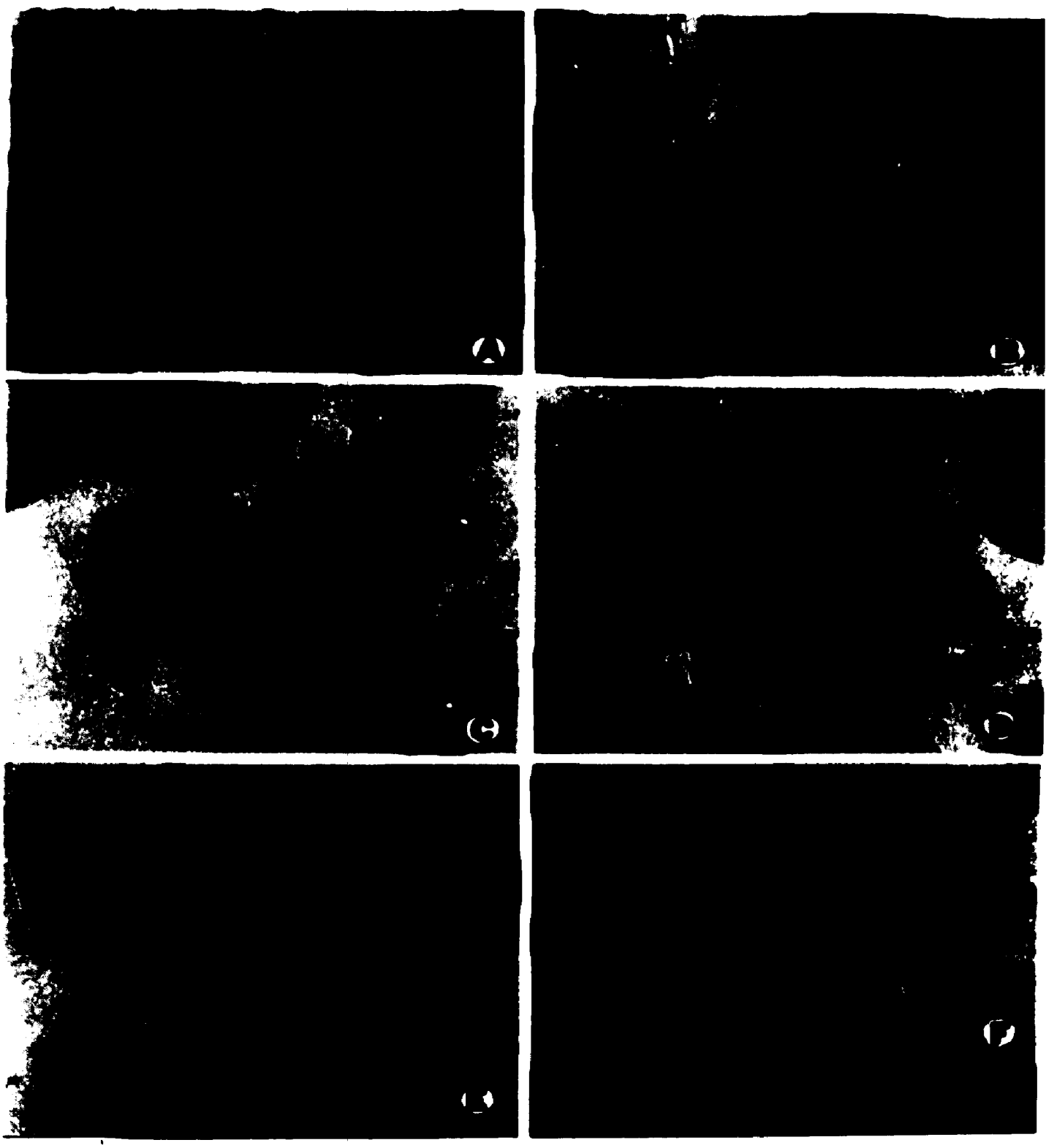
B. Metaphase "reductional" grouping from untreated material.

C. "Scattered" metaphase with chromosome fragment from root tip taken after four hours of continuous treatment with 40 ppm solution of Acti-dione.

D. Split telophase from root tip taken six hours after initiation of short treatment with 5 ppm solution.

E. "Scattered" metaphase (c-mitosis) in which chromatids have "fallen" apart, from root tip taken after nine hours of continuous treatment with 40 ppm solution.

F. Disorganized anaphase from root tip taken four hours after initiation of short minute treatment with 5 ppm solution.



ACKNOWLEDGEMENTS

The writer wishes to express deep appreciation to Dr. G. B. Wilson at whose suggestion the investigation was initiated and under whose direction the project was conducted. His actual counting of a duplicate series of slides made it possible to reduce the subjective element inherent in the method. His constant guidance and encouragement made the completion of the work possible; his patience and good nature made the experience an enjoyable one.

To Mr. Philip G. Coleman the writer wishes to express her sincere thanks for all of the photographic work and especially for the preparation of the Flexichrome Frontispiece which was included at his suggestion and which required so much of his time to complete. In addition, his frequent suggestions during the course of the investigation have, indeed, been most helpful.

The writer is also indebted to the following:

Dr. Andrew D. Perejda for the preparation of the final drafts of the text figures.

The All College Research Committee and the Michigan State College Experiment Station for making funds available for the work.

The People of the United States who, through the G. I. Bill of Rights, made it possible for the writer to pursue her graduate studies of which this investigation was an important unit.

Mary Elizabeth Hawthorne

candidate for the degree of

Doctor of Philosophy

Final examination, August 17, 1951, 2:00 P. M., Botany Seminar Room.

Dissertation: The Cytological Effects of the Antibiotic Acti-dione

Outline of Studies

Major subject: Botany
Minor subject: Zoology

Biographical Items

Born, November 9, 1916, New London, Connecticut

Undergraduate Studies, Rhode Island State College, 1933 - 37

Graduate Studies, The Pennsylvania State College, 1938 - 40, The University of Minnesota, 1948 - 49, Michigan State College, 1949 - 51

Experience: Instructor in Botany and Zoology, Undergraduate Centers Division of the Pennsylvania State College, 1940 - 44, Aviation Physiologist, U. S. Navy (WR) 1944 - 45, Assistant to the Officer in Charge of U. S. Navy Rehabilitation Training School, 1945 - 46, Instructor in Botany and Zoology Undergraduate Centers Division of The Pennsylvania State College, 1946 - 48, Graduate Teaching Assistant, Department of Natural Sciences, University of Minnesota, 1948 - 49, Graduate Teaching Assistant, Department of Basic Biology, Michigan State College, 1949 - 51.

Member of Phi Kappa Phi, Society of Sigma Xi, Sigma Delta Epsilon, The Genetics Society of America, Phi Sigma, Tau Kappa Alpha, Chi Omega Sorority, American Association of University Women, U. S. Navy Research Reserve Unit 9-16, Lansing, Michigan.

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AN ABSTRACT

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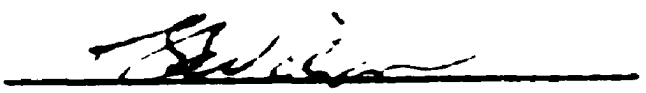
Year 1951

Approved 

Mary Elizabeth Hawthorne

To study the nature, and possibilities of the origin, of the cytological aberrations produced by the antibiotic Acti-dione in the meristematic tissues of onion root tips and to determine the thresholds of toxic as well as of cytological effectiveness were the main objectives of this investigation.

The meristems were treated with solutions of varying concentrations ranging from 1 ppm to 80 ppm continuously for twelve hours and also for a 15 minute period followed by a distilled water leach. From Feulgen stained smear preparations, a study was made of the various types of division figures which appeared at hourly intervals during the first twelve hours after treatment was initiated.

With the exception of the cytologically ineffective 1 ppm short treatment, it was found that the first five hour period following the initiation of treatment was the most critical period for the observation of the effects of Acti-dione upon this meristematic tissue. Ultimately there was a general trend toward a reduction in the numbers of division figures following the short treatment and an eventual stability in these numbers in the continuously treated root tips. Differences in the relative frequencies of the individual stages of mitosis appeared depending upon the concentration and the method of treatment. Evidence was found that the frequencies of the aberrations in the different stages varied; the highest percentages occurred in prophase and the lowest in anaphase. The frequency percentages of the several types of aberrations appeared to have been correlated with time rather than with either the concentration or the method of treatment.

No recoverable aberrations were found.

The possibilities of the interrelationship of the several types of aberrations as well as the cytological and genetical potentialities of each are discussed.

It was suggested that the threshold of toxicity may be very close to that necessary for the production of the cytological aberrations and the threshold of each may be found in some combination of concentration and time between 1 ppm and 5 ppm and between 0 and 15 minutes. It was concluded that Acti-dione appeared to have acted in a manner similar to that of a slow fixing agent with a differential effect upon the nuclear and cytoplasmic constituents of the cell and, as the degree of the fixation was increased, the several types of aberrations appeared. Assuming this analysis of the observed mitotic breakdown to be correct, the following postulates concerning the activities in normal mitosis were offered: (a) a gradual formation of the spindle, (b) cytoplasmic origin of, at least, some parts of the spindle, (c) the possibility of the origin of anaphase movements from some mechanisms arising within the chromatids themselves.

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INTRODUCTION

The cell may be considered a pivot around which the functions of living organisms revolve. When considered in this light, the activities of the living cell assume such fundamental significance that one is stimulated to seek answers to unsolved biological problems at the cellular level.

One of the intriguing processes of cells that, so far, has defied analysis is the mechanism of cell division. From observations of the process and studies of fixed material containing dividing cells there have evolved many hypotheses but few uncontestable theories concerning the mechanisms involved. Since the study of anomalies has been so fruitful in other branches of biology this technique was adopted by cytologists as a promising route toward a better understanding of these perplexing mechanisms. Accordingly, dividing cells have been subjected to almost every conceivable type of chemical and physical treatment and the effects noted. It is the basic assumption in this research that by watching the process break down one can, perhaps, find some of the missing clues to the mechanisms, either in terms of forces or processes involved, or in terms of a better understanding of the activities of the chromosomes themselves. Innumerable investigators have pursued this task and the literature resulting from their labors is becoming increasingly extensive. A complete review of this previous work is beyond the scope of the present report, but such an activity would undoubtedly result in an assemblage of facts which, when placed side by side, might

form the foundation of an entirely new hypothesis.

For purposes of the orientation of the present research, a summary of some of these investigations was prepared and will be found in Appendix I. Some of the references cited there carry extensive bibliographies which should be studied before the complete picture will be seen.

The unavoidable subjectivity that permeates this literature, coupled with a complete lack of standardization of terms, make the task of the preparation of such a table a most difficult one. An observation which one investigator may consider to be paramount in significance another may have neglected or overlooked entirely. In some cases it appears that the same condition has been given different terms. In this table notations were made only in those cases where specific statements were made concerning the particular condition. There is a more or less general agreement that the effects produced are correlated with the concentration used. The table reports the cytological effects throughout the series of concentrations used.

For purposes of this discussion some generalizations may be drawn from the accumulated reports. The phases of this material that immediately impinge upon the present research may be divided into two parts.

1. The general results obtained from other types of treatment.

2. The consensus of opinions concerning the nature of the mechanism involved in the breakdowns.

These diverse treatments, both chemical and physical, produce results that may be classified into two overlapping categories: (1) the mitotic disturbances; and (2) the chromosomal aberrations.

On the basis of the ultimate effects produced the mitotic disturbances may be further partitioned into two sub-groups: (a) Those which can be perpetuated since the cells generally recover and continue to divide according to a more or less regular pattern producing daughter cells which carry on the new condition; (b) Those mitotic disturbances the effects of which, at present, have not been perpetuated since the cells generally do not recover from the disorganization induced by the treatment. The first group would contain the polyploidizing agents such as: colchicine (18, 40, 45, 70 and 99), cyclochlorohexane (33, 72, 106 and 115), veratrine (144), acenaphthene (70, 79 and 99), and chloral hydrate (95 and 101). The second group would contain those substances which are potentially capable of producing polyploidy, of inducing reductions in chromosome numbers and, consequently, resulting in genetical segregation. These potential polyploidizing agents result in the condition that has now become known as "c-mitosis."[1] In the absence of the spindle, the chromosomes are scattered throughout the cell and the delayed division of the kinetochore results in the production of X-shaped chromosomes. Eventually the chromatids may fall apart and restitution nuclei may be formed but, as previously mentioned, these cells generally do not divide again. Thus they are only potentially capable of producing

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[1] "C-mitosis" is the term suggested by Levan (76) to refer to the type of mitosis produced by colchicine. In this respect the term implies the polyploid result. Since the appearance of cells following other types of treatments is practically a duplicate of that following colchicine but (as pointed out above) these conditions do not necessarily lead to polyploidy, the term has now come to refer to the two outstanding effects of colchicine treatment, namely, spindle disruption accompanied by a delayed splitting of the kinetochores of the over-contracted chromosomes.

polyploidy. Tables I and II of Appendix I show the diversified types of chemicals that have been reported as capable of producing this action. Some of these are: phosphates (52), nitrates (82), DDT (135), antibiotics (142), salts of nucleic acid (69), ethylene glycol (107), toluidine blue (8), certain monocyclic organic compounds (86), and certain naphthelene derivatives (86).

As previously mentioned the treatments which may result in mitotic conditions which potentially may lead to the reduction in chromosome numbers and, consequently, genetical segregation, should be placed in this non-recoverable category. The terms "reductional" or "segregational" groupings are frequently used to refer to this situation (1, 63, 64, 113, 142 et al). Because, at first he felt he had found an homology with the regular method of reduction preceding the change from sporophyte to gametophyte numbers, Huskins originally used the term "somatic meiosis" to refer to this situation (63). This type of effect is reported as such after the following treatments: sodium nucleate (63), colchicine (2), and Acti-dione (142). The "exploded c-mitoses" of Barber and Callan (6) and the "distributive c-mitoses" described by Nybom and Knutsson (106) appear to involve the same arrangement as that termed above "reductional" or "segregational" groupings. On this basis some chemicals may be added to the list of treatments that increase the incidence of these arrangements of chromosomes in actively dividing tissue; such as methyl naphthoquinone (106) and the nitrates (82). It should be pointed out here that because of this difference of opinion concerning their significance it is quite likely that this effect has been overlooked by some investigators, and

that many more chemicals may be found to cause the same effect if later workers are alerted to look for them.

Among the chromosome irregularities in Appendix I "stickiness," shortening, lengthening, erosion, and fragmentations are listed. Of these, probably the erosions and fragmentations are of greatest cytological and genetical significance. One is impressed with the similarity between the chemical and physical treatments in producing some of these effects on the chromosomes. Fragmented chromosomes have been reported after treatments with the following chemicals: acenaphthene, 9-aminoacridine and phosphene-5-G (34), methylene and toluidine blue (8), borneol (43), nitrogen mustard (105), radiophosphorous (44), and several phenols (87 and 88). The same result has been obtained through the use of the following physical agents: X-rays (87 and 130), fast neutrons (57), and ultrasonic vibrations (105). It may be of interest to note here that Levan and Tjio (87) pointed out that, unlike X-rays, the phenols seldom induce translocation and reported that the damage caused by the chemical in cutting through the chromosome thread usually does not allow healing. Whether or not this is true of other chemicals could not be discerned from the reports.

Numerous investigators have attempted explanations of the mechanism of c-mitosis. Most of the work was based upon the assumption that, because of the similarity in the appearances of the result, mechanism was similar for all treatments and common factor in all treatments was the main objective in these researches. Both chemical and physical explanations have been proposed. On the chemical side, the early workers attempted to learn which

part of the colchicine molecule was responsible for the activity. As a result of their work, Brues and Cohen (20) pointed out that, of the variety of molecular groupings which compose the colchicine molecule, it was not possible to say that any single group is essential for the activity of colchicine on mitosis and because of this lack of specificity urged an examination of other synthetic compounds of analogous structure. Following this work, a large series of organic compounds was tested and a great many reports appeared. A review of these earlier opinions was presented by Levan and Ostergren (86). From the latter survey one may deduce that the diversified conclusions were about as numerous as the investigators, and the activity was attributed to whatever particular variable the worker happened to choose to vary. For example, the activity was ascribed to the aromatic nucleus by Simonet and Guinochet (127), to the amino groupings by Favorsky (47) and to some chemical structure similar to that of the carcinogenic hydrocarbons by Shmuck and Gusseva (125). Finally, Steinegger and Levan (133), working with iso-colchicine concluded that the "c-ring of the colchicine molecule is of decisive significance for the specific activity on Allium roots, because if the keto- and methoxyl-groups of ring C are interchanged, as it is in the case of iso-colchicine, the specific effect of colchicine disappears."

A few explanations stem from the basic assumption that a physical mechanism is involved in the production of c-mitotic activity. Although he assumed some changes of a more "qualitative" nature to be present, Wada (139) opined that the action of colchicine is mainly due to its ability to reduce the surface tension of the "atrakoplasma" and Shiganaga (121) concluded that the spindle disturbances produced by the various

poisons, chloral hydrate, nicotine, and caffeine, operated in a manner similar to that of hypertonic sugar solution, that is, they caused a dehydration of the "kinoplasm" (spindle or phragmoplast). It might be of interest to note that both of these latter ideas were developed from observations made on living cells. As a great variety of chemicals of widely divergent nature were reported in the literature as capable of inducing c-mitosis, Levan and Ostergren (86) noted that the vast majority of these materials, which were most active, were least soluble in water, and began working on a new hypothesis. In testing the alpha and beta derivatives of naphthalene they found a definite positive, inverse correlation between water solubility and the thresholds of c-mitotic action; i.e., the latter increased as the former decreased. This fact, coupled with the wide divergence in the chemical nature of the c-mitotic substances, as well as the fact that many of the narcotics tested were found highly effective, led them to the opinion that the essential nature of the c-mitotic activity is physical and does not depend directly upon the chemical composition of the substance. Because of the inverse correlation, they arrived at the conclusion that fundamentally the action of these c-mitotic substances was a narcotic one and that the "Meyer-Overton theory of narcosis may hold in the cases of the c-mitotic substances, i.e., the decisive concentration of the substance may be that in the lipoids, not in the water phase of the cells." Ostergren (108) elaborated upon this idea and described the protein chain folding he believed responsible for spindle destruction after the penetration of the substance. He recognized that colchicine does not fit into this hypothesis and because of this suggested that some substances probably react chemically.

The observations that some of these chemicals, in addition to inhibiting the spindle, had other effects started some new lines of thinking. Kodani (69) noted, among the various effects of sodium nucleate solutions, an inhibition of chromosome reduplication and the formation of akinetic fragments. D'Amato (31), after testing a series of chemicals, observed that two different effects must be distinguished: (1) preprophase inhibition and (2) an inhibition of spindle formation giving rise to c-mitosis. He was of the opinion that "the spindle-inhibiting effect, manifested in typical c-mitotic poisons, may be of quite different sort than that by which preprophase poisons induce the c-mitotic effect." Galinsky (53) found that following the phosphate treatments the deviations from normality were observed during prophase in the delay of nuclear membrane "breakdown." He pointed out that this effect had not been observed after the use of the c-mitotic poisons. These substances had been reported to permit mitosis to proceed normally (76) until metaphase when their specific effect on the spindle mechanism was manifested. Accordingly, he concluded that, "the unoriented prometaphase chromosomes must therefore involve forces other than those associated with the oriented spindle mechanism." A distinction between the "mitotic poisons" of which colchicine was considered a type substance and the "physiological substances" exemplified by the nucleate salts was made by Allen, Wilson and Powell (2). Working from the chemical viewpoint, Loveless and Revell (91) attempted to correlate the chemistry and modes of action of certain of the different classes of compounds that had been described as having an effect upon dividing cells. They felt that the "mitotic poisons" could be classified into at least three distinct classes each of which would show a chemical

homogeneity correlated with biological activity: (1) compounds known for some physiological activity which include the bacteriostatic and anti-protozoal agents and anti-growth substances of plant origin which exert a highly toxic, non-specific effect upon the cell, (2) the vesicants, such as sulfur, nitrogen mustards, di-epoxides and proteanemonin, which have a highly specific effect upon the resting nucleus which is manifested in subsequent mitosis by the appearance of breakage and rearrangements of various kinds, (3) the compounds capable of destroying or preventing the formation of the mitotic spindle without undue toxic effect upon the cell, such as colchicine. Hence, the question of the underlying mechanism of c-mitosis remains unanswered, and will probably continue to be unsettled until the underlying issues related to the modes of operation of the several treatments are first decided upon.

The objective of these previous investigations was primarily the determination of the type of effect produced by a particular chemical, the chemical group, or radical responsible for the reaction. The "effective" concentration threshold was stressed. The technique for determining this threshold essentially involved the application of the chemical for an established period of time. Among the several investigators the time period varied. For example, four hours, twelve hours and three days were the established times for examination of test material at the Cytogenic Laboratory at Svalof (131). This was the standard procedure for Levan, Ostergren, Steinegger and all workers at that station. The Wisconsin group mention examination of their material at "various times" (52 and 69). Hence, the bulk of the present literature contains reports of observations made on fixed material after continuous treatment

with diverse chemicals at varying concentrations and examined after rather wide intervals of time.

Some investigators have been studying the effects of chemical treatments on living tissue (100, 121 and 139). In this research, stamen hairs or young petal cells, from the buds of Tradescantia sp., provided the dividing cells for the work. Although these experiments have resulted in some very interesting observations, in using this technique, the worker is limited to the reactions of a single cell at any particular time and, of course, is encumbered by optical limitations when making deductions from unstained cells.

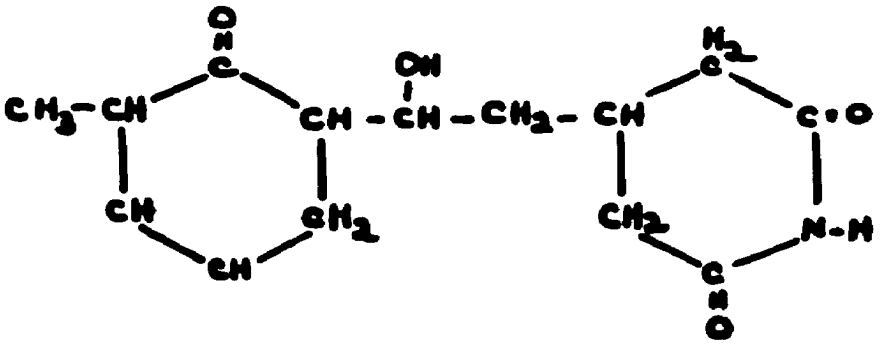
This is a report of a detailed quantitative analysis of the cytological effects of a single chemical, the antibiotic, Acti-dione. In a preliminary report of observations made after 24 hours exposure to 100 parts per million, Wilson (142) showed that the effects of this substance run the gamut of aberrations produced by most other chemicals. Accordingly, he stated that this "is one of the most interesting antibiotics yet investigated since it readily and consistently induces specific aberrations in mitotic behavior." For this reason it was selected for this study.

By varying not only the concentration, but also the time of treatment, and by studying the effects produced in sequential periods of time, particularly in the critical early interval after exposure, it was hoped that data could be obtained that would give some specific clues concerning the origin of some of these aberrations, and would yield some information concerning the nature of, if not the mechanism involved in, these

irregularities. More specifically, by a quantitative study of the aberrations it was hoped that it would be possible to learn whether or not any specific relationship existed between the occurrence of the irregularities and such factors as the concentration, the time and the method of treatment. With these data available it was thought that it would be possible to define, in terms of concentration and time, a particular threshold at which recoverable aberrations might be obtained.

MATERIALS AND METHODS

The fungicidal antibiotic Acti-dione (137 and 141) produced by Streptomyces griseus (49 and 73) and used in this series of investigations, was obtained through the courtesy of the Upjohn Company, Kalamazoo, Michigan. With a melting point of 115 - 115°C. and a solubility of 21 mg./ml. at 2°C. and between 25 to 30 mg./ml. at room temperature, it has a chemical formula as follows:



The root tips developed from the common commercial yellow variety of Allium Cepa L. provided the meristematic tissue for the study of the cytological effects. For practically all of the experiments included in this report, the bulbs were obtained from a single crop produced by Mr. Emil Pontack of Elsie, Michigan.[1]

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[1] Three series 92, 95 and 96 had been completed before the Pontack onions were obtained but this supply was exhausted in subsequent tests so it was not possible to duplicate them. However, since duplicates of these three series produced consistent results it was considered unnecessary to repeat these treatments.

In the earlier series the bulbs were rooted in tap water and the treatment solutions were prepared with distilled water. After treatment and a thorough washing with distilled water, all onions were then placed in distilled water which was changed every hour for the recovery tests. Later, the onions began to produce fewer and fewer roots in the tap water provided in the laboratory and it became necessary to change this technique somewhat. After testing water from a variety of sources, it was found that aerated distilled water was the most successful rooting medium. Accordingly, this procedure was used for all the subsequent series. The rooting medium and the treatment that followed is shown in Table I.

TABLE I

ROOTING MEDIA AND SUBSEQUENT TREATMENTS OF THE VARIOUS SERIES

TAP WATER			AERATED DISTILLED WATER		
Series	Treatment PPM	Duration	Series	Treatment PPM	Duration
			145 & 174A	1	Short (15 min.)
			138 & 152B	5	Short (15 min.)
92	20	Short (15 min.)	140	20	Short (15 min.)
96 & 120	40	Short (15 min.)			
118 & 122	80	Short (15 min.)			
			146 & 151	1	Continuous
			139 & 152A	5	Continuous
128	20	Continuous	134	20	Continuous
95 & 125	40	Continuous			
102 & 115	80	Continuous			
126	40	Intermittent (15 min./2 hrs.)	135	40	Intermittent (15 min./1 hr.)
127		Untreated (Consecutive hrs.)	132		Untreated (Consecutive hrs.)
			137B		Untreated (Same hr.)

MacFarlane and her co-workers (93) studied the effects of water source on the toxicity of some mercurial poisons. They reported that the transfer from tap to distilled water retarded growth of the onion root tips and proved fatal sometimes unless the change was made through mixtures of the two waters. In addition, they pointed out that almost any sudden physical change in the medium impedes growth.

In numerous tests conducted in this laboratory all sorts of water combinations were tried in the general procedure pattern of rooting, treating and leaching after treatment. So far, no appreciable differences have been observed among the numerous combinations investigated. It must be pointed out, however, that in these studies a transfer from either tap water or treatment solution directly to thoroughly distilled water was not made and hence, the possibility of the physical shock of sudden change in water alone can be eliminated. In addition, the distilled water available in this laboratory is once distilled in a metal still and is not completely free of all ions.

Since the roots tolerated more poison in "raw" water taken before purification in the water works, MacFarlane concluded that the imbalance of ions in city water plays an important role in debilitating the tissues and urged that the tonicity effects should be taken into consideration in the interest of the standardization of the Allium test.

It will be observed in Table I that, in this investigation, for those series in which the greatest dilutions were tested, onions were used which had been rooted in aerated distilled water. For the 80 ppm concentration there was one series of each type. Reference to Appendix II, Tables 5 and 8, will reveal that the results obtained from the onions

rooted in tap water were consistent with data obtained from those rooted in the distilled water. In the higher concentration series the transfers were made from tap water to 40 and 80 ppm solutions. One control series for each rooting medium is included in these results. In the highest dilutions (1 ppm and 5 ppm), rooting, treatment and recovery solutions were prepared from distilled water thus precluding the possibility of a distortion of results by tonisity effects.

A more or less standard method of procedure for the treatments was used. For rooting, the bulbs were arranged on small glass containers in such a manner that from one-fourth to one-half inch of the bulb protruded into the rooting medium. When roots were from one and one-half to two and one-half inches in length the root tips were examined and, if high mitotic activity were in evidence, treatments were started by placing the young roots in the treatment solution and supporting the bulb over the container. When treatments were completed all bulbs were placed over distilled water which was changed every hour and a check for recovery of the tissues was made.

Root tips were collected in a three to one absolute alcohol-acetic acid mixture, fixed for fifteen minutes at 60°C, hydrolized in one normal hydrochloric acid for eight to ten minutes, stained with Feulgen's stain and smear preparations made. After dehydration in a 95% ethyl alcohol to which a little fast green for counterstain had been added, the slides then were made permanent with diaphane.

A stock solution of 100 ppm was prepared by dissolving the powdered Acti-dione in distilled water and kept refrigerated until time for use. When dilutions from the stock solution were prepared, care was taken that

the treatment solutions assumed room temperature before the roots were placed in them.

A preliminary series of experiments were conducted in which the root tips were collected every fifteen minutes and examined. Counts made on these slides indicated that this interval was too short to reveal changes occurring and it was decided that during the course of the further series the root tips would be collected every hour. It was further decided, as a result of these exploratory tests, that two main types of treatments would be followed for the detailed, comparative, quantitative study: (1) a short treatment in which the young roots would be exposed to the test solution for a period of fifteen minutes and then transferred to the leach water and (2) a continuous treatment in which the roots would remain in the solution for a period of twelve hours. For each of these, two types of treatments concentrations of 1, 5, 20, 40 and 80 ppm were used. For purposes of comparison, two intermittent series were included with the concentration of 40 ppm for fifteen minutes out of every hour and also the same period every two hours. When it was found that these two series produced results similar to those of the continuous and short treatments respectively, they were abandoned.

Early investigators have reported a periodicity in the mitotic cycle in onions. Lewis (69) concluded that under conditions of normal light and dark, there were two waves of mitotic activity, the maxima of which came at midnight and at noon. Kellicott (66) was of the same opinion but set the maxima at 11 P.M. and 1 P.M. Friesner (51) reported that the maxima and minima depended not upon the time of day but rather

on the time of initiation of metabolic activity. These conclusions were reached after observations were made of samples taken at two hour intervals. As a result of their investigation in which root tips of Allium were collected every hour and the numbers of division figures for a predetermined area were counted, Solomon and Trent (130) concluded that under normal conditions of day and night, there were no definite waves of mitoses but rather an hourly rhythmicity of mitosis in this particular plant. Their graphs show maximal mitotic activity at 5 A.M., 8 A.M., 10 A.M. and 6 P.M.

In order to determine the regular mitotic pattern of the root tips treated in this series of experiments, three types of controls were set up. (1) A consecutive hour control for which root tips were taken from an untreated onion at the same time that they were collected from the treated bulbs. (2) An individual root control which was prepared by taking 25 root tips from the same onion at the same hour and preparing the slides. From this group, twelve slides were selected at random for the study. (3) A series control for which a sample was taken just before the roots were placed in the treatment solutions. The data from all these controls are included in Appendix II. Since the variation among the roots taken from the same onion at the same time was even greater than the variation among those taken at consecutive hours the significance of mitotic cycles may be minimized.

Throughout the entire investigation the onions were kept under normal conditions of day and night. All experiments were conducted at room temperature, and, although this fluctuated a little, the variation

was not great enough to have had any appreciable effect upon the results. During the course of the research the bulbs were started at various hours of the day and night, and the root tips were checked before treatment for high mitotic activity.

After the preliminary, exploratory experiments, in which the various types of effects were observed, discussed and categorized, a standard data sheet was prepared. Table II, page 19, is presented as a sample of the recording method.

For each set of treatments two root tips were taken every hour and placed on separate slides. In order to reduce the subjective element, one slide for every hour of each treatment was counted by Dr. G. B. Wilson, the director of this research, and the duplicate slide by the writer.

The procedure for counting was standardized between the two observers. Each slide was placed on the calibrated mechanical stage, the count was started at a constant, predetermined point on the vertical axis and this reading recorded on the data sheet. Consecutive high powered fields were counted from edge to edge of the 22 x 22 mm cover slip and these totals are recorded in the tables as "axis" totals. The vertical axis was increased by one unit each time and the count continued until a total of approximately 100 division figures[1] were obtained from each slide. In the slides where the 100 figures were achieved in the middle of the axis, the count was continued until the

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[1] There was one exception to this: in order to equalize numbers in the case of the untreated bulb in which several roots were collected at the same hour 200 division figures for each slide were counted.

TABLE II

SAMPLE COPY OF THE DATA SHEETS

TREATMENT _____ SLIDE _____

	PROPHASE					METAPHASE					ANAPHASE				TELOPHASE	
	UNAFFECTED		AFFECTED			ORGANIZED		DISORGANIZED			ORGANIZED		DISORGANIZED		UNAFFECTED	AFFECTED
AXIS	Not segregated	Segregated	Not segregated	Necrotic	Segregated	Normal	Split	Scattered	Clumpings	Segregated	Normal	Split	Oriented	Not Oriented		
1220																
1230																
1240																
1250																
1260																
1270																
Total																

readings on that axis had been completed. In those cases where, in the later hours, the division figures were less numerous, it was frequently necessary to go back over the slide and take readings at the one-half unit intervals. In several cases duplicate counts were made from the same slide to check on the consistency of the results. Summaries were prepared of the two sets of readings and these are presented in Appendix II. Since two slides of the root tips taken at hourly intervals in two series of each treatment were prepared and counted and this data later combined, the hourly percentages and averages in most cases were based on totals ranging from 450 to 500 cells.

It is readily recognized that, aside from the subjective element mentioned above, two factors may influence the counts: (1) the evenness of the distribution of the material and (2) whether or not the most actively dividing areas of the meristem happen to have fallen within the predetermined area of the count. However, since it can be assumed that all areas of the root tip have an equal chance of being evenly distributed within the predetermined area of the count, it is believed that this technique may be used to obtain an indication of trends and should be interpreted accordingly. Since, for the most part, the several series upheld, within reasonable limits, under the consistency test, it was felt that the influence of these two factors in distorting the picture could be minimized.

OBSERVATIONS

Qualitative Aspects

The various types of cytological effects on the onion root tip meristems of the Acti-dione treatments can be seen by examination of the plates. For purposes of comparison, Plate I was prepared. It contains series of mitotic figures which illustrate what is generally considered as normally organized figures. The lateral view of a prophase in Pl. I, Fig. A, shows the chromosomes as they reappear with their telophasic polarity (Pl. I, Fig. H) retained since the previous division. At this stage it is thought (136) that, at the kinetochores, they are still in contact with the nuclear membrane even though, "considerable shifting may take place as the chromosome arms, which filled the nuclear space, come to lodge themselves more or less against the membrane." In the polar prometaphase (Pl. I, Fig. B), the contraction of the chromosomes is approximately completed and their positions just prior to the metaphase plate alignment (Pl. I, Fig. C) are shown. Normal anaphase separation, i.e., kinetochores first, can be seen in Pl. I, Fig. D, while later stages of anaphase movement appear in the two following figures (Pl. I, Figs. E and F). This movement is completed in the early telophase of Pl. I, Fig. G. The reorganization of the daughter nuclei, initiated in Pl. I, Fig. H, is completed in the two sets of adjacent cells in Pl. I, Fig. I, where two interphase nuclei can be seen lying to the right of the late telophase stage.

The cytological effects of the Acti-dione treatments may be subdivided into two major categories: (1) those which are primarily of interest because of their cytological and genetical potentialities and (2) those which reflect varying degrees of toxicity.

At the outset, it must be pointed out, that the cytological aberrations shown in the plates may be found as comparatively rare occurrences in the untreated material (See description of the plates). Furthermore, with the possible exception of those toxicity effects which invariably develop after long periods of continuous treatment, and with the higher concentrations after treatment for 15 minutes, no particular aberrant figure can be said to be exclusively the result of a single type of treatment, their occurrence being more closely correlated with time than with concentration. These quantitative aspects of the problem will be discussed more fully later after a survey of the several types of effects has been made.

There are two main types of effects of particular cytological interest. (1) The organized figures which give the appearance of having a partially inhibited spindle and which, depending upon the degree of spindle suppression, can probably return to the normal condition in the succeeding stage. (2) The disorganized figures which reflect total inhibition of the spindle mechanism and which ordinarily seem unable to return to the normal pattern in that particular division.

The single type of organized figures are those which have been called "reductional" groupings. A series of cells containing "reductional groupings are assembled in Plate II. In general, these configurations occurred most frequently in the early period after treatment, i.e., about

four hours or less.

Probably depending upon the mitotic stage of the cell at the time the Acti-dione becomes effective upon it, there are two possibilities of the origin of these "reductional" groupings. In the first place, it is possible that these separations, in some cases at least, may develop from "normal" figures. For example, the metaphases shown in Pl. II, Figs. C and D may have developed from an inhibition of the middle section of a normal spindle, and, likewise, the split anaphase in Pl. II, Fig. F may have followed a normally organized metaphase plate by a disruption of the usual by-polarity of the spindle. By the same token, the split in Pl. II, Fig. H telophase may have originated during or after normal anaphase movement.

On the other hand, it seems equally plausible that, depending upon such factors as the size of the cell, width of the gap, and the orientations of the groups of chromosomes, the separation may be retained through subsequent stages of the division and thus a divided metaphase plate (Pl. II, Fig. C), which may have originated from a prophase "reductional" grouping (Pl. II, Fig. A), may, in turn, develop into split anaphase and telophase figures (Pl. II, Fig. F, G and H).

These "reductional" groupings involved either equal (Pl. II A, Frontispiece A) or unequal (Pl. II, Figs. B, C and D; Frontispiece B) chromosome numbers. In addition, metaphase plates were observed in which the groups of chromosomes had either similar (Pl. II, Figs. C and D) or dissimilar orientations (Frontispiece B). This orientation may be a factor in determining whether or not the separation would be maintained in succeeding phases of the division. If the two sections of the split

plate are properly oriented (Pl. II, Figs. C and D) it is quite possible the gap between the chromosome groups may be closed (particularly in smaller cells) as they proceed through the next stage forming a normal late anaphase (Pl. I, Fig. F). In the cases where the divided groups have different orientations (Frontispiece Fig. B), it seems logical to assume that the separation is more apt to be maintained in the later stages. A comparison of Pl. II, Fig. F with Pl. II, Figs. H and I will illustrate this point. The wide separation between the upper groups in the first figure may be maintained so that the following telophase may appear similar to, or perhaps even more accentuated than, that shown in Pl. II, Fig. H. Furthermore, depending upon the size of the intergroup space, as well as the size of the cell, the gap may either remain separated or be closed during telophase as shown in Pl. II, Fig. I. If the latter occurs the result will be a return to the regular chromosome number. If the gap persists, however, three nuclei would be formed, one with the regular and two with reduced chromosome numbers. These nuclei might present the appearance of a triad such as that illustrated in Pl. VI, Fig. D.

Evidence that reduced nuclei can be formed in a few cases following unequal separations was found in the occasional occurrence of unbalanced late telophases (Pl. V, Fig. I), of binucleate cells with nuclei of unequal size (Pl. VI, Fig. C) of trinucleate cells (Pl. VI, Fig. D) and the very rarely occurring reduced cells (Pl. VI, Figs. E and F). The latter were found in the material taken too soon after treatment to have been the results of the Acti-dione influence and more than likely represent two naturally occurring "accidents."

In Summary:

1. These "reductional" groupings were characterized by the presence of elements of organization.

2. They may have developed from the normal condition at any stage in the mitotic cycle.

3. The separations involved both equal and unequal numbers of chromosomes.

4. The separated groups had both similar and dissimilar orientations.

5. Depending upon such factors as size of the cell, the orientation, and the width of the gap between chromosome groups the separations may or may not have been retained.

6. As far as this study is concerned they generally resulted in the production of nuclei which had lost their ability to divide.

Two types of disorganized figures that appear to have resulted from the complete inhibition of the spindle, namely, the "segregational" groupings and the "scattered" arrangements may be recognized. In this report the term "segregational" group is used to refer to those disorganized plates in which the chromosomes have been separated into two groups. In contrast to the organized "reductional" groupings which are most characteristically found in the early hours after the beginning of treatment, these more disorganized figures were found more frequently in the later hours after the initial exposure to Acti-dione.

Plate III contains a series of these "segregational" groups. In Pl. III, Fig. A and the upper cell of Fig. B, equal numbers of chromosomes have been grouped. The separation of the constituent chromatids

started in Pl. III, Fig. C has been completed in Pl. III, Fig. D. It will be observed that this movement differs from the normal type, in which the kinetochores move first (Pl. I, Fig. D), since here the chromatids appear to have merely fallen apart. In the absence of an orientation mechanism, it is highly probable that they may remain more or less close together with the result that each group of chromosomes may become included in a single nucleus. If the daughter nuclei are formed in this manner, the return to the original chromosome number will follow with the possible result of genetic segregation <u>without</u> a reduction in chromosome number.

An unequal (10-6) numerical separation of one of these disorganized figures is illustrated in Pl. III, Fig. D. The possible genetical significance of this will be discussed later.

In Summary:

1. These "segregational" groupings may be said to differ from the "reductional" type in their lack of organization, in being incapable of returning to the normal division pattern in the next phase of division, in being found more frequently when a longer period of time has elapsed after the beginning of treatment and in affecting segregation <u>without</u> reduction in chromosome numbers.

Examples of the second type of disorganized figures, i.e., those to which the term "scattered" arrangements has been applied are shown in the figures of Plate IV and Frontispiece Figs. E and F. They are of particular significance because, potentially, they may lead to the polyploid condition.

From the observations it appears that this disorganization may occur

at either prophase, metaphase or anaphase of normal mitosis. Pl. IV, Fig. A, shows the results of a prometaphase disruption (Cf. Pl. I, Fig.B). It is quite possible that these prometaphase disorganizations may have developed from "affected, non-segregated" prophases such as that shown in Pl. V, Fig. C. Neglecting the possibility mentioned above, of groups of chromosomes segregating, there are two possibilities of subsequent development of these disorganizations. In the first place, the prometaphases may give rise to "scattered" metaphases (Pl. IV, Figs. B and C). Also characteristic of these "scattered" metaphases is the delay in the splitting of the kinetochore as shown in Pl. IV, Fig. D. However, eventually the split may occur, the chromatids appear to fall apart (Pl. IV, Fig. F and Frontiepiece Fig. E) and, for a time, lie parallel to one another. If these chromatids should "drift" apart the result could be the disorganized anaphases such as shown in Pl. IV, Figs. H and I and Frontiepiece F. Eventually all of these chromatids may be included within a single nucleus and in this manner the polyploid condition is established. This has been shown to have been the case after treatment of onion root tip meristems with colchicine (76). The second possibility for the subsequent disposition of these disorganized prometaphases, especially of the type shown in Plate IV, Fig. B, and also of the "scattered" metaphases, such as figured in Pl. IV, Fig. C, sometimes become "sticky" and appear to fuse together at the ends resulting in a "clumped" metaphase such as that shown in Pl. V, Fig. F. Eventually the whole group of chromosomes contracts giving rise to a restitution nucleus, which, depending upon whether or not the chromatids separate may or may not become polytene.

It is possible that sometimes the disorganization may take place

during metaphase. This appears to have occurred in the figure shown in Pl. IV, Fig. E. In this case, because of their appararent alignment at an equatorial plate, it seems logical to assume that the spindle inhibitions must have occurred after the chromosomes had maneuvered to their "normal" metaphase alignment. Evidence of the obstruction of the spindle mechanism may be found in the fact that the chromatids appear to have fallen apart and lack the orientation of their "normal" separation (Pl.I, Fig. D). These cells may follow either of the patterns outlined above for division figures affected during prophase.

Spindle inhibition may also occur in an early anaphase cell such as shown in Pl. I, Fig. D. Assuming that chromatids separated in a normal manner, i.e., kinetochores first, and then the spindle ceased to function, the oriented chromosomes may then "drift" in all directions and practically fill the cell, in a manner shown in Pl. IV, Fig. G. This situation may result in the polyploid condition in the same manner as that described above for the unoriented chromatids that arise from the falling apart of "scattered" metaphases. It is equally reasonable, however, that there may be a clumping into more than one group with nuclei formed from each group and, as a result, bi-nucleate (Pl. VI, Fig. C) tri-nucleate (Plate VI, Fig. D) or even multinucleate cells be formed. Nuclei formed in such a manner would be aneuploid.

These "scattered" metaphases occurred in the highest frequency after exposures to the higher concentrations in both types of treatments. Furthermore, there was no recovery from any of the treatments in which the "scattered" metaphases approximated 100% of the total metaphases.

Since, in this investigation, neither polyploid nor aneuploid cells were found that could have occurred as a result of treatment, it may be assumed that, under the conditions provided by the leach water, there were formed restitution nuclei, which varied in their chromosome numbers and which did not divide again.

In Summary:

1. These disorganized figures reflect complete inhibition of the spindle.

2. The disorganization may occur at prophase, metaphase or anaphase stages of mitosis.

3. Subsequent development of these disorganized figures probably depends upon that stage at which the disorganization occurred.

4. Like the "segregational" groupings these "scattered" figures are found more frequently in the later observation periods.

5. Reversions to "resting" condition occurring when cells which have been affected in the early stages of mitosis result in the formation of restitution nuclei which may sometimes be polytene.

The toxicity effects of treatments may be sub-divided into three categories: (a) those which appear to affect the nuclear membrane, (b) those which seem to affect the matrix resulting in a chromosome "stickiness" and (c) deterioration of the nuclear constituents leaving only the spiral structure. Examples of these various toxic effects are shown in Plate V and Frontispiece Fig. D.

Cells in which the nuclear membrane appears to have been affected are shown in Pl. V, Figs. B, C, D and E. When affected in prophase, it

appeared that the membrane may have been retarded in its activity while the chromosomes continued to proceed through their regular morphological changes. Later the membrane appeared to have contracted drawing the contents of the nucleus into a tight ball. At this stage it is referred to as "necrotic" in this study. In Pl. V, Fig. B the chromosomes seem to have achieved almost their metaphase appearance while still occupying their early prophase position (Cf. Pl. I, Fig. A). In Pl. V, Fig. C the chromosomes have reached their metaphase length but they appear to be "held" inside the nuclear membrane, and in addition, some have lost their normal orientation as shown in Pl. I, Fig. B. It seems quite reasonable that nuclei of this type may revert to interphase in a manner similar to that shown in Pl. V, Fig. D. Eventually the nuclei of the types shown in Pl. V, Figs. B, C and D contract drawing the contents of the nucleus together and the "necrotic" condition results (Pl. V, Fig. E). These "necrotic" nuclei sometimes may arise by reversions of "scattered" metaphases by way of the "stickiness" of chromosomes as shown in Pl. V, Fig. F. Stages of this reversion have been observed in this study.

The second toxic effect mentioned above appears to involve a liquifaction or dissolution of the matrix and the chromosomes develop a "stickiness" manifested by their apparent fusions, first at the ends and later at points along their length. This type of toxic effect is illustrated in the metaphase plate of Pl. V, Fig. F, in the anaphase of Pl. V, Fig. G, and the telophase of Pl. V, Fig. H. There is also some of this "stickiness" evident in the split telophase of Pl. II, Fig. G. In the case of the unequally separated telophase groups of Pl. V, Fig. I, it

seems probable that this situation could have arisen from clumpings resulting from "stickiness" in a disorganized anaphase. It is possible that such a figure might result in a bi-nucleate cell such as that shown in Pl. VI, Fig. C.

The deterioration of the nuclear constituents is a third type of toxic effect observed in the course of this study. The spiraled chromonemata alone appears to be resistant to this, and as a result, in the cells affected in this manner often the spiral structure becomes evident. Frontispiece Fig. D and Pl. VI, Fig. G contain telophase figures in which the spirals are evident. The interphases shown in Pl. V, Fig. A give an indication of what happens when toxic conditions manifest themselves at this stage. Here it appears that the constituents of the nucleus have deteriorated until only the uncoiled chromonemata remained giving the nucleus a "thready" appearance not unlike that of the zygotene nucleus. These interphases are quite typical of the later hours after treatment with higher concentrations.

In Summary: The following were considered as indications of toxic conditions.

1. Interference with the nuclear membrane and eventually causing a contraction of it.

2. Chromosome "stickiness" manifested by fusions, by strands extending from chromosome groups and by unequal separations.

3. Disintegration of the contents of the nucleus and/or the chromosome matrix in dividing cells.

A group of miscellaneous observations, most of which are comparatively infrequent, compose Plate VI. With the exception of the binucleate (Pl. VI, Fig. C) and the trinucleate (Pl. VI, Fig. D), the figures in this plate may be said to have been observed only rarely. These figures, as well as the reduced cells (Pl. VI, Figs. E and F) have been discussed.

The polyploid anaphase (Pl. VI, Fig. A) occurred in a root tip taken three hours after exposure to 1 ppm for 15 minutes. Since sufficient time had not elapsed after treatment, this could not be attributed to an effect of Acti-dione. It is significant in this study as an indication that, occasionally, polyploid cells are produced in the "normal" course of events.

Another occasional observation was chromosome fragmentation (Pl. VI, Fig. B and Frontispiece Fig. C). Since these fragmented chromosomes appeared so rarely in the slides, it seems most likely that they, like the single polyploid cell mentioned above, also may have been "natural" accidents.

A destruction of the late anaphase or early telophase polarity and consequent separation of the chromosomes may have been responsible for the peculiar telophase shown in Pl. VI, Fig. G. It might be of interest to note that this figure contains an indication of toxicity in the revelation of the coil structure.

The cell in Pl. VI, Fig. H may be classified as a sort of "transitional" metaphase. It appeared in a root tip which was taken after three hours exposure to 80 ppm of Acti-dione and appears to reflect a

transition between a split metaphase and a completely disorganized one. The delay in the splitting of the kinetochores seemed to accompany the obstruction of the spindle and this delay is evident in the figure. It seems reasonable, therefore, that there may have been a partial inhibition of the spindle at first and the split metaphase developed, that this was followed by the suppression of the activity of the remainder of the spindle. As the split metaphase started to fall apart the root tip was removed and fixed and thus the cell was caught in a stage transitional between an organized split metaphase and a disorganized arrangement of either the "segregational" or "scattered" type.

The lack of orientation of the chromatids is an indication of the disorganization in the cell in Pl. VI, Fig. I. Because of the clumping in the center, it was impossible to count the chromatids massed into the group, but there appeared to be a reduced number. On this basis it is interpreted as an unipolar anaphase of a reduced cell. However, because it appeared in a root tip taken only two hours after exposure to 80 ppm of Acti-dione, only the disorganization, and not the reduction, may be considered as a result of the antibiotic treatment.

In Summary:

1. Polyploid cells, fragmentation of chromosomes, reduced cells and transitional stages between the several categories described were very rare occurrences in the observations.

Quantitative Aspects

The results obtained in the quantitative aspects of this study are presented in the tables of Appendix II. For purposes of presentation the data will be grouped into two categories: (1) Those which reflect the general effects of the Acti-dione on the mitotic cycle and include a consideration of the total numbers of division figures as well as the comparative frequencies of the individual stages; (2) Those which indicate the more specific effects of the treatment as manifested by the occurrences of each of the types of aberrations discussed in the preceding section. The combined results of the first five hours as well as the hourly variations of the effects of the Acti-dione treatments will be compared. In several series of treatments, (particularly the short) after a period of five hours, there were insufficient division figures[1] found in the entire slide to obtain the minimum count of 100 (Appendix II, Tables 1 through 15). Therefore, a comparison of the combined results of all treatments may only be made on the basis of the first five hours after exposure. Accordingly, to equalize the data for such a comparison, separate Tables III, IV and V have been prepared.

A. General Effects of Acti-dione

The average numbers of division figures per axis[2] obtained from the combined data of the first five hours are shown in Table III. When

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[1] The reader is referred to Pl. I, Figs. A through H to observe the range within which the cells were considered as being in the state of division.

[2] "Axis" refers to the consecutive series of high powered fields taken from edge to edge of the cover slip.

the results from all untreated material are examined, (Appendix Tables 26 through 28C), it will be observed that 16114 division figures were found on 926 axes giving the average of 17.5 dividing cells per axis. This compares favorably with the mean (20.3) of the untreated roots taken at consecutive hours for the first five hours.

TABLE III

AVERAGE NUMBER OF DIVISION FIGURES PER AXIS OBTAINED FROM THE COMBINED DATA OF FIRST FIVE HOURS

Concentration	Type Treatment	Total Count	Axes	Avg.
1 PPM	Short	2422	192	12.6
	Continuous	2524	191	13.2
5 PPM	Short	2421	223	10.8
	Continuous	2574	182	14.1
20 PPM	Short	2269	195	11.6
	Continuous	2446	100	24.5
40 PPM	Short	1774	246	7.2
	Continuous	2215	155	14.3
80 PPM	Short	2344	187	12.5
	Continuous	2121	179	11.8
Untreated (Consecutive hours)		2421	119	20.3
All Untreated Material		16158	926	17.5

The data of Table III are graphically presented in Text Fig. 1. From this histogram it is evident that, on the basis of the average numbers of division figures per axis obtained from the combined data of the first five hours, the results of the two types of treatment may be said to have been similar at either end of the concentration series with a divergence occurring in the center and the greatest difference between

the results of the two types of treatment appearing at the 20 ppm concentration.

Before a consideration of the hourly variation in the average numbers of division figures per axis could be undertaken, it was necessary to obtain information concerning the variation among several roots taken from the same onion at the same hour. Accordingly, the individual root control was prepared (See description of method, page 17). The data taken from the root which happened to have been chosen first was arbitrarily used as a base and the deviations of other averages from it were calculated (Appendix II, Table 32). When these deviations are graphed (Text Fig. 8), one can readily see that not only is there a considerable variation among the 12 roots in the mean number of division figures per axis, but also there is a wide range from lowest to highest point (+16.8 to -7.3). Attention is called to the fact that the hourly variations, shown in the graphs (Text Figs. 2 through 7, 9 through 13), are superimposed upon an underlying pattern of wide variation from root to root and this fact must be kept in mind when interpretations of the data are being made.

In order to study the variation in the average number of division figures per axis, the mean of the zero hour (i.e., just before the treatment was applied) was used as a base and the deviations from this point were calculated (Appendix II, Tables 29 through 32). When the deviations from the zero hour average of the untreated material are plotted in their hourly sequence (Text Fig. 2), one may observe that the range of fluctuation was less (+10.1 to -7.0) than it had been for the root tips collected from the same onion at the same hour (+16.8 to -7.3). In addition, the

hourly variation in most cases was less than that occurring among roots taken at one time. Although both of these graphs were made on the basis of approximately the same numbers of cells, each point in Text Fig. 8 is based on readings taken from a single root (See method, footnote, page 18), while the data for Text Fig. 2 were accumulated from four roots of two different onions. Increasing the number of roots and of onions was the method used to lower some of the radical peaks that generally occur as a result of individual variation.

Text Figs. 3 through 7 and 8 through 15 have been constructed in a manner similar to that described for Text Fig. 2. In those cases where the division figures were insufficient for the counts to be made, the condition is indicated by a dotted line ending at a point below the abscissa equal to the average number of divisions found before the initiation of treatment.

After the short (15 minute) treatment with the 1 ppm solution, the results produced (Text Fig. 3), at first glance, appear to have been scarcely different from those obtained from the untreated root tips (Text Fig. 2). However, the peak that developed after eight hours in this series is rather interesting. Most of these divisions were within the normal range. This sudden spurt of normal division was rather striking and one that occurred in both roots of both series (Appendix II, Table 16). Although this difference was not greater in range than that occurring between two separate roots in the individual root control series (Text Fig. 8), it was larger than that observed between any two untreated roots taken at consecutive hours (Text Fig. 2).

Aside from the results obtained from exposure to 1 ppm for 15

hourly variation in most cases was less than that occurring among roots taken at one time. Although both of these graphs were made on the basis of approximately the same numbers of cells, each point in Text Fig. 8 is based on readings taken from a single root (See method, footnote, page 18), while the data for Text Fig. 2 were accumulated from four roots of two different onions. Increasing the number of roots and of onions was the method used to lower some of the radical peaks that generally occur as a result of individual variation.

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Aside from the results obtained from exposure to 1 ppm for 15

minutes which resembled those obtained in control series, and, despite the two underlying varying patterns observed in untreated material, the two types of treatment may be separated on the basis of the trends observed in the hourly changes in the average numbers of division figures per axis. Following the short treatment a general trend toward a reduction in the average numbers of division figures was evident (Text Figs. 4 through 7). On the other hand, under the conditions of continuous exposure to Acti-dione, two distinct patterns were manifested (Text Figs. 9 through 13): The 1 ppm and 5 ppm concentrations (Text Figs. 15 and 16) displayed the same trends as generally appeared following the short treatments. In the 20 ppm and 80 ppm concentrations of the continuous series, the general tendency was an increase in the numbers of division figures initially and later a fluctuation around the average number that were present at the beginning of treatment. The 80 ppm series followed this general pattern during the first eight hours after which time the division figures disappeared.

Since the same concentrations were used in both the short and continuous treatments, little differences might be expected to have occurred at the end of one hour. When the graphs of the same concentrations in the two types of treatments are compared, one finds this to be true in all cases excepting the 5 ppm series where the two types of applications produced opposing results.

When comparisons of trends resulting from the two types of treatments are made on the basis of the hourly variation during the first five hours after exposure to the different concentrations of Acti-dione, differences in tendencies become apparent. In the case of the 1 ppm series,

the same general trends appeared for the first three hours but at the fourth hour they diverged and the average numbers of division figures increased after the short treatment while they started a marked decline in the continuously exposed series. In the 5 ppm treatments, the reverse of this situation was found. Opposing trends appeared during the first two hours and, after the third hour, the same pattern was followed in each of the treatments. With 20 ppm differences in the results obtained from the two methods of application appeared. Following the short treatment, the greatest numbers of division figures were reached at the end of the first hour; this increase was followed by a steady decrease until, at the end of the fifth hour, there were insufficient figures to obtain the minimum count of 100 dividing cells on the entire slide. Under the conditions of continuous exposure, on the contrary, the average numbers of division figures steadily rose for two hours and then fell more gradually, but did not decline much below the average of the zero hour. When the 40 ppm concentration was used, it will be observed that these general tendencies were carried further, i.e., there was a steady decline in the numbers of division figures after the short treatment while, in those roots continuously exposed, the mean number of dividing cells, after an initial increase in the first two hours, returned to approximately the same as it had been at the beginning of treatment. The trends observed during the first five hours following original exposure to the 80 ppm solution were somewhat similar in both series of treatments, the major difference being in the time the peak of division figures was reached. It will be observed to have been attained one hour earlier after the

short treatment than it was in the roots continuously in contact with the same concentration of Acti-dione.

From these results it will be observed that, on the basis of average numbers of division figures per axis, the first five hours may be considered to have been the critical period for the comparative observation of the differences in effects of the several concentrations of each type of treatment.

In Summary:

1. For untreated material, there was a considerable variation in the average numbers of division figures per axis in the individual root control as well as in the consecutive hour control series.

2. Despite the underlying pattern of variation in the controls, the treated material showed definite trends in the means of the numbers of dividing cells.

3. Following the short treatment with 1 ppm, for all practical purposes, the results obtained were not unlike the controls.

4. There was a general decline in the numbers of dividing cells following exposure to all concentrations of the Acti-dione for 15 minutes as well as during the period of continuous contact with 1 ppm and 5 ppm solutions.

5. There was an initial rise which was followed by a decline and a leveling off at approximately the starting point under conditions of continuous treatment to the 20 ppm, 40 ppm and 80 ppm concentrations. In the later hours of the 80 ppm series division figures disappeared.

6. The first five hours after the initiation of treatment was

the most critical period for the observation of the effects of the several treatments on the frequency of division figures.

Another approach to the study of the general effects of the Acti-dione treatments may be found in comparisons of the frequencies of the individual stages. For this phase of the study the percentages of the total number of division figures were calculated for prophase, metaphase and post-metaphase stages. These percentages are presented in Appendix II, Tables 1 through 15.

In the "Summary of all untreated material" included in the study (Appendix II, Table 15C) it may be observed that prophase composed 49%, metaphase 24.8%, and post-metaphase 24.8% of the 16,114 dividing cells counted in the untreated material. In order to obtain the proportionate hourly variations in these stages, all percentages were converted to a common base of 50% (Appendix II, Tables 1D through 10D, Table 13D and Tables 11B, 12B and 14B). These hourly conversion figures were plotted and the graphs will be found in Text Figs. 14 through 27.

Text Fig 14 shows the proportionate hourly variations observed in the untreated bulbs. In this graph it will be seen that each of the stages fluctuated around the base of 50. The same was true in the case of the untreated roots which were taken from the same onion at the same time (Text Fig. 25).

When the proportionate hourly variations in these stages are compared, differences will be observed, not only between the two types of treatment, but also among the concentrations of each method of application. The general result of the short treatment was an increase in the

prophases, but the pattern of the increase differs somewhat among the concentrations used.

As a result of exposure to 1 ppm and to 5 ppm for 15 minutes (Text Figs. 15 and 16) the very marked increase which occurred during the first two hours after the treatment was initiated, was followed by a decline in both cases. At the fourth hour, however, these two concentrations seem to have produced different effects: In the 5 ppm solution series the prophase proportion continued to decrease until the divisions ceased at the end of the seventh hour, while in the 1 ppm material there was a second rise in prophase frequency which began at the fifth hour and was more or less retained until the end of the eleventh hour. The general rise in the prophases assumed a different pattern in the remaining three concentrations (Text Figs. 17 through 19). In these cases, the prophase increase was a more gradual one, and the highest peak in each, which is practically the same for each of these higher concentrations in the later hours, was achieved at the last hour, i.e., just before the divisions ceased entirely.

It is to be expected that the metaphase and post-metaphase patterns would fluctuate with the prophases. It is interesting to notice, however, that, depending upon the concentrations used in these short treatments, there was a divergence in the effects upon the frequencies of these stages. The 1 ppm and 5 ppm solutions of Acti-dione resulted in an initial decline in metaphases and assumed different patterns in the later hours (Text Figs. 15 and 16). In the 1 ppm treatment, after the initial drop, the metaphase fluctuations were comparatively slight and there was a tendency for a leveling off about 10% below the

percentage present prior to the treatment. In the 5 ppm concentration, however, after the initial drop, there was a rather steady rise in metaphases until the fifth hour and, following a sharp decline (21.9% in one hour), there was an equally acute increase (22.8% in one hour).

The metaphase picture began to change somewhat in the 20 ppm series (Text Fig. 17). In this case, it may be said that the general pattern which resulted from the 5 ppm treatment was followed but the changes were more gradual ones. Initially there was an almost negligible rise (4.1%) in the frequency and the decline, which took an hour longer, was similarily followed by a second increase.

A radical change in the effects of the short treatment with Actidione on the metaphase frequencies occurred when a 40 ppm solution was used (Text Fig. 18). There was a very marked increase in the frequency of this stage (from 50.0% to 96%) during the first two hours following the initiation of treatment and this was followed by an even sharper drop (from 96.0% to 16.4%) in the second two hours. It will be observed that these trends of metaphase frequencies is exactly opposite to those which developed during exposures to lower concentrations for the short interval (Text Figs. 16 through 18). This early dominance of metaphases, although not continued, is considered a rather important characteristic of the results of this treatment.

After the short treatment with the 80 ppm the metaphases followed the same general trends but the hourly changes, although still somewhat marked, were more gradual ones (Text Fig. 19).

In the two lowest concentrations of the short treatment it is of interest to observe that the post-metaphases followed the same general

frequency course as that of the metaphases. In other words, as the prophases increased or decreased, both of these stages, for the most part, fell and rose more or less together. Since in the untreated roots these two phases were found in almost equal percentages, it appears that both of these later stages of the mitotic cycle were almost equally sharing the effects of the Acti-dione in these lower concentrations. After the short treatment with the 20 ppm solution, however, this was not the case and the metaphase and post-metaphase frequencies began to diverge with a sharp rise in one being accompanied by a correspondingly sharp decline in the other. This trend, which first appeared in the 20 ppm series, and was even more accentuated in the root tips which were treated with the 40 ppm solution for the short period was also observed following the 80 ppm treatment. After the first two hours in 20 ppm of Acti-dione, a steady decline in the post-metaphase frequency was observed, but in the 40 ppm and 80 ppm groups during this same period, sharp increases of this stage occurred.

In summary, this study of the proportionate hourly variation of the individual stages following the short treatment with Acti-dione revealed the following:

1. After exposure to the 1 ppm solution, differences in the relative frequencies of the individual stages began to appear from five to six hours after initiation of treatment.

2. The general effect of the short exposure was a rise in the relative frequency of the prophases.

3. This prophase rise was rather acute in the early hours following exposure to the lower concentrations of the antibiotic and more

gradual in the higher ones.

4. Metaphase frequency fluctuated considerably as a result of the 15 minute exposure to treatment solutions and became the dominant feature in the early hours following the short treatment with the 40 ppm solution.

5. Post-metaphases tended to follow the metaphase pattern following exposure to the lower (1 ppm and 5 ppm) concentrations but diverged considerably after higher (20 ppm, 40 ppm, and 80 ppm) concentrations and dominated the scene between the third and fifth hour after contact with the 80 ppm solution for 15 minutes.

Text Figs. 20 through 24 show the proportionate hourly variation in the percentage frequencies of the individual stages in material continuously treated with the several concentrations of Acti-dione. When this set of graphs is compared with those of the short treatment (Text Figs. 15 through 19), the 1 ppm diagram of this group immediately stands out as being more similar in its general trends to those of the lowest three concentrations of the short exposure series. In this case (Text Fig. 20) the predominance of prophases and its more or less steady, gradual rise will be noticed to be quite similar to that observed in the 20 ppm short treatment graph (Text Fig. 17). The metaphase and post-metaphase lines in these two graphs also have resemblances in their general courses. It will be observed that, although they follow the same pattern, in the case of the continuous treatment the changes were more gradual ones. This tendency toward more gradual change which was manifested first in the highest concentration of the short treatment is the

most predominating feature of the continuous treatment series after the first hour (Text Fig. 19).

The 5 ppm continuous treatment (Text Fig. 21) appears to have had not only some effects similar to those produced by the higher concentrations after 15 minutes exposure, but also some results resembling those observed in continuously treated material, and could, perhaps, be considered intermediate in this respect. The effects simulated those resulting from the short treatment in the following features: (a) the disappearance of divisions within twelve hours and (b) during the first three hours, the gradual rise in prophases, the sharp decrease in metaphases and the almost equally sharp decline in post-metaphases are features which were also pointed out in the results of the exposure to the 40 ppm solution for 15 minutes. On the other hand, the effects that differ from those of the short treatment are: (a) the more gradual changes, (b) the dominance of metaphases in the last three hours when divisions were present, and (c) after their initial drop, the slight variation in the relative frequencies of post-metaphases. In other words, of this particular treatment it might be said that during the first two hours the results showed tendencies that appeared similar to those of the higher concentrations of short treatment and, in the later hours, the effects more nearly resembled those obtained in the continuously treated group.

The similarities in the trends of each stage in the graphs of the 20 ppm, 40 ppm and 80 ppm series of the continuous exposure to Actidione (Text Figs. 22 through 24) are most striking. It may be of interest to observe that, after the first hour, there was little hourly change in the frequencies of any stage and that the tendencies established at the

end of the first two hours were more or less retained throughout the remaining period of the observation. The outstanding features of these higher concentrations of continuous treatment were the dominance of the metaphases, the radical reduction in the post-metaphases and the more or less stable proportion of prophase figures. Changes appeared in the 80 ppm series of this group (Text Fig. 24): (a) The divisions disappeared at the end of eight hours; (b) The metaphase rise was not as great as it had been during the comparable period in the 40 ppm solution; (c) There was a greater general drop in the prophases.

When the results obtained in the series using the highest concentrations of the continuously exposed group are compared with the same concentration of the short treatment set, it will be observed that the same general trends for each phase appeared during the first two hours after initial exposure. In the continuously treated group, with the exception of comparatively minor fluctuations, there were no radical increases or decreases after the initial reaction to Acti-dione. On this basis, the two methods of treatment separate and it may be said that the effects during the entire period of observation in the continuously treated group are comparable to those which appeared during the first two hours of the short treatment. Here, again, the most critical changes occurred during the early hours after the initiation of treatment.

In summary, with respect to the hourly changes in the frequency of the individual stages, the following observations were made:

1. The results observed in the 1 ppm series were more similar in their general trends to those found in the three lowest concentrations

of the short treatment than they were to the effects observed in other continuously treated material.

2. The results found in the 5 ppm series simulated those observed in the higher concentrations of the short treatment in the early hours and, in the later hours, resembled other series in the continuously exposed group.

3. The outstanding features of the higher concentrations (20 ppm, 40 ppm and 80 ppm) of the continuous series were the dominance of metaphase, the more or less stability of prophases, the great decrease in post-metaphase and the conspicuous lack of any radical change after the first hour of exposure.

4. When these three highest concentrations are compared with the same series in the short treatment group, the same trends are found to occur within the first hour, but radical changes are found in the results of the two types of treatment in the later hours following initial exposure.

5. The most critical changes in all concentrations occurred during the first five hours after the initiation of treatment.

The hourly variations in the frequencies of the individual stages resulting from the two types of intermittent treatments are shown in Text Figs. 26 and 27. It will be observed that when the root tips were exposed to a 40 ppm solution for a period of 15 minutes every two hours (Text Fig. 26) the results produced were similar to those observed following treatment with the same concentration for a single exposure of the same duration (Text Fig. 18). The only difference between the effects

of the two methods of application was an earlier disappearance of the division figures in the material that had been in contact with Acti-dione only once. In the meristems exposed to a 40 ppm solution of the antibiotic for fifteen minutes every hour (Text Fig. 27), the hourly variation in the frequencies of the individual stages was, in its general aspect, similar to those changes observed in the material exposed continuously to the same concentration (Text Fig. 25).

B. Specific effects of Acti-dione

The specific effects of the Acti-dione treatments were manifested in the aberrant figures. A description of them and a consideration of their genetical and cytological potentialities have previously been presented. It is of significance to note that these aberrations were not found exclusively in treated material but occasionally did occur (less than 10%) in untreated root tips (Appendix II, Tables 49C, 62C, 77C and 90C).

The hourly frequency percentages of these aberrations throughout the period of observation may be found in Appendix II, Tables 33 through 90. From these tables the data of the first five hours were combined (Table IV) and the percentages of aberrant figures in each phase of mitosis were calculated. These percentages are graphically shown in Text Figs. 70 through 72. By comparing the histograms it is possible to make some general comparisons of the results of treatments for the combined period of time on the three stages of the mitotic cycle and, in addition, to obtain some indications of the similarities and differences in the effects of the several treatments tested.

TABLE IV

PERCENTAGES OF ABERRANT FIGURES OBTAINED FROM COMBINED DATA OF FIRST FIVE HOURS

Conc.*	Treatment	Prophase Aber.	Prophase Total	Prophase %	Metaphase Aber.	Metaphase Total	Metaphase %	Anaphase Disorg.	Anaphase Total	Anaphase %
1 PPM	Short	63	1265	5.0	9	547	1.6	7	231	3.0
	Continuous	515	1468	35.1	112	384	20.4	12	188	6.4
5 PPM	Short	635	1479	42.9	101	456	22.1	25	208	12.0
	Continuous	1127	1556	72.3	207	588	35.2	17	243	7.0
20 PPM	Short	705	1413	49.6	188	430	43.7	36	256	14.1
	Continuous	1022	1378	74.2	374	923	40.5	22	106	20.8
40 PPM	Short	622	1021	60.9	101	424	23.8	43	177	24.3
	Continuous	620	1133	54.7	433	911	47.5	44	121	36.4
80 PPM	Short	995	1361	73.1	132	480	27.5	32	233	13.7
	Continuous	587	1187	49.5	257	838	30.7	9	62	14.5
Untreated		70	1128	6.2	21	582	3.6	30	331	9.1

* Concentration

By reference to the three histograms (Text Figs. 70 through 72) a comparison of the effects of all treatments on the individual stages may be made. It will be observed that the percentage of aberrant prophase, metaphase and anaphase figures was higher in the untreated series than it was in the lowest concentration of the short treatment. It will be noted also that the greatest frequencies of aberrant figures occurred in the prophase group (Text Figs. 70 through 72). As shown in Table IV, with the exception of the 1 ppm series, more than 40% of the prophases were affected in all concentrations of each type of treatment and, in some cases the number exceeded 70%. The lowest percentages of aberrations appeared in anaphase (Text Fig. 72). As shown in Table IV all percentages in this group were below 36.4%. In addition, it may be observed that the greatest

difference between the two treatments consistently appears in the prophase histogram (Text Fig. 70), while the most similarity between the percentages of aberrant figures appear in the anaphase group (Text Fig. 72). Finally, the 1 ppm short treatment appears to have been ineffectual in increasing the percentages of aberration in each of the three stages of mitosis (Text Figs. 70 through 72).

A comparison of the effects of the several concentrations and of the two types of treatment on each of the phases of mitosis during the combined five hour period may be made by a study of each of the histograms separately. Following the short treatment there was a steady increase (42.9% - 73.1%) in the frequencies of aberrant prophases (Text Fig. 70) as higher concentrations were used. This differed from the results obtained in the continuously treated group where, as shown in Table IV, the aberrant prophase frequency dropped in the 40 ppm and 80 ppm series (19.9% and 6.8% respectively). The greatest differences between the effects of the two types of treatment were found in the 1 ppm and 5 ppm sets where the differences between the short and continuous applications were 30.1% and 29.4% respectively. The 5 ppm and 20 ppm continuously treated and the 80 ppm 15 minute exposure were the most effective in producing aberrations in prophase.

Following the short treatment there was a rise in the frequencies of aberrant metaphases (Text Fig. 71) as the concentration was increased to 20 ppm when the percentage fell back to the level it had been at 5 ppm. In the continuously treated root tips, however, the percentage continued to increase in the 40 ppm solution, but dropped in the 80 ppm treatment. The most effective conditions for the production of

"scattered" metaphases were provided by the 80 ppm and 40 ppm continuous and the 20 ppm short treatments. The percentage differences between the types of treatments in these three sets of results is probably not significant. The greatest difference in effectiveness between the same concentrations of the two methods of application appeared in the 40 ppm series.

The most effective treatments resulting in aberrant anaphases (Text Fig. 72) were the 40 ppm concentrations of both types of treatment. Both the 1 ppm and 5 ppm solutions may be considered to have been ineffective since the aberrations in the treated root tips differed by 3% or less from their frequency in the controls. This difference is probably within the range of natural variation.

In summary it might be said that from the histograms constructed from the combined data of the first five hours the following points become evident:

1. The greatest percentages of aberrant figures resulting from all the Acti-dione treatments appeared in the prophase stage.

2. The greatest differences between the same concentration of the two types of treatments also occurred in the prophase stage.

3. The lowest percentages of aberrations occurred at the anaphase stage.

4. Following the 80 ppm short treatment, the results produced, in terms of frequency of aberrant prophases, were similar to those appearing in the 20 ppm and 40 ppm continuously treated material.

5. The 20 ppm solution of both treatments as well as the 40 ppm

solution continuously applied were equally effective in producing the highest percentages of aberrant metaphases.

6. The 40 ppm continuous treatment appears to have been most effective in producing disorganized anaphases.

The percentages of aberrant prophases are plotted in their hourly sequence in Text Figs. 28 through 41. For purposes of comparison the total prophase percentages are included as dotted lines in these graphs. It will be observed that in the untreated root tips (Text Figs. 28 and 35) the percentage of aberrant prophases remained consistently below the 10% level and the 1 ppm concentration of the short treatment (Text Fig. 29) was virtually ineffective in increasing the incidence of this aberration.

In the study of the comparative quantitative effects of Acti-dione upon the frequencies of the individual stages, one of the differences observed between the two types of application was the radical hourly variation in the prophase and metaphase frequencies following the short treatment, while in the continuously exposed series no sharp hourly changes occurred after the third hour and practically none after the fifth hour. If one scans the graphs showing the hourly percentages of aberrations in prophase (Text Figs. 30 through 33, 37 through 39) and also in metaphase (Text Figs. 44 through 47, 51 through 53), one may observe that the irregularities increased radically during the first five hours as a result of both treatments. In the later hours of the two lower concentrations in the continuously exposed group (Text Figs. 36, 37, 50, 51) division figures had disappeared and, as a result, the

graphs are similar to those representing the short treatment. During the same period in higher concentrations (Text Figs. 37 through 39, 51 through 53) there was, however, a gradual rise in the frequency of aberrations in both prophase and metaphase.

One of the general effects of Acti-dione was a decrease in the relative percentages of anaphase figures. On the basis of the combined data of the first five hours (Text Fig. 72), it has been shown that anaphase seemed to have been the least affected stage. From the graphs of the hourly distribution of percentages of aberrant anaphases (Text Figs. 57 through 69) it will be observed that the peaks of the anaphase disturbances appeared in the later hours of the observation. In this stage (Appendix II, Tables 63 through 77C) the percentages are based on much smaller numbers and hence the very radical changes are perhaps not as significant as they are in the graphs of the other phases of mitosis.

Turning now to a separate consideration of the prophase aberrations, it will be recalled that one of the characteristic effects of the short treatment was the general increase in the frequency of the prophases. The course of this increase is indicated by the dotted line on the graphs. Omitting the ineffectual 1 ppm series, when one scans the graphs of the results following the other short treatments (Text Figs. 30 through 33) one observes a general positive and direct correlation between the increase in the percentages of aberrations and the increase in the frequencies of the total prophase figures. The 5 ppm, 20 ppm and 40 ppm solutions may be considered to have been equally effective in the maximal points reached, in the achievement of these maxima in the early hours

after exposure (three to four hours) and, following exposure to the two lower concentrations of this series, in a decline in the percent of aberrations after the peak has been reached. The graph of the results obtained following a 15 minute period in the 80 ppm solution (Text Fig. 33) stands alone in the series of this method of application as having been most effectual since there was 100% aberration at six hours when the total prophase figures comprised 75.9% of the total division figures. In other words, since, at the end of the period of observation, the dominant element in the observations were these aberrant prophases, the 80 ppm series differed from the results found in root tips exposed for 15 minutes to the other concentrations.

When one observes the graphs of the data obtained from the continuously treated root tips (Text Figs. 36 through 40), one is inclined to conclude that this type of treatment was more effective in producing a high percentage of aberrations at an earlier hour than the short treatment method. A closer inspection will reveal one exception to this: In the 80 ppm series (Text Fig. 40) it will be noted that the percentage of aberrant prophases reached neither the peak achieved in the other concentrations of this group nor the maximum achieved in the same concentration of the short treatment series. Furthermore, it may be noted that this general trend toward an increase in the percentage of aberrations occurred regardless of the total prophase pattern.

In the 1 ppm concentration of the continuously treated group (Text Fig. 36) there appeared a tendency for the total prophase percentage to increase. This is shown by the dotted line in the graph. It will also be observed that there was a positive and direct correlation

between the increase in aberration and the increase in total prophases. In this respect the 1 ppm concentration of this group corresponds to the general tendencies found in the short treatment series. Because of the rapidity of the increase in the percentage of prophase aberrations (95.4% in four hours), the results of the 5 ppm concentration of the continuously exposed group (Text Fig. 37), appear most similar to those found following the 80 ppm short treatment (94.9% in four hours).

As the total prophase percentages declined, the frequency of the aberrant figures increased very markedly in root tips continuously exposed to the 20 ppm and 40 ppm solutions (Text Figs. 38 and 39) and hence a direct negative correlation was established. As a result, at the end of the twelve hour period of observation, the percentage of aberrant figures was close to 100% but, at this time the prophase stage composed between 40% and 50% of the total division figures and this may be said to be an outstanding difference between the results observed in the two types of treatment.

The 80 ppm continuous series stands alone within this group in having shown radical hourly changes in the percentages of affected prophases as well as in the fact that the frequency of the aberrations does not reach the peak achieved in the lower concentrations of the same type of treatment.

It will be recalled that, on the basis of the frequency percentages of the individual stages, the results of the intermittent treatments appeared similar to the short and continuously treated materials depending upon the length of the interval between the applications. When the hourly percentage of aberrant prophases is examined (Text Figs. 34 and

41), however, both of these series in the early hours appear to have had effects most nearly similar to the 80 ppm concentration of the short treatment and, in the later hours to have diverged in their effects. When the interval between the exposures was one hour and 45 minutes the results resembled those found in the short treatment series, i.e., division figures disappeared; when the interval between the exposures was only 45 minutes, the results in the later hours of the observation period were more nearly similar to those observed in continuously exposed root tips, i.e., the division figures persisted with little change until the end of the eleventh hour.

When one examines the graphs showing the hourly distribution of percentages of "scattered" metaphases (Text Figs. 42 through 55), one should first become aware of the frequencies of total metaphase. If these graphs are compared with those of prophase (Text Figs. 28 through 41), one will observe that in those concentrations of the short treatment series (Text Figs. 30 through 33) in which aberrant prophase percentages were relatively high there were comparatively high frequencies of total prophase figures at the same time. In the comparable metaphase graphs (Text Figs. 44 through 47) the total metaphase figures declined considerably so that, by the end of the observation period, although 100% aberration may have been seen, this particular type of effect can not be considered as a dominant feature of the results of the short treatments with these solutions. In the three higher concentrations of the continuous treatment, the total metaphase frequency rose, and, at the same time, the percentages of the aberrations also increased. As a result, by the end of the observation period, the aberrant metaphases may be said to

have been the significant elements in the results of the continuous treatment with the higher concentrations.

In the lower concentrations of this series, the results produced were more nearly similar, in general, to those obtained in the short treatment series. Although the increase in metaphase aberrations during the first five hours was more gradual in the group continuously exposed to the 1 ppm solution of Acti-dione (Text Fig. 50), the general effects may be said to more nearly resemble those exposed to the 5 ppm concentration for 15 minutes during the same period (Text Fig. 44). In the later hours, when this frequency sharply declined in the short treatment series, it continued to rise in those which remained continuously in the Acti-dione solution. In this respect, the lowest concentration of the continuously treated group may be said to have exhibited the tendencies of both types of treatment consecutively. On the other hand, on thie basis of increasing metaphase aberrations, the results found in the 5 ppm series (Text Fig. 51) closely resemble those noted in the 80 ppm short treatment (Text Fig. 47) and other continuously treated roots (Text Figs. 52 and 53). However, the <u>total</u> metaphase frequency did not increase greatly and hence the relative frequency at the end of the observation period more nearly resembles the situation found after the short treatment.

The distribution of the hourly percentages of "scattered" metaphases occurring during the intermittent treatment using 40 ppm for 15 minutes every two hours (Text Fig. 48) closely followed that observed following a single exposure to a solution of the same concentration (Text Fig. 46). When the interval was shortened (Text Fig. 55), the

results, during the first five hours, simulated those obtained after a single exposure to the same concentration (Text Fig. 46), but in the later hours the division figures remained in evidence, a feature characteristic of the 20 ppm and 40 ppm continuously treated group (Text Figs. 52 and 53).

The following effects on anaphase have already been pointed out: (1) One of the general effects of the Acti-dione treatments was a reduction in the relative numbers of anaphase; (2) On the basis of the combined data of the first five hours (Text Fig. 72), anaphase was the stage least affected by the Acti-dione treatments; (3) A relatively high percentage of disorganized anaphases developed in the later hours, particularly in the root tips continuously treated with the higher concentrations of the antibiotic (Text Figs. 66 through 68).

When the graphs of the hourly distribution of disorganized anaphases are examined (Text Figs. 56 through 69) for a general comparison between the effects of Acti-dione under various conditions, one finds relatively the same general tendencies as those previously mentioned for the metaphase groups. The following similarities will be observed: (1) The 1 ppm short treatment (Text, Fig. 57) was virtually ineffective in disorganising anaphase; (2) During the first five hours, the results of the 1 ppm continuous treatments (Text Fig. 64) were most nearly similar to those found in the same period in the 5 ppm short treatment series (Text Fig. 58), but in the later hours they simulated the results obtained in continuously treated material; (3) During the first five hours of continuous treatment with the 20 ppm and 40 ppm solutions (Text Figs. 66 and 67), the effects were somewhat similar to those observed in the same period

following exposure to the same concentrations for the 15 minute period but, in later hours, differences appeared and rather radical changes in the percentages of disorganized anaphases occurred; (4) In the intermittent treatment, when the interval between exposures was long (Text Fig. 62) the anaphase disorganization formed a pattern similar to that found following the same concentration after a single treatment (Text Fig. 60).

In these same graphs the following differences between anaphase and metaphase effects can also be noted: (1) During the first five hours in the 5 ppm continuously treated group (Text Fig. 65) there was a steady rise in the amount of anaphase disorganization but the level reached did not equal that found in untreated material. In the case of the metaphase figures, on the other hand, this solution was highly effective when continuously applied (Text Fig. 51); (2) The 80 ppm solution for the 15 minute period (Text Fig. 61) seems to have produced less disorganization in anaphase than occurred when the next lower concentration was used for the same time (Text Fig. 60), a situation contrary to that found in the metaphase group (Text Figs. 46 and 47); (3) During the first five hours of the intermittent treatment when the roots were treated for 15 minutes every hour (Text Fig. 69) the results in terms of anaphase disorganization were more nearly similar to those found after exposure to the 80 ppm solution for a single 15 minute period (Text Fig. 61). In the later hours the aberrant anaphase percentages steadily increased in a manner similar to that observed when root tips were continuously treated with the 80 ppm solution (Text Fig. 69). These results more closely resembled the effects of this intermittent treatment on the prophase stage (Text

Figs. 47, 54 and 55) than on metaphase (Text Figs. 33, 40 and 41).

In Summary:

1. The 1 ppm solution when applied for a period of 15 minutes was ineffective in producing aberrations in any of the mitotic stages.

2. On the basis of the combined data of the first five hours the highest percentages of aberrations appeared in prophase and the lowest in anaphase.

3. The first five hours after the initiation of treatment was the period in which the greatest percentages of aberrations were produced in prophase and metaphase.

4. After the first five hours the most radical hourly variation in aberrant anaphases occurred.

5. The greatest differences between the two types of treatments appeared after the first five hours.

6. During the first five hours of the intermittent treatments the aberration percentages resembled those obtained from meristems to which the same concentration was applied a single time. In the later hours of the observation, the results of these types of application diverged and, in those series where the interval between exposures was short, they were similar to those obtained in the continuously treated material, when the interval was longer the results simulated those found after the short treatments.

7. Although the same general tendencies were shown in the effects of all concentrations of the continuous treatment, there appeared a drop in percentages of aberrations in prophase and metaphase in the 80 ppm series.

In Appendix II, Tables 78 through 90C the numbers of prophase and metaphase cells containing "reductional" or "segregational" groupings are presented in their hourly sequence together with the percentage they occupy of the total of the prophase and metaphase divisions. From the percentages obtained during the first five hours the mean and its standard error were calculated. These average percentages are graphically shown in Text Fig. 73. In this figure it will be seen that the 40 ppm solution of both methods of treatment appeared to have been most effective in increasing the frequency of these types of aberrations. In Table V these average percentages are presented together with their standard errors. Here it may be observed that the mean percentage obtained following the 40 ppm short and in the 20 ppm, 40 ppm and 80 ppm continuous treatments were found to have been statistically significant.

TABLE V

MEAN PERCENTAGES OF PROPHASE AND METAPHASE CELLS CONTAINING "REDUCTIONAL" AND "SEGREGATIONAL" GROUPINGS

Concentration	Treatment	Mean %	Standard Error
1 PPM	Short	1.1	± 0.15
	Continuous	1.8	± 0.51
5 PPM	Short	2.17	± 0.56
	Continuous	3.11	± 0.64
20 PPM	Short	2.45	± 0.60
	Continuous	3.54	± 0.29*
40 PPM	Short	5.52	± 0.68*
	Continuous	6.10	± 0.63*
80 PPM	Short	2.46	± 0.66
	Continuous	5.48	± 1.48
Untreated		1.20	± 0.44

* Statistically significant at the 5% level

DISCUSSION

A. General Effects of Acti-dione

On this basis all of the data accumulated in this series of experiments one may venture the hypothesis that the general effect of the Acti-dione treatments appeared to have been similar to that of a slow fixing agent. When the data are examined in the light of this hypothesis the results observed become understandable.

When the average numbers of division figures were examined, contrasting trends were found to characterize the two main types of Acti-dione treatments studied in this investigation. Following the short treatments there was a general decline in the average numbers of dividing cells, while in those root tips continuously exposed the ultimate effect was a stability in frequency of division figures. In the short treatments, in which the root tips were exposed to Acti-dione for 15 minutes, the assumed fixation effect was incomplete and when removed from the test solution the cells were still capable of reverting to a "resting" condition. Consequently, there were no division figures in the later hours of the observation period. In those root tips continuously exposed to the more effective concentrations of Acti-dione, however, the numbers of figures at the end of the observation period were approximately the same as they had been at the time treatment was initiated. Furthermore, after the first five hours (Text Figs. 11 and 12) there was little change in the average numbers of dividing cells. On the basis of this evidence it appears that, in

these concentrations, the cells virtually become fixed after a period of five or six hours. The amount of Acti-dione in the lower concentrations of the continuous treatment (Text Figs. 9 and 10) also appeared to have been insufficient to have completed the assumed fixation and, as a result, by the end of eight hours the cells had reverted to a "resting" condition. On the other end of the concentration series, i.e., in the highest concentration (Text Fig. 13), the amount of Acti-dione probably had reached a level where the fixing effect was nearly completed to a point just below lethality.

When the relative frequencies of individual stages was examined this hypothesis was corroborated (Text Figs. 14 through 27). Following exposure to the short treatments, there was a more or less general rise in the relative frequency of the prophases. In the early hours this rise was inversely proportional to the concentration, while in the later hours the relationship was almost a direct one. If we assume the same numbers of cells may enter and leave each phase then, with Acti-dione operating as a slow fixing agent this relationship is to be expected. In the early hours following the lower concentrations of both treatments (Text Figs. 15, 16, 17 and 20) the fixing action was quite weak and very slow resulting in an accumulation of prophase, while in higher concentrations (Text Figs. 18, 19, 22, 23, 24, 26 and 27) it appeared that the stalling effect on the changes had been increased to the extent that the numbers of cells passing into prophase were being affected, thus, with fewer cells coming into division, and those present being slowly fixed an apparent stability was reflected. It appeared that the cells were not completely fixed in lower concentrations with Acti-dione but, as

previously indicated, reverted to interphases. Later stages of these reversions have the general aspects of "affected" prophase nuclei, accordingly, the rise in prophases at the end of the period of observation (Text Figs. 15 through 20) appeared to have been a reflection of this reversion and an explanation of the direct correlation between concentration and frequency of prophase following short treatment. On the other hand, in those root tips continuously exposed to higher concentrations, the fixing effect eventually seemed to have been nearly completed (probably at the end of about five hours) and the result was a relative stability in the frequency of this stage (Text Figs. 22, 23, 24 and 27) throughout the remaining period of the observation.

If we assume the slow stalling effect in prophase as causing the increase in the relative numbers of this stage, a decrease in the relative frequencies of later stages of mitosis would be expected. The data show this to have been the case (Text Figs. 15, 16, 17 and 20) in the early hours of and following treatments with the lower concentrations of Acti-dione. On the other hand, if, in the higher concentrations, there is sufficient Acti-dione present to have not only lengthened the duration of the stages, but also to have increased in effect to the extent that the result is a lowering of the number of cells coming into division, and if we also assume that the stalling effect on the several stages is a differential one, then whichever stage is least affected would be expected to show a decline in relative frequency. Presumably, therefore, an increase in the relative frequency of a given stage at a given time would indicate that it is the one most highly affected by the stalling action at the moment. For example, in the first three hours following

the short treatment with the 40 ppm solution, metaphase appeared to have been the most affected. During the early hours of the observation period this was found to have been the case in the root tips exposed to all the higher concentrations (Text Figs. 18, 22, 23, 26 and 27) where there was a sufficient amount of Acti-dione present to have increased the intensity of the stalling action to this extent. The 15 minute treatment was apparently not long enough for a complete fixation and, as a result, depending upon the degree of the effect, dividing cells could either complete their division or revert to interphase. In the continuous treatment, however, the cells, being almost completely fixed by the end of four hours, there is, as would be expected, little change in the relative frequency of the individual stages after this time.

If the graphs of all treatments (Text Figs. 13 through 27) are divided at the point representing the end of the fifth hour it will be observed that the relative frequency of post-metaphases declined in the first hours considerably and, depending upon whether or not the influence of the Acti-dione had been great enough to completely "fix" the cells, the two types of treatment diverge at this point. In short treatment series (Text Figs. 13 through 19) and the lowest concentration of the continuously exposed group, apparently only a slight fixing of the cells had occured. In the other series of continuous treatment, however, the post-metaphase cells appear to have been almost completely fixed with the result that there was little change in their relative frequency after the first five hours.

The results of the intermittent treatments also support this

hypothesis of a fixation action of Acti-dione. In cases where the root tips were exposed to a 40 ppm solution of Acti-dione for 15 minutes every two hours, during the first five hours the relative frequencies of the individual stages were similar to those observed when the same concentration was used for a single 15 minute period. After five hours, the divisions disappeared in the latter series, but in the intermittently treated material the reversions appear to have taken a little longer and division figures remained in evidence for an additional three hours. When the interval between the treatments was shortened to 45 minutes the results obtained (Text Fig. 27) were similar to those observed when the roots were continuously exposed to the same concentration.

In summary, the data examined so far support the assumption that Acti-dione has a differential slow fixation effect which, in lower concentrations, results in lengthening of prophase and metaphase and in high concentrations this effect increases resulting also in lowering the numbers of cells coming into and going through these division figures.

B. The Specific Effects of Acti-dione

The types of aberrations, their interrelationships and significances. The more specific effects of Acti-dione may be considered to have been reflected in the various aberrations that were observed. In the presentation of the results the many types of aberrant figures were described. Essentially the irregularities reported are similar to some of those previously observed by other investigators (Appendix I). The practical importance of the completely disorganized figures (c-mitosis) is well

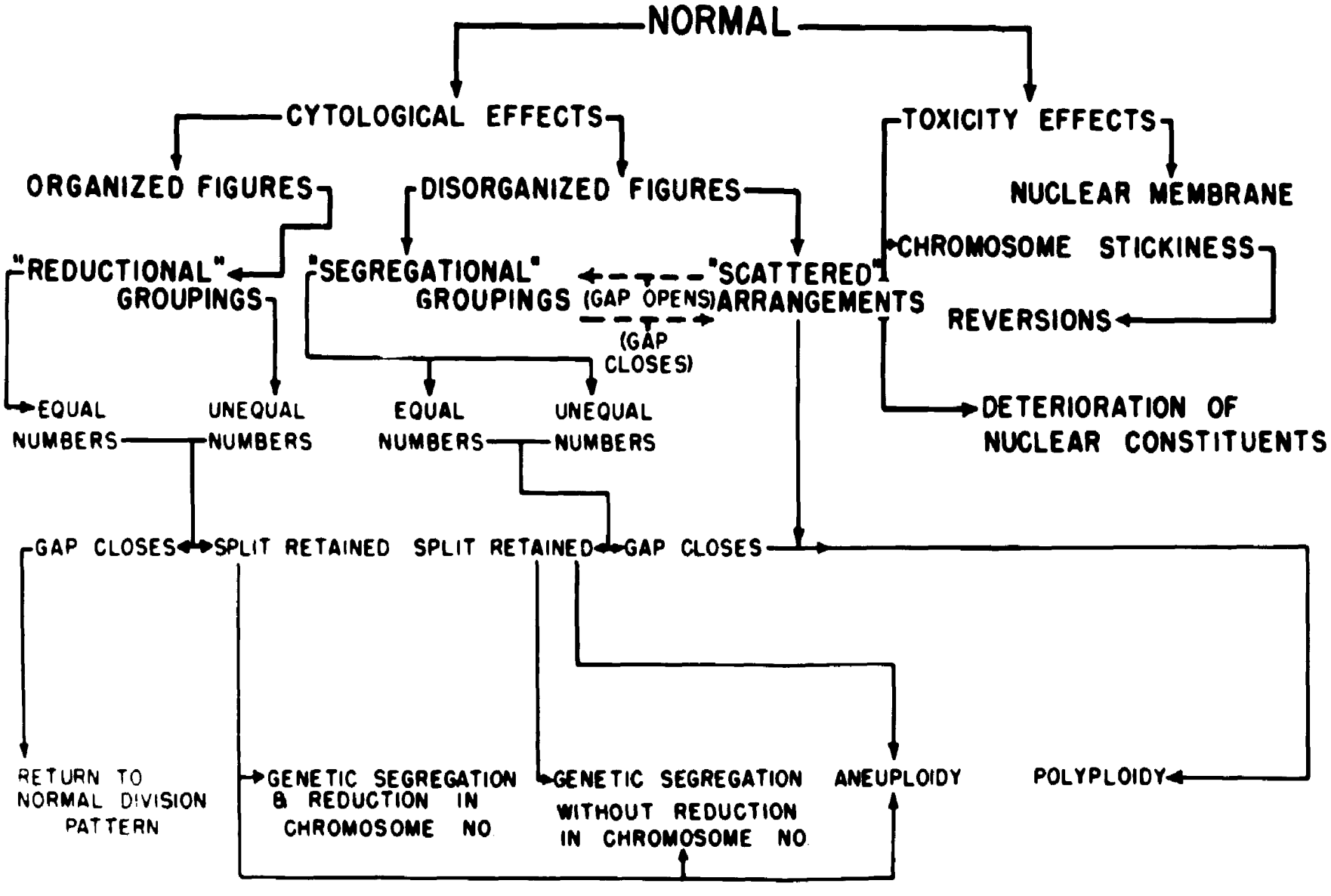
known (18, 45, 47, 50, 70, 79, 99 and 144). Some of the more theoretical potentialities of the organized "reductional" groupings have been previously discussed (62, 85 and 142). All this work was based upon continuously treated material observed at varying intervals ranging from four to twelve hours.

In this study the effects of a 15 minute treatment period were contrasted with those continuously exposed and the quantitative data were collected at hourly intervals. Based upon the results of this study, a general summary of the types of effects, possible interrelationships and the potentialities of each is presented in Table VI. In this table it will be seen that, depending upon whether or not there is an element of organization present, the "reductional" groupings (Plate II) may be separated from the "segregational" type (Plate III) and the "scattered" arrangements (Plate IV).

Although the "reductional" groupings are primarily of interest because of the possibility of their resulting in a reduction in the chromosome number with the consequence, in many cases, of providing for genetical segregation (62), other speculations on their further development are shown in Table VI. As previously mentioned, either equal or unequal numbers of chromosomes may be involved in these "reductional" groupings. When, in heterozygous stock, homologues are separated in this manner, these aberrations assume genetical significance. In those cases of prophase and metaphase "reductional" groupings where equal numbers are involved (Pl. II, Fig. A, also Frontispiece Fig. A), the daughter chromatids within each group must be separated into two individual nuclei if the original number is not to be restored. This would result

TABLE VI

CYTOLOGICAL AND GENETICAL POTENTIALITIES OF THE SEVERAL TYPES OF ABERRATIONS



in the production of four nuclei each containing eight chromosomes. If this should occur, genetic segregation and reduction in chromosome numbers would be accomplished as shown in Table VI. One, perhaps questionable, case of this type of separation is shown in Frontispiece Fig. D, where four groups of eight chromatids have been formed in the same cell. Here the likelihood of the formation of a tetrad of four nuclei each with eight chromosomes seems highly probable. However, none of the few reduced cells found during the course of this study contained eight chromosomes. It seems logical, therefore, that, the actual result following these "reductional" groupings may be either an elimination of the separating gap and a return to the normal condition, or the formation of nuclei with the reduced chromosome number which, for the most part, cannot divide again. Because of the element of organization in the figure, the return to the normal condition may be affected in the stage following that in which the separation occurred. For example, the chromosomes composing the two groups in Frontispiece Fig. A may close the gap between them during the course of their movement to the metaphase plate and a normally organized metaphase figure might result. It seems reasonable that the return to the normal division pattern would depend upon the orientation.

As shown in Table VI when the prophase and metaphase separations are unequal the same two possibilities present themselves: Either they may return to the normal division pattern in the succeeding stage or the gap may be retained until the division ceases. If the latter occurs there are two further probabilities, both of which lead to aneuploidy. Each group of whole chromosomes may form a separate nucleus. For

example, if the two groups in the 10:6 separation shown in Pl. II, Fig. B were to form separate nuclei and the constituent chromatids should fall apart in situ the result would be two nuclei with 20 and 12 chromosomes respectively. On the other hand, if the chromatids of the 10:6 "reductional" grouping should be separated into nuclei, the 4 daughter nuclei would contain 10, 10, 6 and 6 chromosomes respectively.

Some evidence substantiating the retention of the gap in cases involving separations of unequal chromosome numbers may be found in the occasional observations of unbalanced telophases, of binucleate and trinucleate cells with unequal sized nuclei and, in the very rare appearance, of reduced cells. But it is not possible to decide whether they arose in this manner, from more or less organized figures, or from the completely disorganized type. The point to be stressed here is that, although the evidence of actual reduction was rare in this study, the genetical significance of the "reductional" group is, nonetheless, not diminished.

As shown in Table VI two types of disorganized figures were observed. These have been described in the presentation of the results and are designated as the "segregational" groupings and the "scattered" arrangements. In the previous literature these figures collectively are referred to as "c-mitotic" divisions. The "segregational" groupings have been called by various terms, "exploded metaphase" (6), "distributed c-mitosis" (106) and "distributive c-mitosis" (85). As shown in Table VI these "segregational" groupings may involve the separation of either equal or unequal numbers of chromosomes. When equal numbers

of chromosomes have been separated they are of particular interest since they represent the possibility of resulting in genetical segregation without involving a reduction in chromosome number. When two numerically unequal groups are separated, just as in the case of the "reductional" groupings, aneuploidy may result. Alternatively, however, there is the additional possibility of the intergroup gap closing and resulting in "scattered" arrangements, which may eventually lead to polyploidy.

The second type of disorganized figures (See Table VI, page 69) is that which here has been designated as "scattered" arrangements. These were illustrated in Plate IV and in Frontispiece Figs. E and F. Their potential capacity for resulting in the polyploid condition is well recognized but it might be of interest to point out, as indicated by dotted arrows Table VI, that, once established by a "drifting" of whole chromosomes, these "scattered" arrangements may, in some cases give rise to "segregational" groupings. In this respect they also present a possibility of resulting in either aneuploidy or genetic segregation without reduction in chromosome number. It was pointed out, that, like the "segregational" groups, these "scattered" arrangements were also most characteristic of the latter stages of continuous treatment or the latter hours after exposure to the short treatment and appeared to reflect the complete inhibition of the spindle.

In summary of the cytogenetical potentialities of these aberrations:

1. The ultimate result of the organized division figures may be: (a) a return to the normal division pattern, (b) genetic segregation with reduction in chromosome numbers, and (c)aneuploidy.

2. The disorganized configurations may lead to: (a) genetic segregation without reduction in chromosome numbers, (b) aneuploidy, and (c) polyploidy.

The cytological aberrations, their relative frequencies and relationships to types of treatment. To discover any possible relationships between the frequencies of these aberrations and the concentration, the time as well as the method of treatment was one of the more specific objectives of this investigation. It was hoped that it would be possible to obtain a specific combination of these factors which would result in the production of one or more recoverable aberrations and if this were not possible to define the thresholds of cytological and toxic effects.

In the discussion of the general effects of treatment it was pointed out that the most critical period for the observation of the cytological effects of Acti-dione was the first five hours after the initiation of treatment. This early interval had not been studied quantitatively prior to this investigation. In the study of the hourly variation in the frequency of division figures it was shown that during this time the greatest changes in relative numbers of division figures occurred. A slow stalling action, differential upon the individual stages was postulated. It is considered to be of significance that, in terms of aberrations also, there was a differential quantitative effect. This was shown in Text Figs. 70 through 72 where the percentages of aberrations from the combined data of the first five hours were graphically presented. Here it was indicated that the highest percentages of aberrations occurred in prophase and the lowest in anaphase.

It will be observed, that, the relationship of the degree of effect on the relative frequencies of individual stages with the concentrations and the type of treatment is not the same as that existing between the percentages of aberrations and these variables. On the basis that the relationship does exist one may postulate that the assumed stalling action may be the precursor of the production of the aberrations.

In the quantitative study of the cytological aberrations the first five hours also appeared to be the most critical period. Following the short treatment there was a direct correlation between frequencies of division figures and percentages of aberrations in prophase (Text Figs. 29 through 33), but an inverse relationship in metaphase (Text Figs. 44 through 47) and in anaphase (Text Figs. 58 through 61). In all concentrations of the continuously treated root tips the aberrant divisions in all mitotic phases continued to rise even after a stability in numbers of division figures had been reached, (Text Figs. 36 through 40, 50 through 54, 64 through 68).

Furthermore, it will be observed from the graphs that the percentages of aberrations were not in direct proportion to the concentration, but rather were more closely correlated with time and method of treatment. It is of interest to note that the peak of prophase and metaphase aberrations in the 80 ppm of continuous treatment was lower than that observed in more dilute concentrations used in the same manner. Furthermore it is considered somewhat significant that, in terms of percentages of aberrations the 1 ppm short treatment (Text Figs. 29, 45, 57) was virtually ineffective. On the basis of the relative frequencies of the

individual stages the 1 ppm short treatment appeared to have been effective in the later hours.

It seems reasonable to conclude from this that Acti-dione, even in this dilution, had caused some disturbances in the regular activities within the nucleus that resulted in the accumulation of prophases. Since these divisions could be considered to have been within the range of normality, the effect was not sufficient to cause a breakdown of the process but merely seems to have had the stalling effect mentioned above.

All of these results tend to support the hypothesis made at the beginning of this discussion that the Acti-dione appears to act in a manner similar to a slow fixing agent. It appears that, as Acti-dione penetrates the dividing cell, its first effects are slight physiological disturbances which manifest themselves in two easily detectable ways:

1. There appeared to have been a slowing down of the rate of some of the morphological changes that generally occur. One of these activities that seemed to have been affected was related to the nuclear membrane. It appeared to have been retained as a membrane and the result was the appearances of the "affected" prophases (Pl. V, Figs. B, C and D). In these cases the chromosomes themselves seemed to have been capable of proceeding through their usual structural development toward metaphase while the nuclear membrane did not change. The result was unoriented chromosomes of metaphase length enclosed within the nuclear membrane.

2. There seemed to have been a rather slow and gradual interference with the spindle mechanism. The partial inhibition manifested itself in the "reductional" groupings which appeared in the early hours

of observation and the complete suppression was indicated by the "segregational" and "scattered" arrangements which appeared in their greatest frequencies in the later hours.

None of the concentrations used in the short treatment series of this investigation seemed to be sufficiently high to have completely fixed the cells during the 15 minute interval, hence, when removed from the solution the reversals to resting stage could occur.

From all treatments including the continuously treated group there was recovery in only two of the series; the 1 ppm and 5 ppm short treatments. The 1 ppm was found to have been cytologically ineffective (Text Figs. 29, 43 and 57). However, in terms of the relative frequencies of the individual stages (Text Fig. 15), as previously pointed out, some effects were manifested in the accumulation of prophases and the drop in relative frequencies of post-prophase stages which occurred after five hours. The comparatively normal divisions in the recovered material indicate that the changes involved in the stalling action of Acti-dione may be preparatory to the cytological aberration. Furthermore, since there was no evidence of any undue cytological aberration in the recovery material after the 5 ppm short treatment, it seems reasonable to assume that the cells observed in this recovery material had either escaped the effects of Acti-dione or, at least, had not been radically changed by it. Since the 1 ppm short treatment was cytologically ineffective but did reflect some changes having occurred and the 5 ppm of the same group produced cytological effects but appeared to have been toxic to the cells so affected, it seems logical to assume that the toxicity threshold is very close to that required for the

production of cytological aberrations. If recoverable aberrations are to be obtained from Acti-dione it seems most likely that they would be found in some combination of time from between 0 and 15 minutes and at some concentration between 1 ppm and 5 ppm. At any rate it seems highly probable that by further investigation of this range of treatment, the answers might be found to some very pertinent questions related to the method of breakdown, the recoverability of aberrations and possibly the mechanisms involved.

The question that next arises is, why it is that the cells in which Acti-dione has been active have lost their ability to divide. The only observations which might possibly contain some clues in answer to this question were the nature of the interphases. After exposure to the antibiotic, the interphase nuclei assumed a different appearance. This was particularly noticeable in the higher concentrations of both types of treatment and, of course, was most accentuated in those fixed after long periods of continuous treatment. In Plate VI, Fig. A two of these deteriorated interphases are shown. As previously mentioned, it was observed that there seemed to have been some interference with the coiling and the nucleus had the general appearance of a Zygotene. Now, it is well recognized that in the case of the illustrated cell this was most likely a toxicity effect. However, since none of the cells which had manifested cytological effects of Acti-dione were capable of later mitoses and since disruption of the spindle mechanism in one division does not necessarily inhibit succeeding divisions, it seems reasonable that interphases are also vulnerable to the Acti-dione.

In this respect the action of Acti-dione differs from that of

colchicine and gives support to the contentions of Galinsky (52) Allen, Wilson and Powell (2) Loveless and Revell (91) and others, who contend that all chemicals capable of producing the c-mitotic effect do not necessarily operate in the same manner. It is opposed to the opinion of Levan and Lotfy (85) who appear to be working on the alternate assumption. They mention (85) that in substances, "such as sodium nucleate which in strong concentrations give an incomplete c-mitosis, all kinds of deviating types of c-mitosis and intermediate stages between C- and normal are frequent." Accordingly, they consider the prophase "reductional" groupings as "deviating" types and suggest that they are prophase in nuclei resulting from preceding "exploded c-mitosis" or "distributive c-mitosis."

From the results observed in this study the Levan-Lotfy concept cannot be applied in the case of Acti-dione. First of all, these "segregational" groupings occurred most generally in the later period of observations and in those series where they occurred in greatest frequency none of the cells recovered. In those cases where recovery did occur the "segregational" groupings were rare. In the 5 ppm short treatment series, for instance, only 6 of the 47 chromosomal groupings were of the "segregational" type. The remaining 41 of the "reductional" nature could not possibly have arisen from preceding "exploded c-mitosis" since these disorganized figures do not occur in such frequency in untreated material.

One of the significant points revealed by this study was the apparent correlation between the time and the frequency of the aberration of a particular type. It has been pointed out that the "reductional

groupings of the organized type occurred in highest frequencies in the early hours after initiation of treatment and that the more disorganized figures were found in largest numbers later. This would seem to support the hypothesis that Acti-dione acts in a manner similar to that of a slow fixing agent and gives a further clue as to the reason for the weakness in the effect. It appears likely that Acti-dione penetrates the cell very slowly and results in a sequence of events:

1. A blocking or stalling of the usual nuclear activities before any aberration appears. This was manifested in the 1 ppm short treatment where there was a change in the relative frequencies of the individual stages after the first five hours but no undue aberration.

2. An apparent delay in the usual activity of the nuclear membrane without an affect on the normal changes of the chromosomes. This was shown in the early appearances of the "affected" prophases which contained chromosomes that appeared to have been capable of passing through their normal morphological changes prior to metaphase. In these cases it would seem that Acti-dione either had not reached the interior of the nucleus owing to the slow penetration or was ineffective upon the chromosomes themselves.

3. A delay in the formation of whatever structures had not been formed after sufficient Acti-dione has accumulated to start "fixing" the constituents of the cells. This is manifested by the early appearances of the "reductional" groupings and later development of the more disorganized "segregational" and "scattered" figures. In this event the latter could have arisen at the stage before the nuclear membrane has

been completely fixed. In these cases, the chromosomes, having already attained their metaphase length, but lacking in orientation, simply spread throughout the cell when the nuclear membrane finally "breaks down." Hence the disorganized figures would arise, not from an erosion of the spindle but as a result of its failing to form. Whether or not the disorganization would take the form of a "segregational" grouping would then depend either upon chance or whether or not a sufficient amount of Acti-dione had been present to have disrupted the polarity of the cell.

4. In the cases of all of the short treatment apparently none of the cells had been completely fixed and hence "reversals" were the general rule.

In other words, below the toxicity level, Acti-dione appears to have a tendency to halt processes rather than to destroy elements already present, i.e., to be a "fixer" rather than an "initiator" of activities. The testing of the effects of other antibiotic materials in the light of this hypothesis might yield some important clues concerning the modes of actions of, at least, some of these anti-growth substances. From the practical viewpoint it would seem that such information would be necessary for making decisions concerning the use of these substances as bacteriocides, fungicides, anti-protozoal poisons, etc. For example, cognizance of the fact that Acti-dione, in concentrations above 5 ppm, destroys the mitotic activity of the meristematic tissues would appear to be essential for determinations of concentrations and methods of application of the antibiotic as a fungicide.

Furthermore, if the hypothetical analysis of the mitotic breakdown caused by Acti-dione described above is assumed to be correct, then some postulates concerning the events in normal mitosis may be proposed:

1. If the appearance of the "affected" prophases indicates that Acti-dione can produce this stalling effect upon the nuclear membrane without apparently stalling the development of the chromosomes toward their metaphase condition, two assumptions may be made; either, because of the slow penetration of the antibiotic it has not penetrated the nucleus or Acti-dione has a differential effect upon these two structures. Since these "affected" prophases occurred regularly in root tips continuously treated after a rather long period of time, the first of these assumptions seems less probable and a differential effect (most likely in degree) seems more acceptable.

2. In later stages of these "affected" prophases (Pl. V, Fig. C) it was pointed out that many (sometimes all) of the metaphase-appearing chromosomes which seemed to be still within the nuclear membrane, were unoriented. This permits the conclusion that it may be quite possible that the orientation of the chromosomes may be maintained, and possibly metaphase alignment directed by the spindle.

3. It will be recalled that the "reductional" groupings occurred in the early hours after the initiation of treatment. If Acti-dione is considered to behave like a slow fixing agent with a difference in the degree of affect exerted upon the chromosomes and the other constituents of the cell, then these "reductional" groupings give us some evidence that: (a) The spindle may be gradually formed; the gap representing the

undeveloped section; (b) At least some part of the spindle may be of cytoplasmic origin for, if differential activity of Acti-dione is assumed, then, when the cytoplasm has been affected and its activities retarded, the remainder of the spindle cannot form. This is contrasted with the view of Wada (140) of the intranuclear origin of spindle substances.

4. Since, in the completely disorganized figures, which reflect the total inhibition of the spindle, the chromatids eventually do "fall" apart, the generally accepted concept that the splitting of the kenetochore per se is not dependent upon any spindle influence is supported.

5. In the disorganized anaphases (Frontispiece Fig. F, Pl. IV, Fig. G through I) both oriented and unoriented chromatids have been observed spread throughout the cell. It is recognized that this distribution may have resulted from their "drifting" in the partially "fixed" cytoplasm. On the other hand, however, they may be considered as evidence in support of the possibility that at least some forces associated with chromatid movement may be located within the chromosomes themselves.

SUMMARY

1. Onion root tip meristems were treated with the antibiotic Acti-dione in solutions of varying concentrations ranging from 1 ppm to 80 ppm.

2. The several concentrations were applied to the meristems by two main types of methods:

(a) A continuous treatment for 12 hours.

(b) A short treatment for 15 minutes after which time the root tips were transferred to a distilled water leach.

3. Included for purposes of comparison were two intermittent treatments using a 40 ppm solution for 15 minutes; in one it was applied every hour and in the other it was applied every two hours.

4. There were three types of controls:

(a) An individual root tip control for which the untreated root tips were taken from a single onion at a single time.

(b) Consecutive hour series for which the untreated root tips were taken at the same time as the treated material.

(c) An individual series control for which the root tips were taken from the bulb just before treatment was initiated.

5. From smear preparations of the root tips a study was made

of the various types of division figures which appeared at hourly intervals for a period of twelve hours.

6. There was a general trend toward a reduction in the numbers of division figures following the short treatment and an eventual stability in these numbers in the continuously treated root tips.

7. With the exception of the cytologically ineffective 1 ppm short treatment series, the first five hour period following the initiation of treatment was found to have been the most critical period for the observation of the effects of Acti-dione upon meristematic tissue.

8. Differences in the relative frequencies of the individual stages appeared depending upon the concentration and the method of treatment.

9. On the basis of the combined data of the first five hours, differences were observed in the frequencies of the aberrations in the individual stages with the highest percentages occurring in prophase and the lowest in anaphase.

10. Possibilities of the interrelationship of the several types of aberrations as well as the cytological and genetical potentialities of each were discussed.

11. On the basis of the hourly variation study the frequency percentage of the several types of aberrations appeared to be correlated with time rather than with either the concentration or the method of treatment.

12. It was suggested that Acti-dione appeared to have acted in a manner similar to that of a slow fixing agent upon the dividing cells; that is, to halt processes rather than to initiate them. The assumed slow fixing action appeared to have manifested itself as a stalling of the normal changes that take place during mitosis resulting in the prolongation of the duration of each stage.

13. It was suggested that the degree of the fixing action was proportional to the time as well as the concentration and, as the degree of the fixation was increased, the several types of aberrations became apparent.

14. Since no recoverable aberrations were found, it was suggested that the threshold of toxicity may be very close to that necessary for the production of the cytological aberrations. Furthermore, the threshold of each appears to lie in some combination of concentration and time between 1 ppm and 5 ppm and between 0 and 15 minutes.

15. In several of the types of aberrant figures it appeared that the chromosomes seemed to have been capable of proceeding through their regular morphological changes when, at the same time, there was evidence of partial or total spindle suppression. On this basis it was suggested that Acti-dione may have had a differential effect upon the cytoplasmic and nuclear constituents of the cell.

16. Assuming the slow fixation action of Acti-dione to be correct, the following postulates concerning activities in normal mitosis may be proposed:

(a) A gradual formation of the spindle.

(b) Cytoplasmic origin of, at least, some parts of the spindle.

(c) Possibility of the origin of anaphase movements from some mechanisms arising within the chromatids themselves.

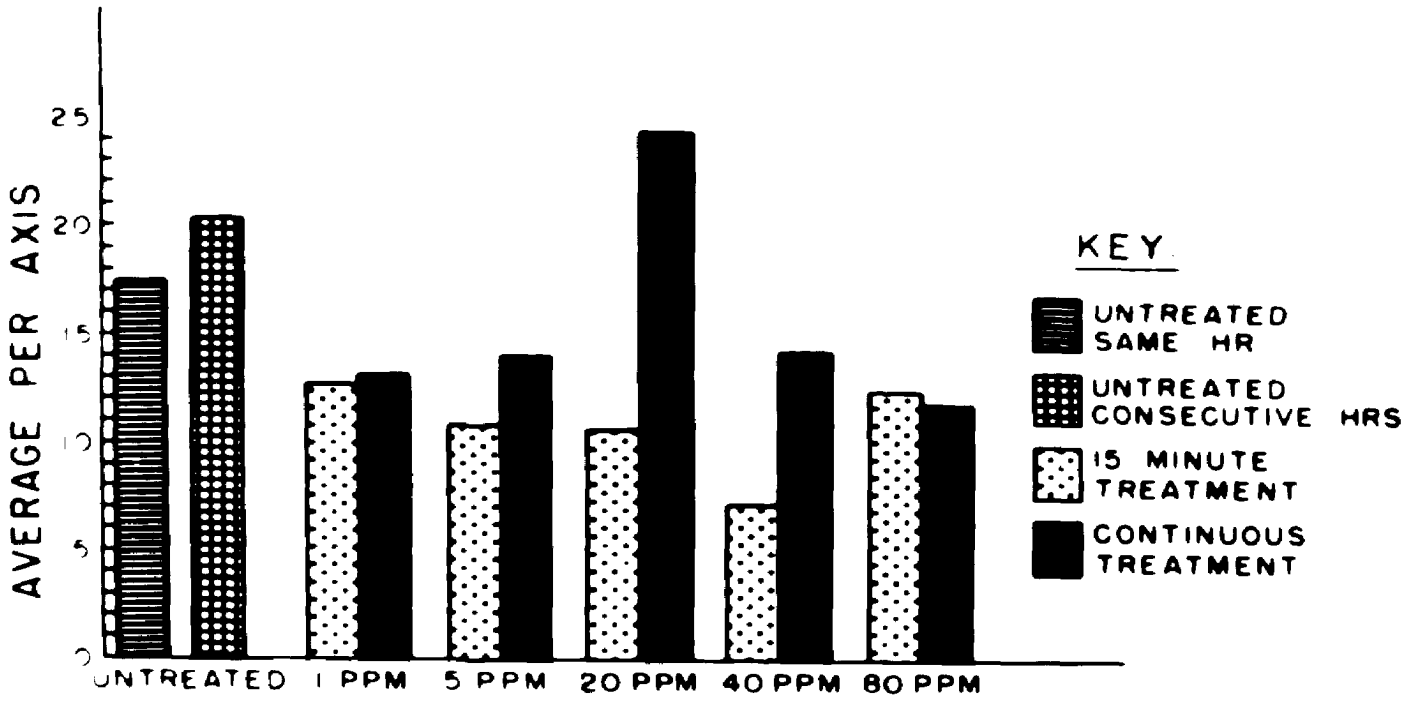
17. Three possible approaches of further investigations are suggested:

(a) The search for recoverable aberrations from Acti-dione should be continued by testing combinations of concentrations between 1 ppm and 5 ppm and time between 0 and 15 minutes. Success in this endeavor would probably yield results of considerable practical significance.

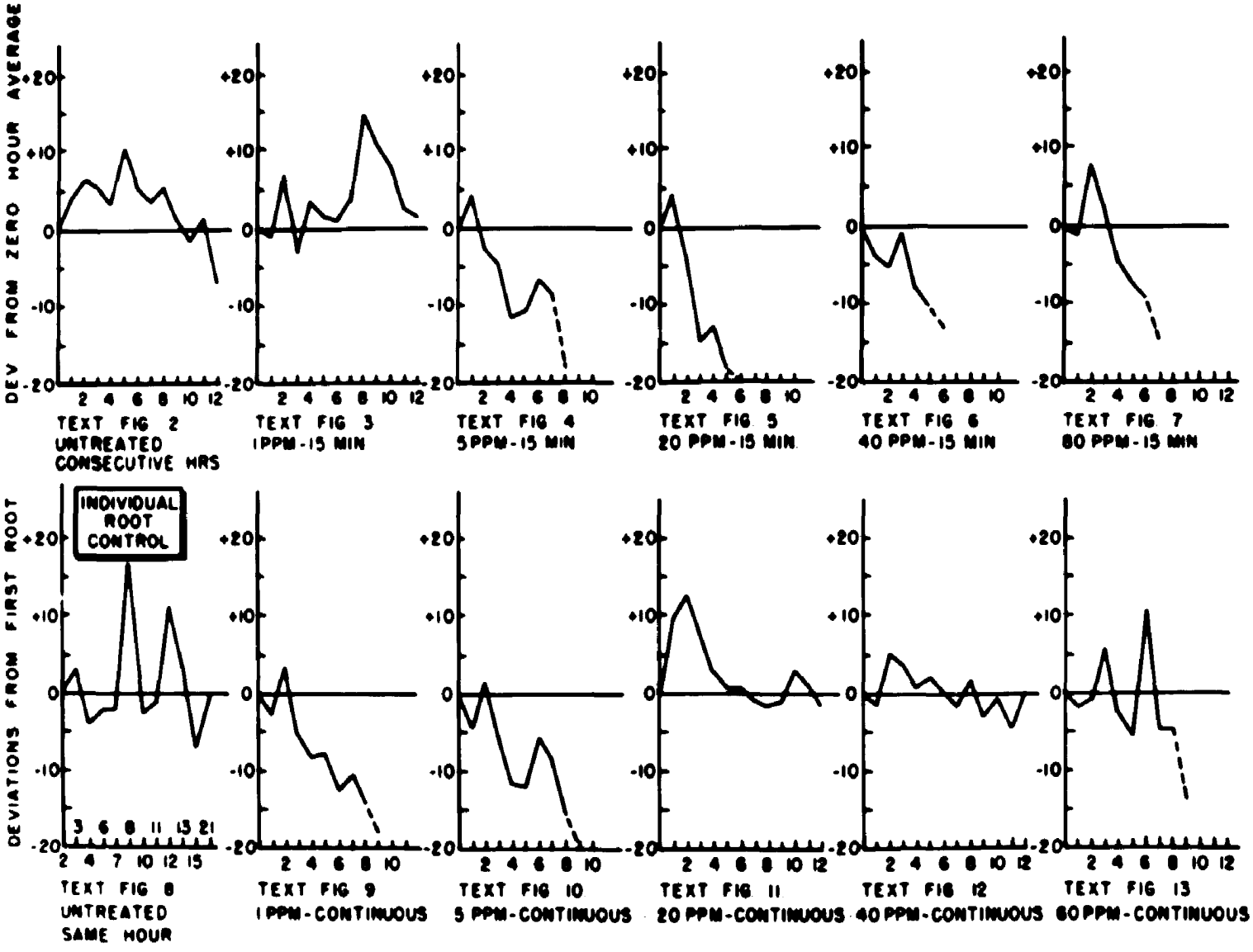
(b) The hypothesis of the slow fixing action of Acti-dione should be tested using other chemicals, particularly the antibiotics. Results of such an investigation might lead to a better understanding of the mode of action of anti-growth substances and could result in some important changes in the methods of practical utilization of these materials.

(c) Since the aberrations appeared to have developed during the early hours following initiation of treatment, it appears that continued observations made during this particular time would yield data from which a better understanding of the fundamental processes of mitosis could be developed.

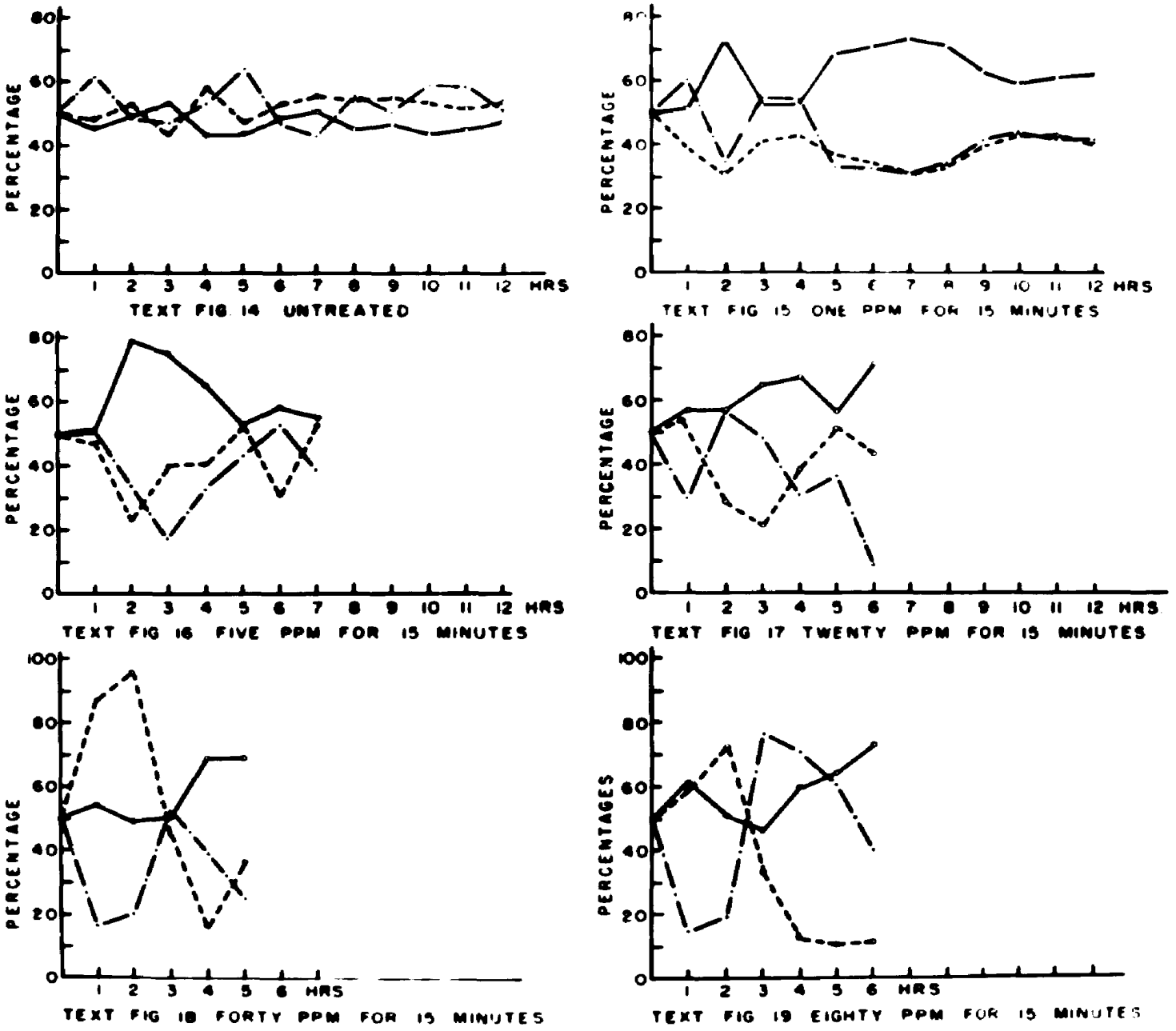
TEXT FIGURES



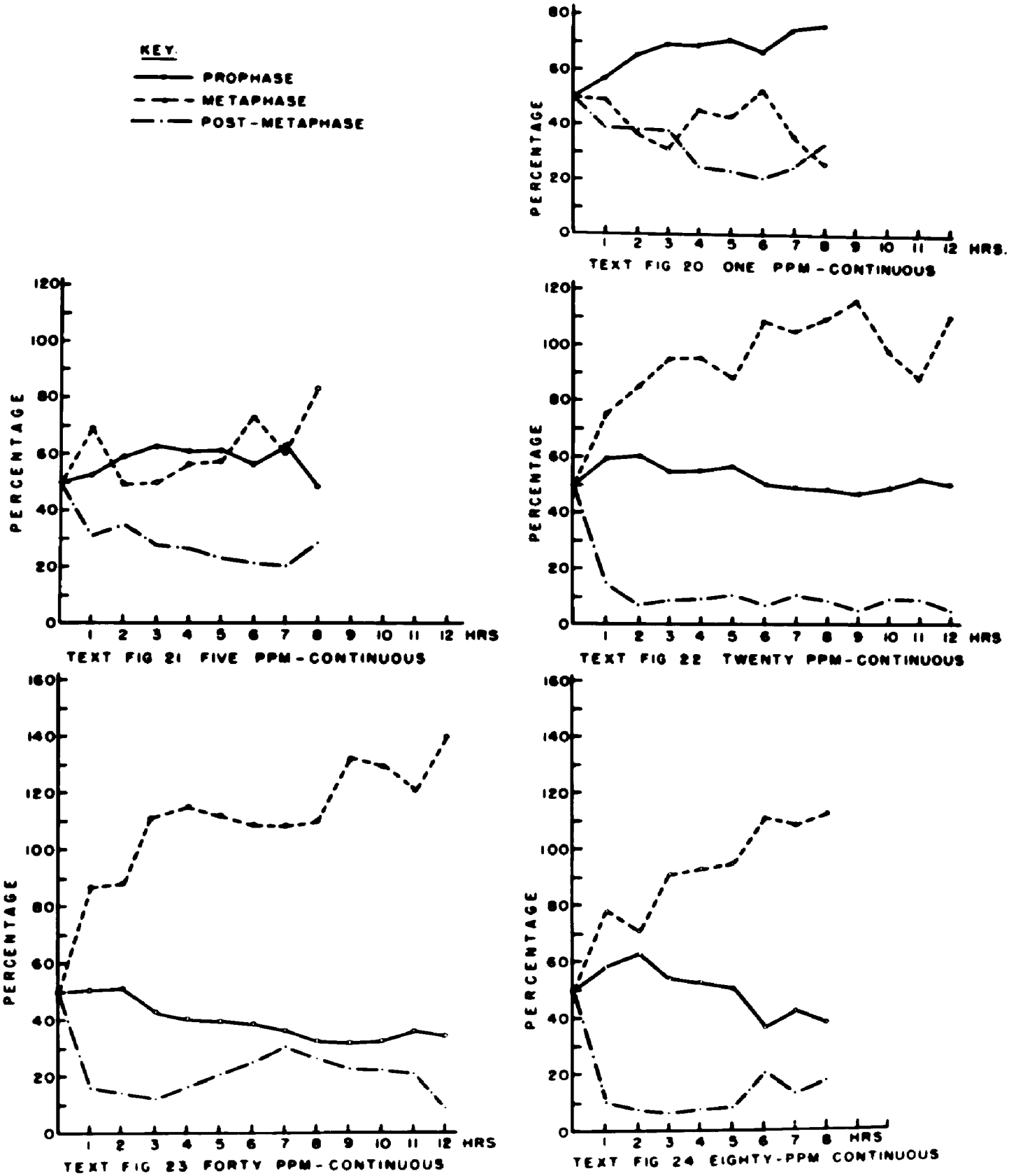
TEXT FIG 1 AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS OBTAINED FROM COMBINED DATA OF FIRST 5 HOURS.



TEXT FIGS 2-13 HOURLY CHANGES IN AVERAGE NUMBER OF DIVISION FIGURES PER AXIS

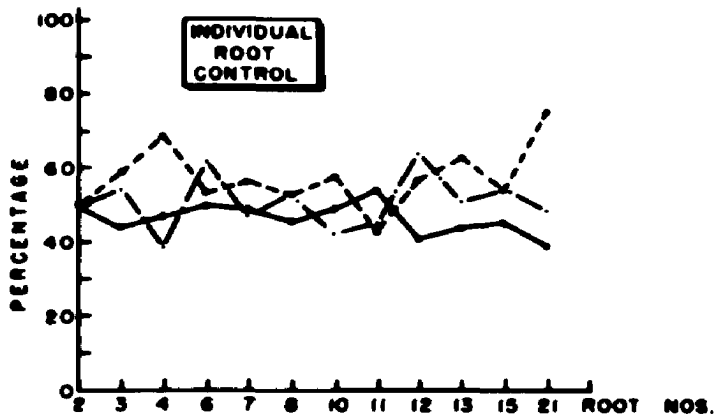


TEXT FIGS. 14-19. HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES.

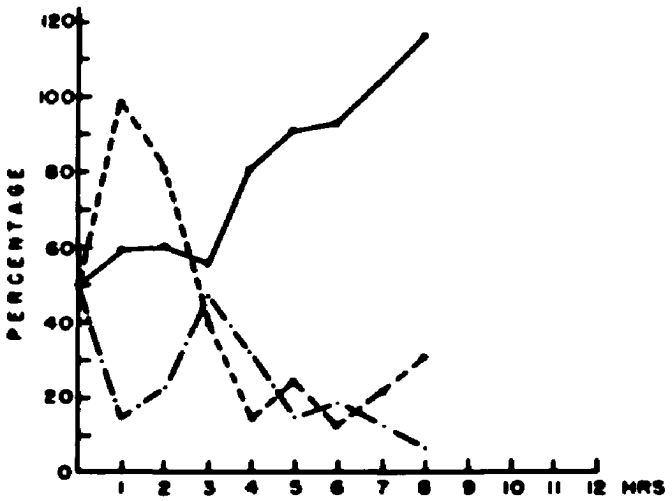


(PERCENTAGES CONVERTED TO BASE OF 50)

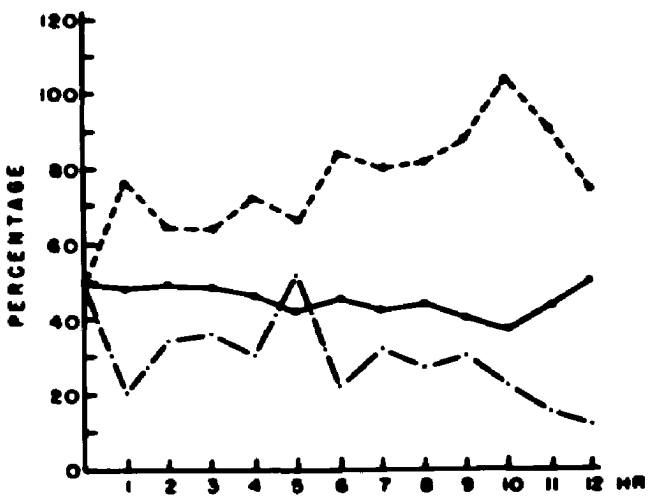
TEXT FIGS. 20-24. HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES IN CONTINUOUS TREATMENT.



TEXT FIG 25 UNTREATED ROOTS FROM SAME ONION AT SAME HOUR



TEXT FIG 26. FORTY PPM/15 MIN/2 HRS

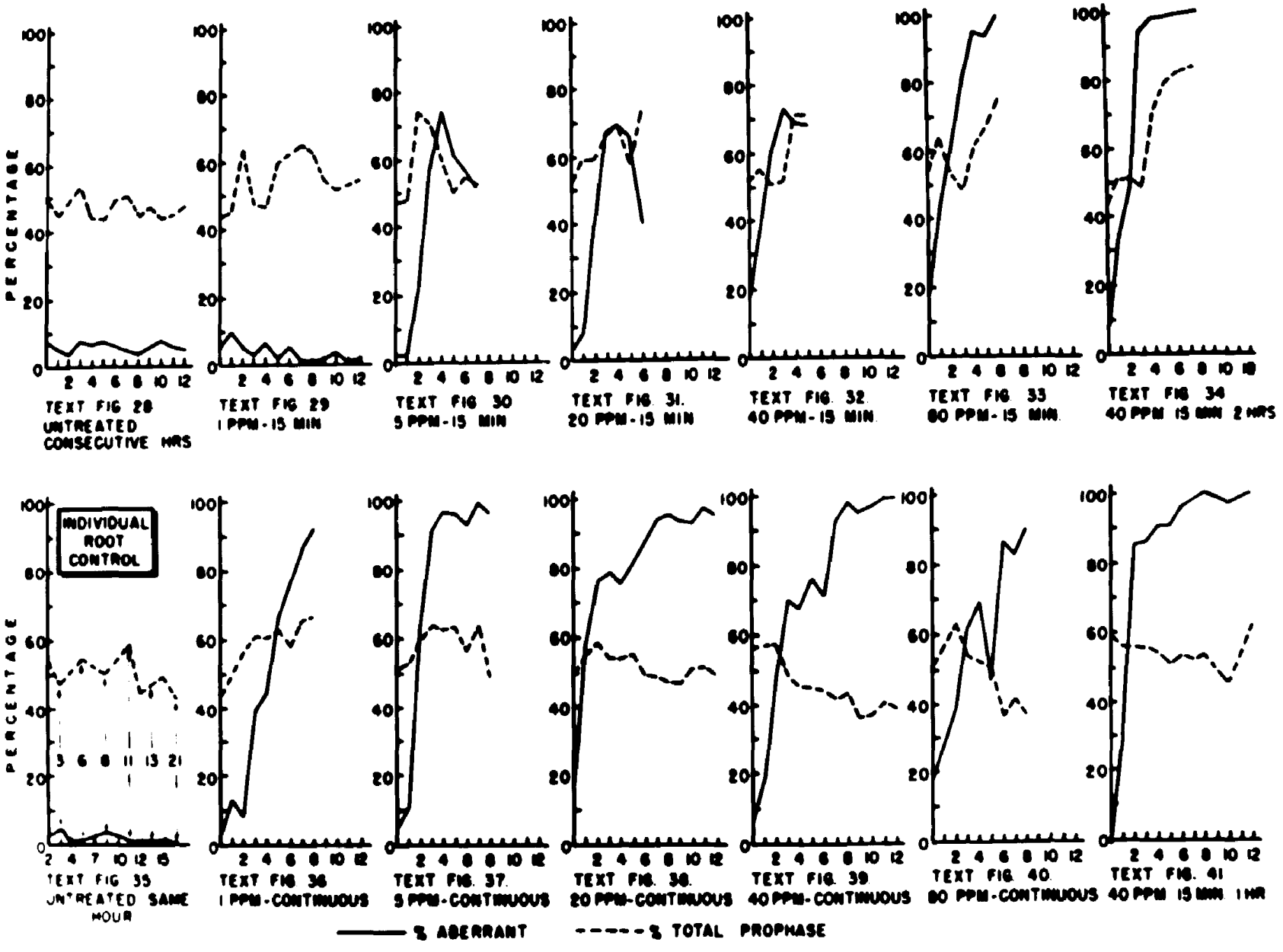


TEXT FIG 27 FORTY PPM/15 MIN/1 HR

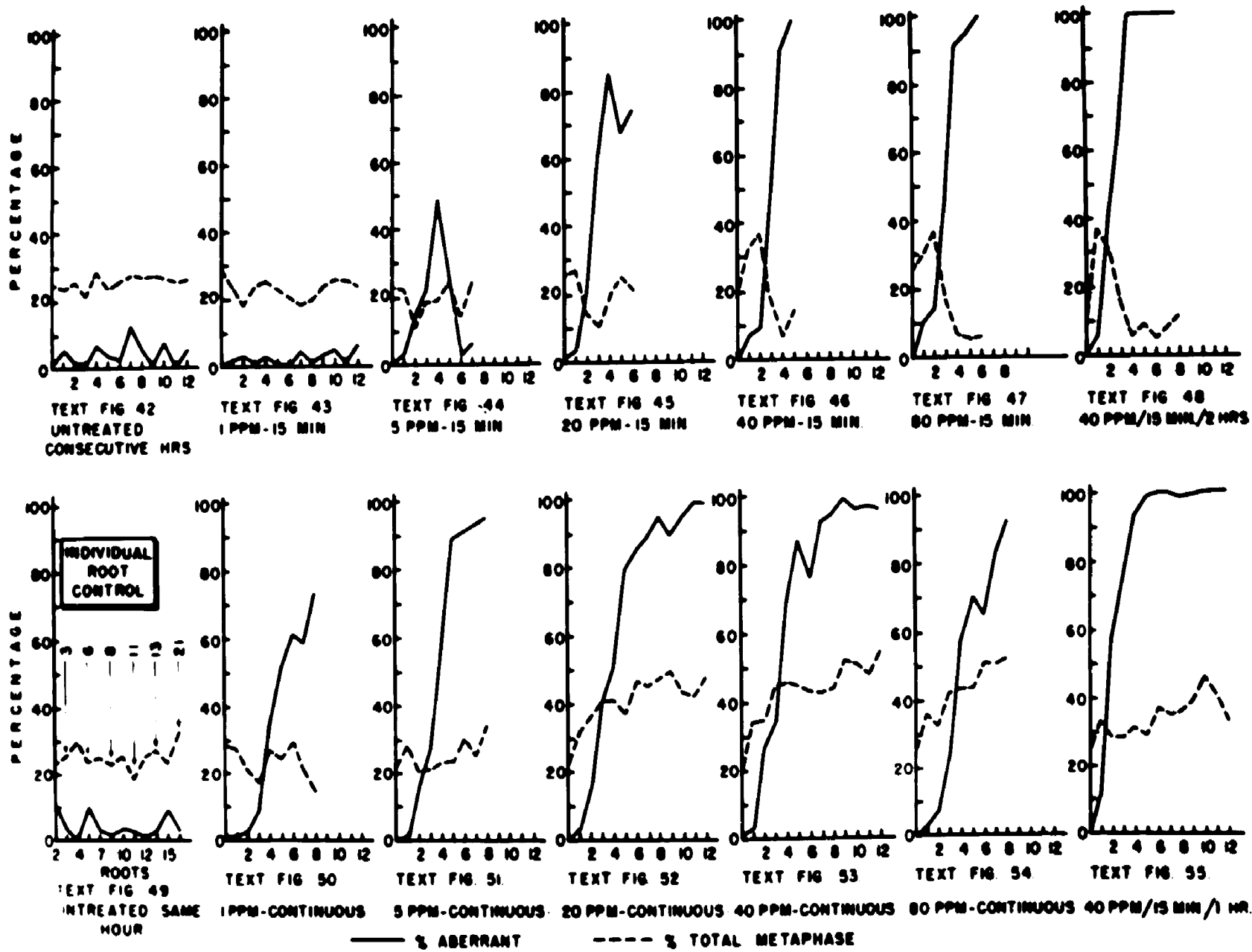
KEY: —●— PROPHASE — —○— — METAPHASE —·— POST-METAPHASE

(PERCENTAGES CONVERTED TO BASE OF 50)

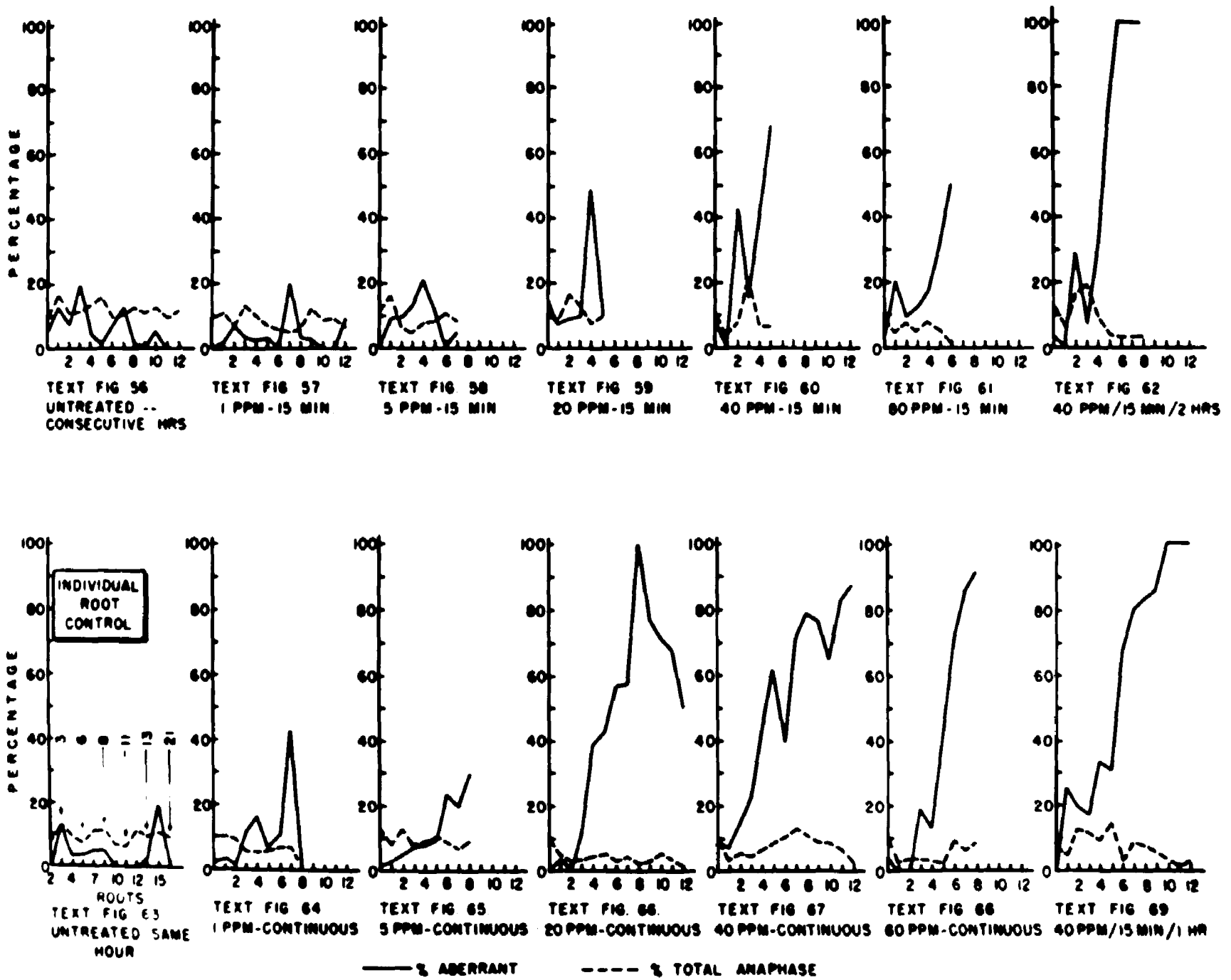
TEXT FIGS. 25-27. HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES.



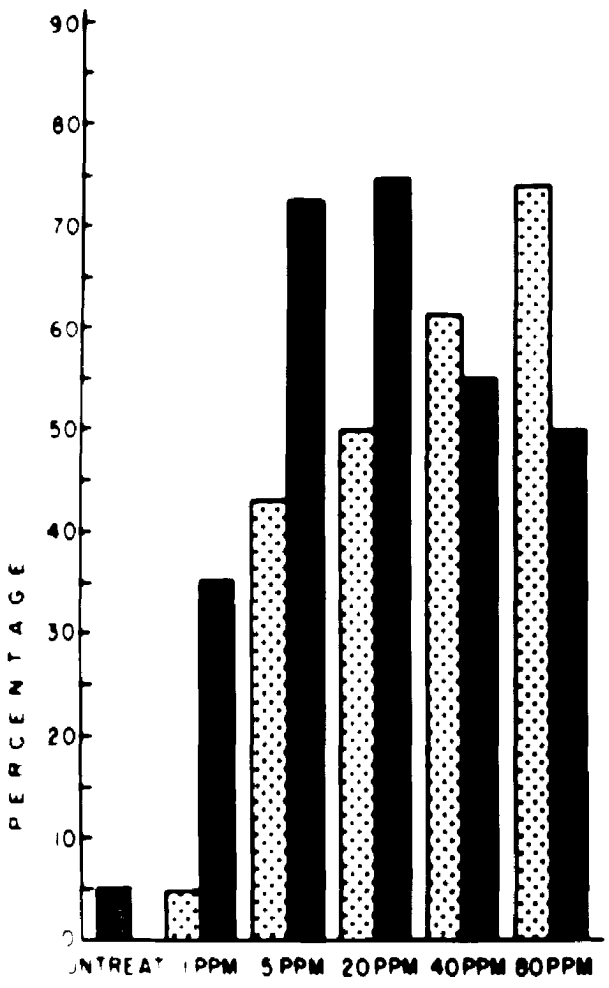
TEXT FIGS 28-41. HOURLY PERCENTAGES OF ABERRANT PROPHASE COMPARED WITH PERCENTAGES OF TOTAL PROPHASE.



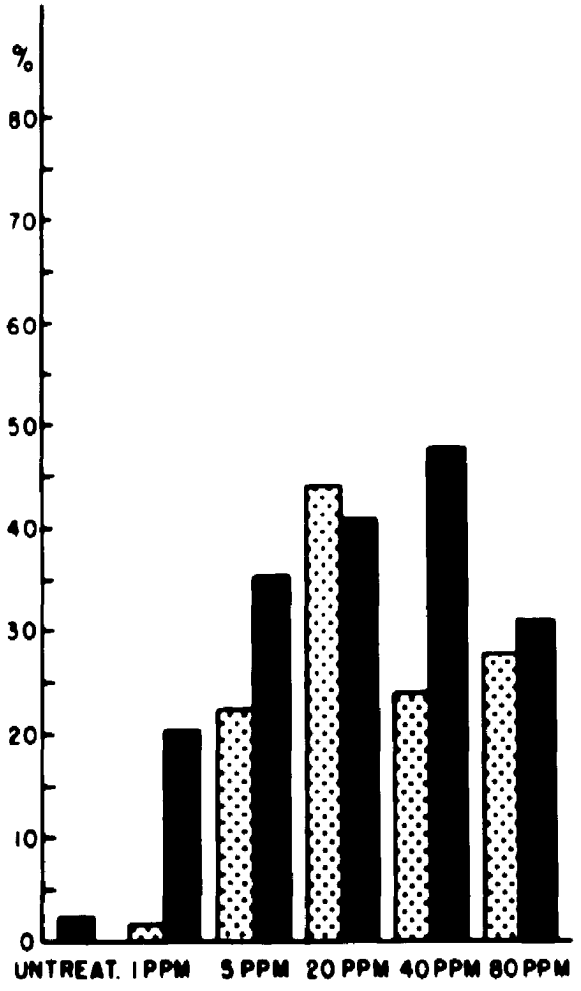
TEXT FIGS. 42-55. HOURLY PERCENTAGES OF ABERRANT METAPHASES COMPARED WITH PERCENTAGES OF TOTAL METAPHASE



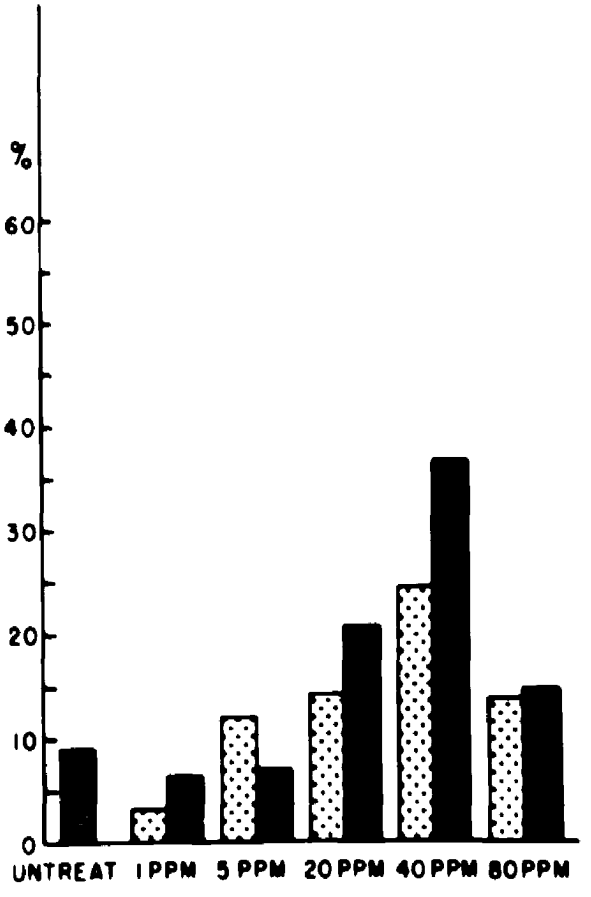
TEXT FIGS. 56-69. HOURLY PERCENTAGES OF DISORGANIZED ANAPHASES COMPARED WITH PERCENTAGES OF TOTAL ANAPHASES



TEXT FIG 70 ABERRANT PROPHASES



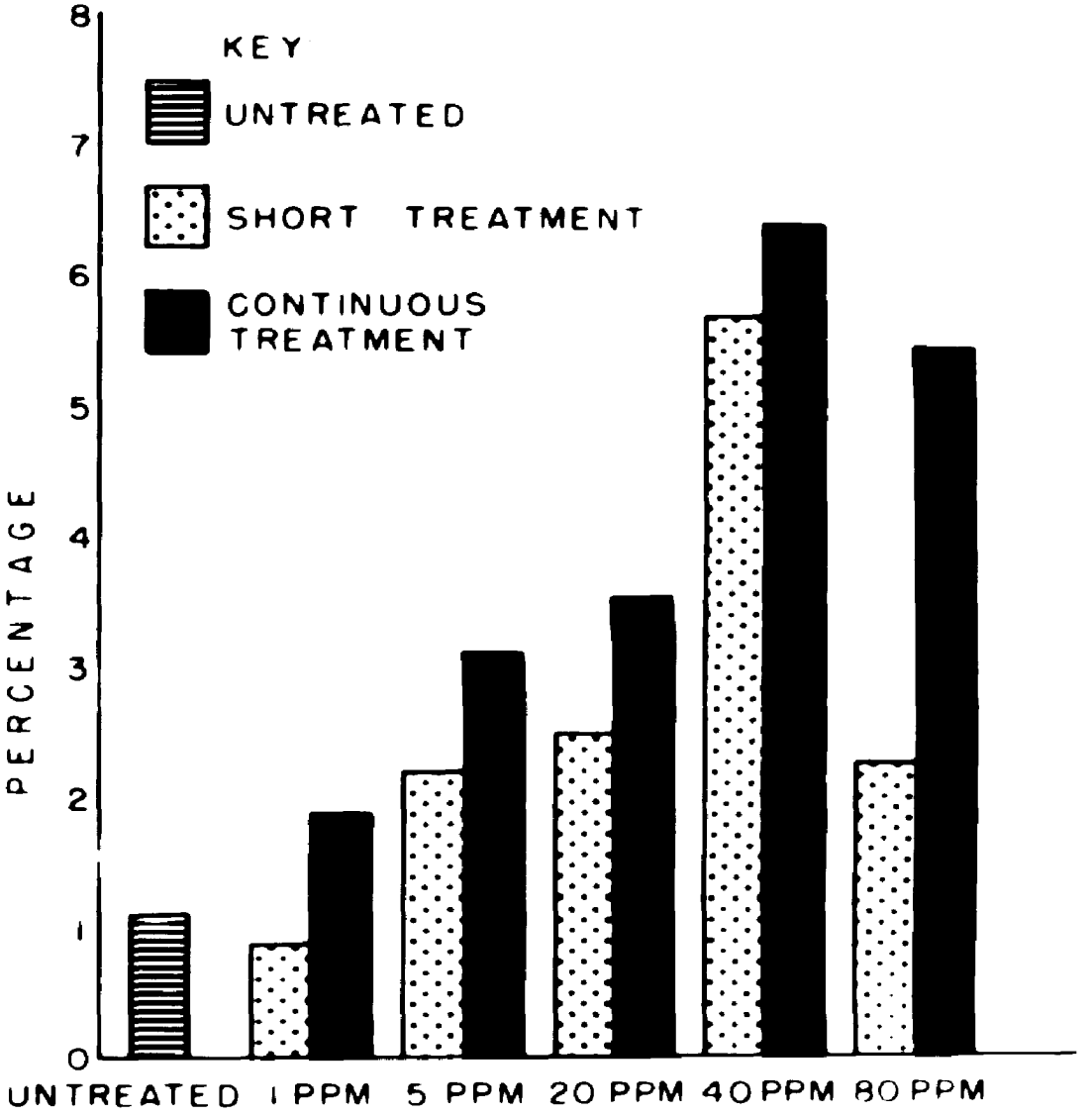
TEXT FIG. 71 "SCATTERED" METAPHASES



TEXT FIG 72 DISORGANIZED ANAPHASES

UNTREATED SHORT TREATMENT CONTINUOUS TREATMENT

TEXT FIGS 70-72. PERCENTAGES OF ABERRANT FIGURES OBTAINED FROM COMBINED DATA OF FIRST FIVE HOURS.



TEXT FIG. 73. MEAN PERCENTAGES OF PROPHASE & METAPHASE CELLS CONTAINING "SEGREGATIONAL" OR "REDUCTIONAL" GROUPINGS FOUND DURING FIRST FIVE HOURS.

PLATES

PLATE I

"NORMALLY" ORGANIZED FIGURES

FIGURE

A. Prophase from root tip taken eight hours after initiation of short treatment with 1 ppm solution.

B. Prometaphase from root tip taken after two hours of continuous treatment with 20 ppm solution.

C. Metaphase from untreated material.

D. Early anaphase from untreated material.

E. Middle anaphase from root tip taken one hour after beginning of short treatment with 1 ppm solution.

F. Late anaphase from untreated material.

G. Early telophase from root tip taken after one hour of continuous treatment with 5 ppm solution.

H. Later telophase from root tip taken one hour after beginning of short treatment with 1 ppm solution.

I. Late telophase accompanied by two interphase cells from untreated material.

PLATE I

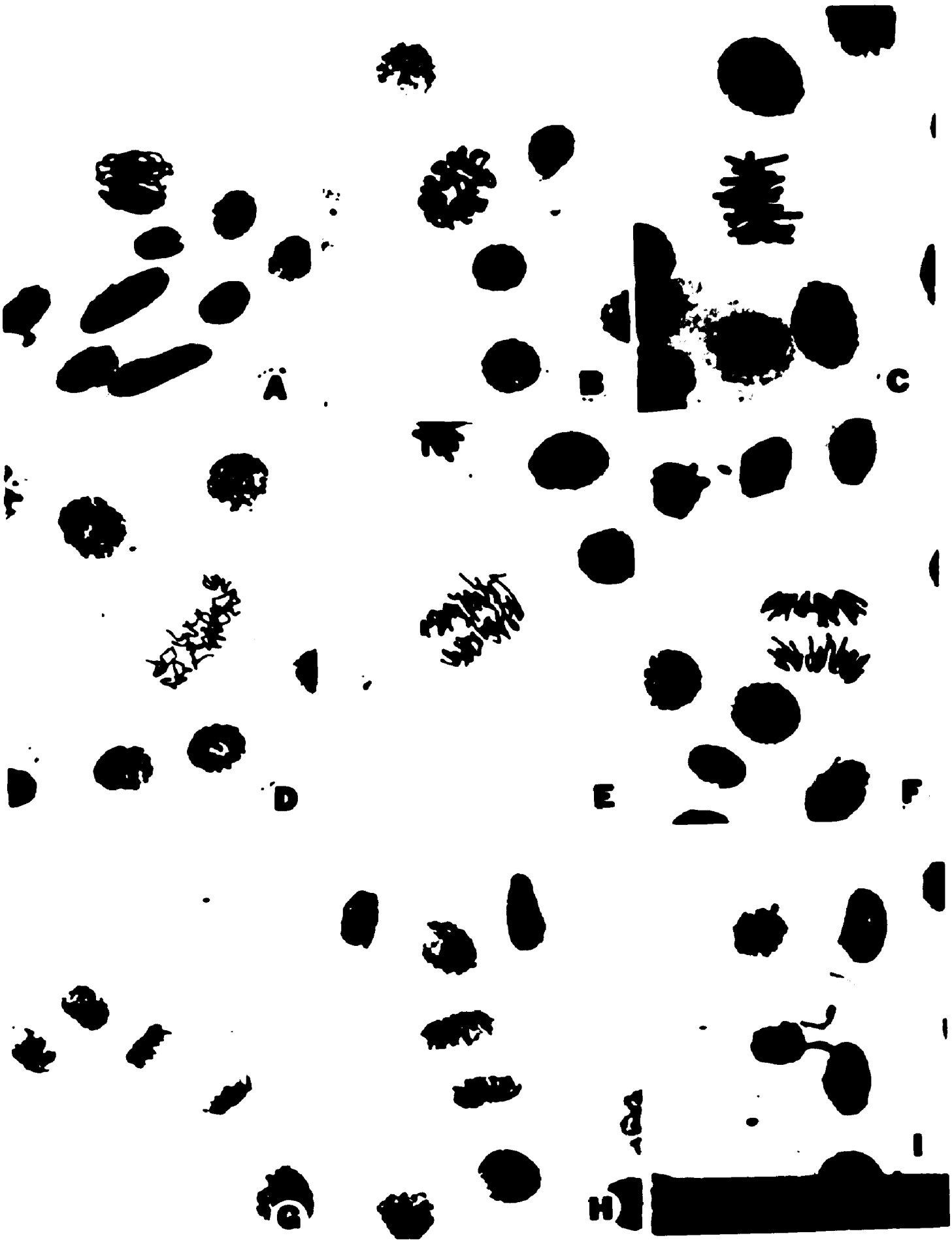


PLATE II

A SERIES OF "REDUCTIONAL" GROUPINGS

FIGURE

A. Prophase "reductional" grouping from root tip taken after one hour of continuous treatment with 40 ppm solution.

B. Prometaphase "reductional" grouping from untreated material.

C. Split metaphase from untreated material.

D. Split metaphase from root tip taken after two hours of continuous treatment with 40 ppm solution.

E. Split anaphase from root tip taken after two and one-half hours of continuous treatment with 40 ppm solution.

F. Split anaphase from untreated material.

G. Split telophase from root tip taken after six hours of continuous treatment with 20 ppm solution.

H. Split telophase from untreated material.

I. Split telophase from root tip taken three hours after beginning of short treatment with 5 ppm solution.

PLATE II

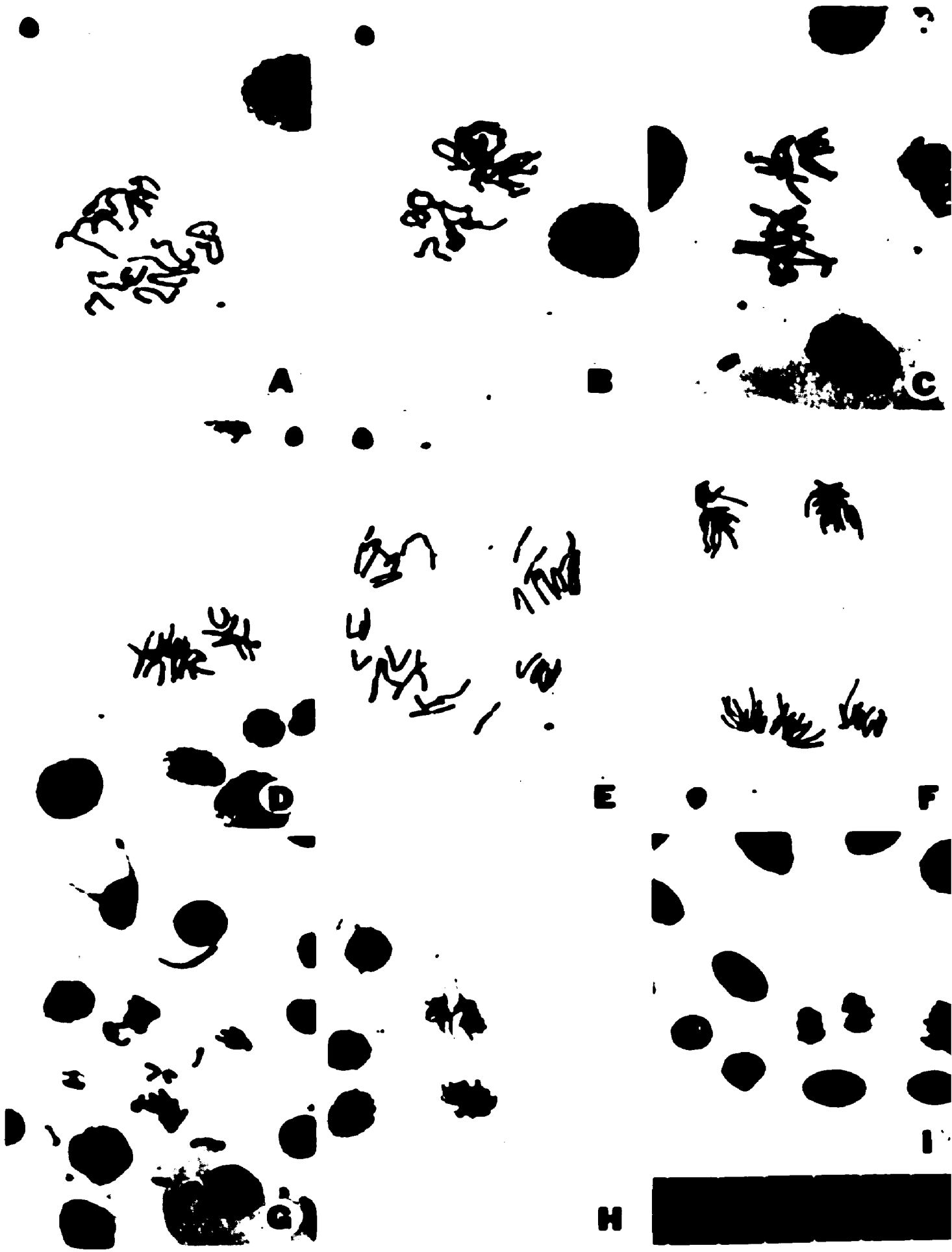


PLATE III

A SERIES OF "SEGREGATIONAL" GROUPINGS

FIGURE

A. Metaphase "segregation" involving equal chromosome numbers from root tip taken after four hours of continuous treatment with 80 ppm solution.

B. Two adjacent metaphase cells, one (upper) involving equal, the other unequal chromosome numbers, from root tip taken after four hours of continuous treatment with 80 ppm solution.

C. Metaphase "segregation" involving equal chromosome numbers from root tip taken after four hours of continuous treatment with 80 ppm solution.

D. Unequal metaphase "segregation" in which chromatids have completely "fallen" apart from root tip taken after three and one-half hours of continuous treatment with 40 ppm solution.

PLATE III

PLATE IV

A SERIES OF "SCATTERED" FIGURES OF THE DISORGANIZED TYPE

FIGURE

A. Disorganized prometaphase from root tip taken after six hours of continuous treatment with 20 ppm solution.

B - C - D.

"Scattered" metaphases from root tips taken after four hours of continuous treatment with 20 ppm solution.

E. Normally organized metaphase showing evidences of spindle suppression in the type of kinetochore splitting, from root tip taken after four hours of continuous treatment in 20 ppm solution.

F. "Scattered" metaphase in which chromatids have "fallen" apart, from root tip taken six hours after the beginning of the short treatment with 20 ppm solution.

G. Disorganized anaphase involving oriented chromatids taken from a root tip after four hours of continuous treatment with a 1 ppm solution.

H. Disorganized anaphase involving both oriented and unoriented chromatids taken from root tip six hours after the initiation of the short treatment with a 40 ppm solution.

I. Disorganized anaphase involving mostly unoriented chromatids taken from root tip after four hours of continuous treatment with 20 ppm solution.

PLATE IV

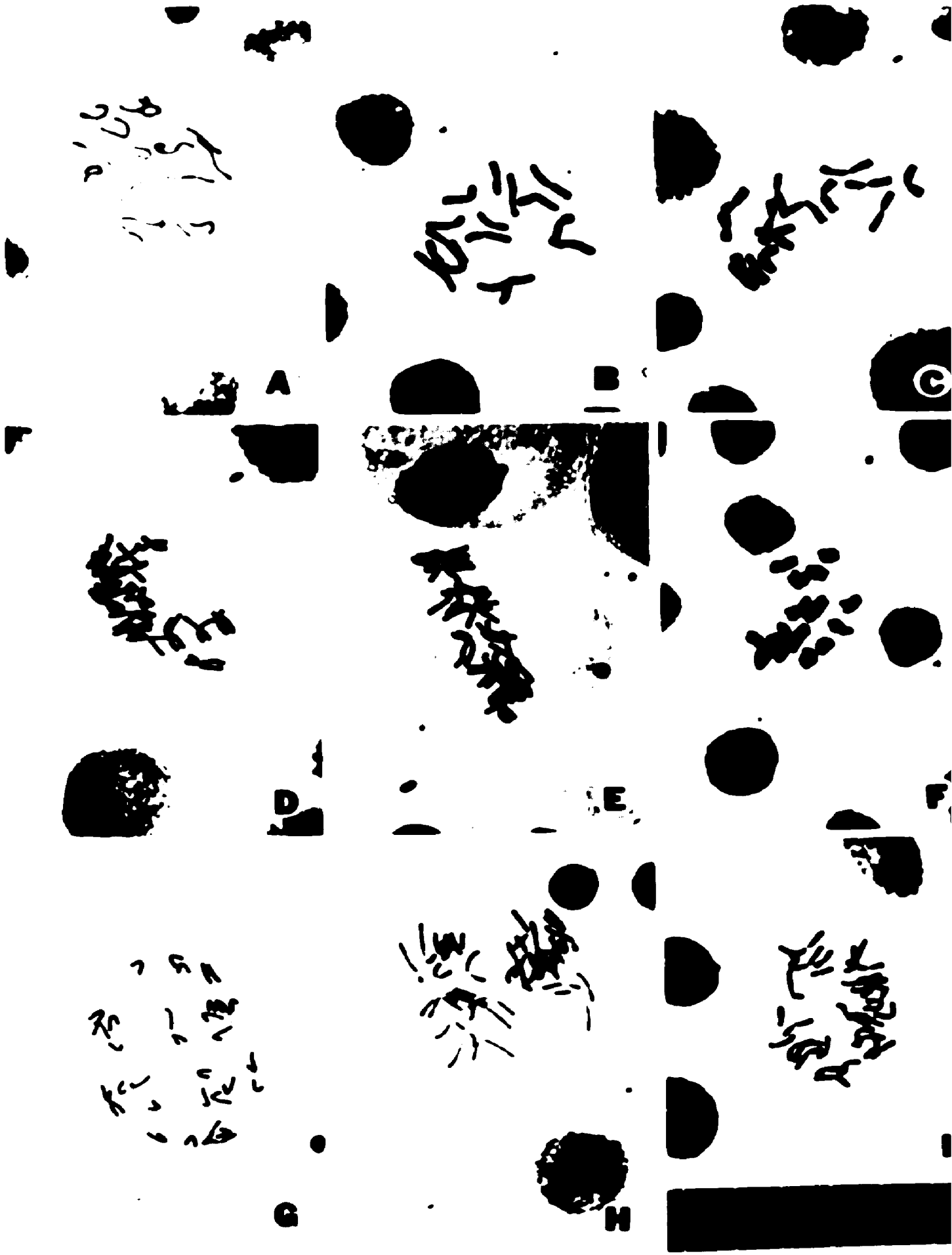


PLATE V

A SERIES OF CELLS SHOWING INDICATIONS OF TOXICITY

FIGURE

A. Deteriorated interphases from root tip taken after eleven hours of continuous treatment with 20 ppm solution.

B. "Affected" prometaphase from root tip taken after four hours of continuous treatment with 20 ppm solution.

C. "Affected" prophase from root tip taken after five hours of continuous treatment with 20 ppm solution.

D. "Reverting" prophase from root tip taken five hours after initiation of short treatment.

E. "Necrotic" prophase from root tip taken after three hours of continuous treatment with 1 ppm solution.

F. "Clumped" metaphase from root tip taken after six hours of continuous treatment with 20 ppm solution.

G. Late anaphase with lagging chromosomes from root tip taken after ten hours of continuous treatment with 20 ppm solution.

H. "Affected" telophase with chromosome "bridges" from root tip taken after three hours of continuous treatment with 40 ppm solution.

I. Telophase with unequal separation of chromatids from root tip taken after nine hours of continuous treatment with 40 ppm solution.

PLATE V

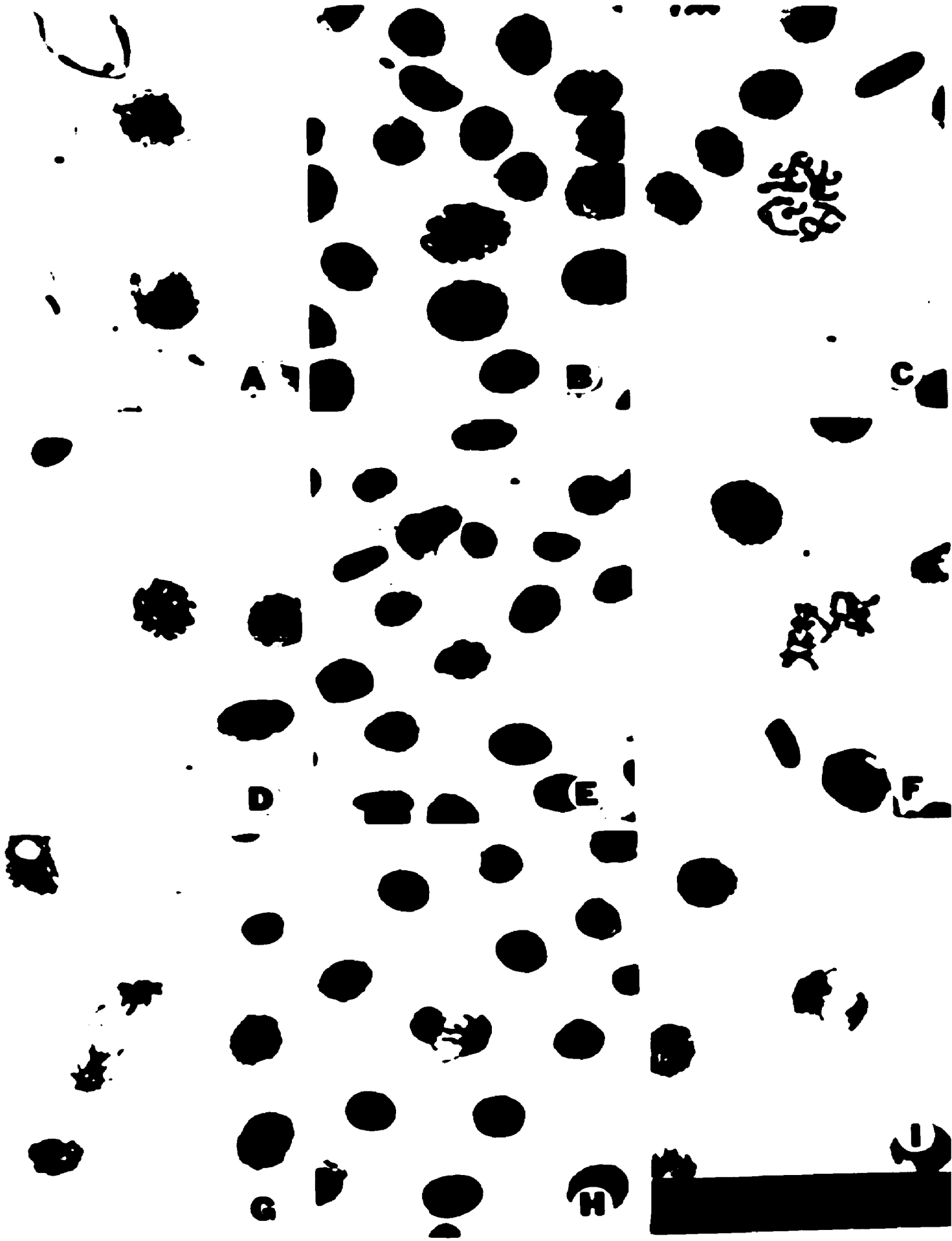


PLATE VI

MISCELLANEOUS

FIGURE

A. Anaphase of a polyploid division from root tip taken three hours after initiation of short treatment with 1 ppm solution.

B. Metaphase with chromosome fragment from root tip taken one hour after initiation of short treatment with 40 ppm solution.

C. Binucleate cell from root tip taken after five hours of continuous treatment with 20 ppm solution.

D. Trinucleate cell from root tip taken after twenty-one hours of continuous treatment with 80 ppm solution.

E. Reduced cell from root tip taken after two hours of continuous treatment with 20 ppm solution.

F. Reduced cell from root tip taken after six hours of continuous treatment with 40 ppm solution.

G. Disorganized telophase from root tip taken four hours after initiation of short treatment with 5 ppm solution.

H. "Transitional" metaphase from root tip taken after three hours of continuous treatment with 80 ppm solution.

I. Unipolar anaphase from root tip taken after two hours of continuous treatment with 20 ppm solution.

PLATE VI

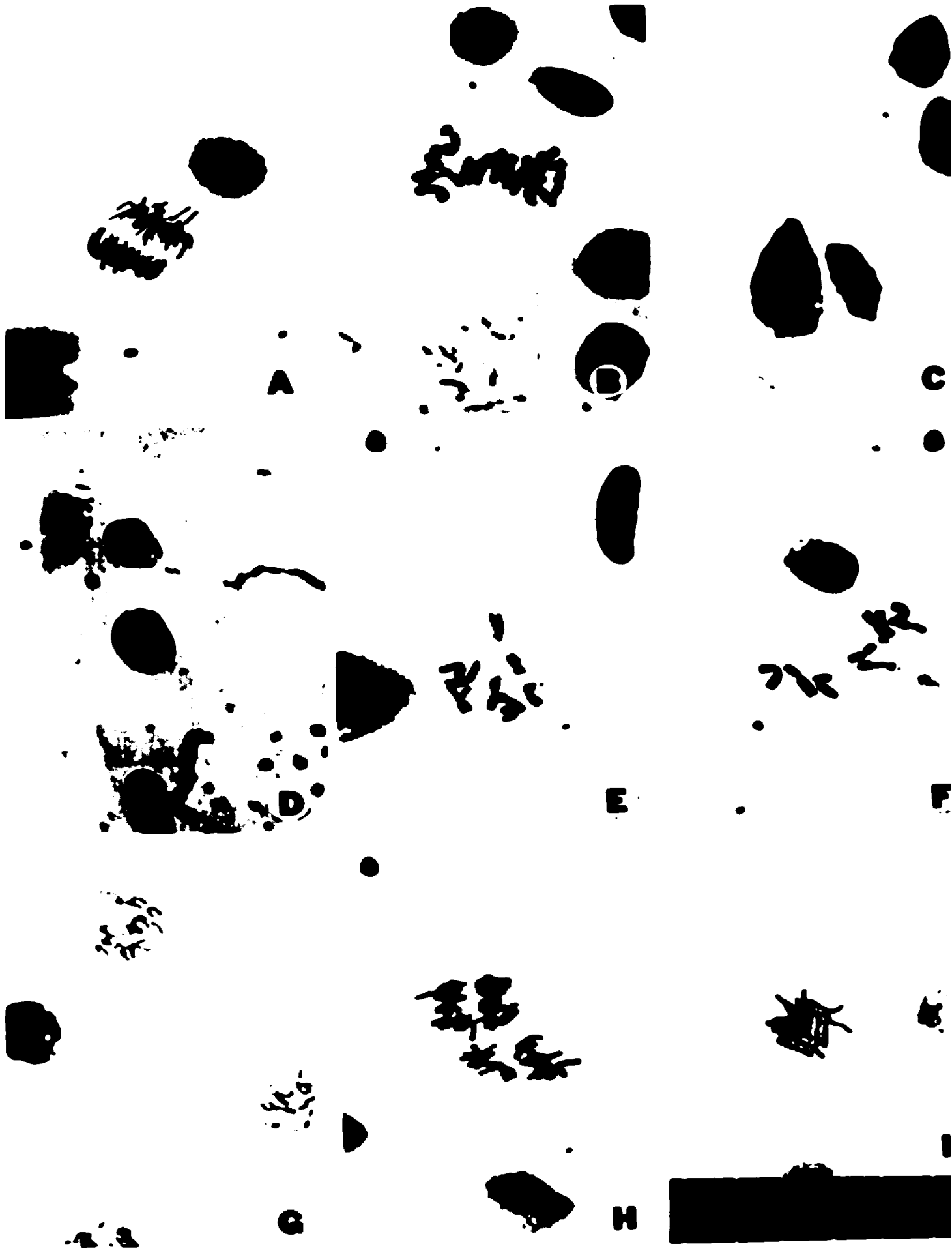


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TABLE I

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE												CHROMOSOME								REFERENCE
			PRO			META			ANA		TELO											
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid cells	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
ACENAPHTHENE	ALLIUM CEPA					X							X					X				13,32,36,70, 71,79,99
ACENAPHTHENE	CREPIS												X									95
ACETYLCHOLINE	ALLIUM CEPA																				X	97
ACRIDINE and amino derivatives	ALLIUM CEPA	X												X				X				35
ACTIDIONE	" "				X	X				X					X							142
ALIZARIN	" "						X				X			X				X				87
AMIDOL	" "																				X	87
9-AMINOACRIDINE	" "										X			X				X				34
p-AMINOPHENOL	" "																X	X				88
ANILINE	" "					X												X				87,88
ANILINE BLUE	" "																				X	9
ATABRINE	" "	X																				35
BENZENE	" "					X							X									13
BENZEDRINE	" "																	X				35
p-BENZOQUINONE	" "					X											X	X				88
BORNEOL	" "					X																43,133,111
BROMOCYCLOHEXANE	" "					X																111
CAFFEINE	" "					X					X		X	X				X	X			67,68
CAFFEINE	TRADESCANTIA											X	X									122

TABLE I (continued)

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE													CHROMOSOME								REFERENCE
			PRO			META			ANA			TELO												
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges		Bi-to Multi-nucleate cells	Polyploid "	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Cone mutations	Ineffective	
CAMPHOR	ALLIUM CEPA					X																	111
CHLORAL HYDRATE	CREPIS sp											X		X									95
CHLORAL HYDRATE	PISUM					X								X									101
CHLORAL HYDRATE	TRADESCANTIA												X	X									121
CHLORAL HYDRATE	ALLIUM CEPA													X									13
CHLOROFORM	TRADESCANTIA												X	X									121
CHLOROFORM	CREPIS													X									95
CIRCULIN	ALLIUM CEPA																					X	142
COLCHICEINE	" "					X								X		X						X	133
COLCHICINE	" "					X			X					X		X							13,27,40,76,79
COLCHICINE	CREPIS											X		X									95
COLCHICINE	WHEAT													X									70
COUMARIN	ALLIUM CEPA and LILIUM					X							X	X		X							24
CRESOL	ALLIUM CEPA					X													X				88
CYCLOHEXANE	" "					X																	111
CYCLOHEXANOL	" "					X									X								111
D-D-T	" "									X	X				X	X							135
DIBROMOTHIOPHENE	" "					X																	111
p-DICHLOROBENZENE	CREPIS													X									95
2,6-DICHLOROPHENOL	ALLIUM CEPA					X																	87
DIIODOTHIOPHENE	" "					X																	111

TABLE I (continued)

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE												CHROMOSOME								REFERENCE
		PRO				META			ANA			TELO											
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindle	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid "	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
ENDOMYCIN	ALLIUM CEPA				X										X							142
EPINEPHRINE	" "																				X	97
ERYTHROSINE	" "																				X	9
ETHER							X						X									117,132
ETHYL ALCOHOL	VICIA FABA								X	X				X								91
ETHYLENE GLYCOL	ALLIUM CEPA						X	X	X					X						?		27,107
4,-ETHYLPYROCATECHOL	" "					X																87
8, ETHOXYCAFFEINE	" "			X														X				67
FUCSIN (BASIC)	" "																				X	9
GALLIC ACID	" "																				X	87
HAEMATOXYLIN	" "																				X	9
HEXACHLOROBENZENE	" "					X																36
HEXACHLOROCYCLOHEXANE	" "					X							X		X							33,72,106,115
HISTAMINE	" "																				X	97
HYDROQUINONE	" "																X	X				87,88
HYDROXYHYDROQUINONE	" "					X	X										X	X				87,88
HYDROXYPHENYLGLYCINE	" "																				X	88
INDOLYL-3-ACETIC ACID	" "																				X	77
INDOLYL-3-BUTYRIC ACID	" "																				X	77
m-INOSITUL	" "																				X	21,30
INSULIN	" "																				X	97
METABROMOTOLUENE	" "					X																111
METACHLOROTOLUENE	" "					X																111

TABLE I (continued)

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE												CHROMOSOME								REFERENCE
			PRO			META			ANA		TELO											
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid "	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
METADICHLOROBENZENE	ALLIUM CEPA					X																111
METHOL	" "					X	X											X				87,88
METHOXYPHENOL	" "					X									X							87,88
METHOXYHYDROQUINONE	" "					X																87,88
METHYLENE BLUE	" "					X	X											X				9
METHYLNAPHTHOQUINONE	" "				X								X									106
METHYLNAPHTHOHYDRO-QUINONE DIACETATE	" "								X													106
MONOCHLOROBENZENE	" "					X																111
MONOBROMOBENZENE	" "					X																111
MONOIODOBENZENE	" "					X																111
MORPHINE	VICIA sp					X												X				3
MUSTARD GAS	ZEA MAYS																	X				55
MUSTARD GAS	BARLEY SEED																			X		60
NAPHTHALENE ACETIC ACID	ALLIUM CEPA																				X	13,16,41,77,85
NAPHTHOL	" "					X																87
NAPHTHORESORCINOL	" "																					87
NEOMYCIN	" "						X							X								142
NICOTINE	TRADESCANTIA											X	X									121
NITROGEN MUSTARD	DORMANT SEEDS OF BARLEY																			X		39,105
NITROGEN MUSTARD	ALLIUM CEPA										X					X		X				39,105
NITROGEN MUSTARD	VICIA									X	X			X				X	X			91
NITROPHENOL	ALLIUM CEPA					X								X								87
OILS (essential oils)	" "		X				X	X										X				36

TABLE I (continued)

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE													CHROMOSOME								REFERENCE
		Interphase	PRO		"Reductional" Groupings	META			ANA		TELO	Bi-to Multi-nucleate cells	Polyploid "		Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
			Membrane	Constituents		"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges												
ORCINOL	ALLIUM CEPA					X	X									X							87
ORSEILLIN BB	" "																					X	9
ORTHODICHLOROBENZENE	" "					X																	111
ORTHOCHLOROTOLUENE	" "					X																	111
PARASORBIC ACID	ALLIUM LILIUM																					X	24
PARATHYROID POWDER	ALLIUM CEPA																					X	97
PENICILLIN G	" "				X																		142
PERVITINE	" "																					X	35
PHENANTHRENEQUINONE	" "																					X	88
PHENOL	" "					X																	88
PHENOTHIAZINE	" "																					X	29
PHENYL-ACETIC ACID	" "																					X	77
PHENYLENEDIAMINE	" "																	X	X				88
PHENYLMERCURIC HYDROXIDE	" "					X								X									93
PHENYLMERCURIC NITRATE	" "					X								X									93
PHENYLPROPRIONIC ACID	" "																					X	77
PHLOROGLUCINOL	" "					X		X															88
PHOSPHINE 5-G	" "										X			X					X				35
PICRIC ACID	" "					X								X									88
PROCATECHOL	" "					X												X	X				88
PROFLAVINE	" "					X								X									35

TABLE I (continued)

REPORTED EFFECTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE													CHROMOSOME									REFERENCE
			PRO			META			ANA			TELO													
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid "	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
PROTOANEMONIN	ZEA MAYS													X									46
PURPUROGALLINE	ALLIUM CEPA							X			X							X					88
PYROGALLOL	" "						X										X	X					88
QUINHYDRONE	" "			X										X				X					88
QUINOLINE BLUE	" "																				X		9
RESORCINOL	" "					X																	88
RIVANOL (BAYER)	" "	X																					34
RODINAL	" "					X												X					88
SAFRANIN																					X		9
SANGUINARINE NITRATE	CREPIS sp												X										95
SODIUM-METHYLNAPHTHO-HYDROQUINONE DIPHOSPHATE	ALLIUM CEPA																				X		106
SODIUM NUCLEATE	ALLIUM CEPA TRADESCANTIA				X	X			X						X								1,2,63,69
SODIUM NUCLEATE and LOW TEMPERATURE	TRILLIUM				X										X								143
STREPTOMYCIN	ALLIUM CEPA				X		X							X	X								142
STREPTOTHRICIN	" "				X	X				X					X								142
SUGAR SOLUTION	TRADESCANTIA											X	X										121
SULFATHIAZOL	ALLIUM CEPA																				X		29
SULFANILAMIDE	" "												X										13

TABLE I (continued)

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH ORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE													CHROMOSOME								REFERENCE
			PRO			META			ANA			TELO												
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid "	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
SULFANILAMIDE	CREPIS												X									95
SULFANILAMIDE	EPHEDRA					X		X							X							94
SULFANILAMIDE	PHASEOLUS											X	X									134
THEOBROMINE	ALLIUM CEPA																	X	X			68
THEOPHYLLINE	" "																	X	X			68
THIOPHENE	" "					X																111
THYMOL	" "					X									X							88
THYMOHYDROQUINONE	" "					X	X	X														88
THYROID POWDER	" "					X					X				X							96,97
TOLUIDINE BLUE	" "					X									X				X			9
TRIBROMOPHENOL	" "					X																88
TRICHLOROBENZENE 1,2,3	" "					X																111
TRIMETHYL COLCHICINIC ACID	" "						X		X					X								133
TRYPAFLAVIN	" "					X			X		X	X		X								10,35
URANIN	" "													X							X	9
URETHANE	" "					X																7
VERATRINE	" "										X		X									13
VIOLET (crystal)	" "																				X	9
3,5,XYLENOL	" "					X									X							88
YEAST EXTRACT	" "					X									X							93

TABLE II

REPORTED RESULTS OF TREATMENTS OF MERISTEMATIC TISSUES WITH INORGANIC CHEMICALS

CHEMICAL	MATERIAL	MITOTIC CYCLE														CHROMOSOME									REFERENCE
			PRO			META			ANA			TELO														
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-Mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges	Bi-to Multi-nucleate cells	Polyploid cells	Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective		
BROMINE	ALLIUM CEPA												X												23
CHROMIUM TRIONDE	" "										X			X											25
MERCURIC CHLORIDE	CREPIS										X		X												95
NITRATES (of 40 metals)	ALLIUM CEPA					X		X			X			X											82
PHOSPHATES	" "			X		X																			52,53
PHOSPHOROUS (RADIOACTIVE)	WHEAT SEEDS																	X							4
PHOSPHOROUS (RADIOACTIVE)	BARLEY AND WHEAT SEEDS													X			X	X							44
PLATINUM TETRACHLORIDE	ALLIUM CEPA										X			X											25
SALTS of Li, Na, K, NH_4	" "						X								X										90

TABLE III

REPORTED RESULTS OF APPLICATION OF CERTAIN PHYSICAL STIMULI TO MERISTEMATIC TISSUE

STIMULUS	MATERIAL	MITOTIC CYCLE													CHROMOSOME								REFERENCE
			PRO			META			ANA		TELO		Bi-to Multi-nucleate cells	Polyploid cells									
		Interphase	Membrane	Constituents	"Reductional" Groupings	"Scattered" (C-mitosis)	Clumpings	Reversals	Uni-, to Multi-polar Spindles	Lagging Chromosomes	Bridges				Stickiness	Shortening	Lengthening	Erosion	Fragments	Interchanges	Gene mutations	Ineffective	
ATOMIC RADIATIONS	MAIZE SEED																		X	X			116
FAST NEUTRONS FROM URANIUM FISSION	TRADESCANTIA MICROSPORES																		X	X			57
NEUTRONS FROM CYCLOTRONS	DORMANT SEEDS OF BARLEY																		X				60
TEMPERATURE (low) and SODIUM NUCLEATE	TRILLIUM				X											X							142
TEMPERATURE (high)	TRADESCANTIA						X		X				X										122
TEMPERATURE (low)	TRADESCANTIA								X														122
TEMPERATURE (low)	ALLIUM CEPA				X	X				X						X							64
ULTRACENTRIFUGING	GERMINATING WHEAT SEED								X				X						X				12
ULTRA SONIC VIBRATIONS	NARCISSUS																		X				103
X-RAYS	ALLIUM CEPA																		X	X			87
X-RAYS	FLAX-SEEDS																				X		80
X-RAYS	SPINACIA ZEA HORDEUM																		X				54
X-RAYS	TRADESCANTIA																		X				17

APPENDIX II

TABLE 1

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
A. SHORT TREATMENT

(One part per million)

TABLE 1A

SERIES 145

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	105	38.6	75	27.5	91	34.1	267
1	115	40.4	68	24.3	99	35.4	280
2	167	65.7	46	18.1	41	16.1	254
3	116	46.8	69	27.8	63	25.4	248
4	106	42.2	77	30.7	68	27.1	251
5	149	64.8	47	20.4	34	14.8	230
6	163	64.4	53	20.9	37	14.6	253
7	170	65.2	64	25.8	35	13.0	269
8	159	67.9	44	18.8	31	13.2	234
9	158	56.2	67	23.8	56	19.9	281
10	124	48.8	71	28.0	59	23.2	254
11	132	48.4	82	30.0	59	21.6	273
12	125	47.2	72	27.2	68	25.7	265
Total Treated	1682	54.4	760	24.6	650	21.0	3092

TABLE 1B

SERIES 147A

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	117	49.6	76	32.2	43	18.2	236
1	109	50.9	46	21.5	59	27.6	214
2	129	60.8	38	17.9	45	21.2	212
3	104	47.3	43	19.5	73	33.2	220
4	122	50.0	47	19.3	75	30.7	244
5	150	55.8	64	23.8	55	20.4	269
6	119	59.2	39	19.4	43	21.4	201
7	173	66.5	34	13.1	53	20.4	260
8	132	57.4	48	20.9	50	21.7	230
9	130	53.1	57	23.3	58	23.7	245
10	128	54.5	52	22.1	55	23.4	235
11	130	58.6	42	18.9	50	22.5	222
12	138	62.7	43	19.5	39	17.7	220
Total Treated	1564	56.4	553	19.9	655	23.6	2772

TABLE 1 (Continued)

(One part per million)

TABLE 1C

SERIES 143 AND 147A COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	220	43.7	149	29.6	134	26.6	505
1	222	44.9	114	23.1	158	32.0	494
2	296	63.5	84	18.0	86	18.5	466
3	220	47.0	112	23.9	136	29.1	468
4	228	46.1	124	25.1	143	28.9	495
5	299	59.9	111	22.2	89	17.8	499
6	282	62.1	92	20.3	80	17.6	454
7	343	64.8	98	18.5	88	16.6	529
8	291	62.7	92	19.8	81	17.5	464
9	288	54.8	124	23.6	114	21.7	526
10	252	51.5	123	25.2	114	23.3	489
11	262	52.9	124	25.1	109	22.0	495
12	263	54.2	115	23.7	107	22.1	485
Total Treated	3246	55.4	1313	22.4	1305	22.3	5864

TABLE 1D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	43.7	50.0	29.6	50.0	26.6	50.0
1	44.9	51.2	23.1	39.0	32.0	60.2
2	63.5	72.4	18.0	30.4	18.5	34.8
3	47.0	53.6	23.9	40.4	29.1	54.7
4	46.1	52.6	25.1	42.4	28.9	54.3
5	59.9	68.3	22.2	37.5	17.8	33.5
6	62.1	70.8	20.3	34.3	17.6	33.1
7	64.8	73.9	18.5	31.3	16.6	31.2
8	62.7	71.4	19.8	33.5	17.5	32.9
9	54.8	62.5	23.6	39.9	21.7	40.8
10	51.5	58.7	25.2	42.6	23.3	43.8
11	52.9	60.3	25.1	42.4	22.0	41.4
12	54.2	61.8	23.7	40.1	22.1	41.5

TABLE 2

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
A. SHORT TREATMENT

(Five parts per million)

TABLE 2A

SERIES 138

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	125	45.8	80	29.3	68	24.9	273
1	141	53.4	58	22.0	65	24.6	264
2	205	77.2	25	9.5	35	13.3	265
3	177	69.4	57	22.4	21	8.2	255
4	120	53.8	63	28.3	40	17.9	223
5	60	46.5	40	31.0	29	22.5	129
6							
7							
8							
9							
10	Insufficient Division Figures						
11							
12							
Total Treated	701	61.8	243	21.4	190	16.8	1134

TABLE 2B

SERIES 152B

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	113	49.1	37	16.1	80	34.8	230
1	113	41.7	60	22.1	98	36.2	271
2	168	71.2	30	12.7	38	16.1	236
3	185	72.3	38	14.8	33	12.9	256
4	160	69.9	22	9.6	47	20.5	229
5	152	51.5	63	21.4	80	27.1	295
6	137	55.0	35	14.1	77	30.9	249
7	140	52.4	66	24.7	61	22.8	267
8							
9							
10	Insufficient Division Figures						
11							
12							
Total Treated	1055	58.5	314	17.4	434	24.1	1803

TABLE 2 (Continued)

(Five parts per million)

TABLE 2C

SERIES 138 AND 152B COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	238	47.3	117	23.3	148	29.4	503
1	254	47.5	118	22.1	163	30.5	535
2	371	74.3	55	11.0	73	14.6	499
3	362	70.8	95	18.6	54	10.6	511
4	280	61.9	85	18.8	87	19.2	452
5	212	50.0	103	24.3	109	25.7	424
6	137	55.0	35	14.1	77	30.9	249
7	140	52.4	66	24.7	61	22.8	267
Total Treated	1756	59.8	557	19.0	624	21.2	2937

TABLE 2D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	47.3	50.0	23.3	50.0	29.4	50.0
1	47.5	50.4	22.1	47.5	30.5	51.9
2	74.3	78.8	11.0	23.7	14.6	24.8
3	70.8	75.0	18.6	40.0	10.6	18.0
4	61.9	65.6	18.8	40.4	19.2	32.6
5	50.0	55.0	24.3	52.2	25.7	43.7
6	55.0	58.3	14.1	30.3	30.9	52.5
7	52.4	55.5	24.7	53.1	22.8	38.8

TABLE 3

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
A. SHORT TREATMENT

(Twenty parts per million)

TABLE 3A

SERIES 92

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	113	54.6	44	21.3	50	24.2	207
1	142	63.1	45	20.0	38	16.9	225
2	126	62.7	28	13.9	47	23.4	201
3	114	56.2	26	12.8	63	31.0	203
4	132	66.3	22	11.1	45	22.6	199
5	146	69.9	45	21.5	18	8.6	209
6	94	74.0	27	21.3	6	4.7	127
7, 8, 9, 10, 11, 12	Insufficient Division Figures						
Total Treated	754	64.8	193	16.6	217	18.6	1164

TABLE 3B

SERIES 140

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	128	49.0	72	27.6	61	23.4	261
1	143	55.6	84	32.7	30	11.7	257
2	164	56.6	41	14.1	85	29.3	290
3	185	75.5	21	8.6	39	15.9	245
4	163	71.5	59	25.9	6	2.6	228
5	98	46.2	59	27.8	55	25.9	212
6							
7, 8, 9, 10, 11, 12	Insufficient Division Figures						
Total Treated	753	61.1	264	21.4	215	17.5	1232

TABLE 3C

SERIES 92 AND 140 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	241	51.5	116	24.8	111	23.7	468
1	285	59.1	129	26.8	68	14.1	482
2	290	59.1	69	14.1	132	26.9	491
3	299	66.7	47	10.5	102	22.8	448
4	295	69.1	81	19.0	51	11.9	427
5	244	58.0	104	24.7	73	17.3	421
6	94	74.0	27	21.3	6	4.7	127
Total Treated	1507	62.9	457	19.1	432	18.0	2396

TABLE 3D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	51.5	50.0	24.8	50.0	23.7	50.0
1	59.1	57.5	26.8	54.1	14.1	29.8
2	59.1	57.3	14.1	28.5	26.9	56.8
3	66.7	64.7	10.5	21.2	22.8	48.1
4	69.1	67.0	19.0	38.4	11.9	25.1
5	58.0	56.3	24.7	49.9	17.3	36.5
6	74.0	71.8	21.3	43.0	4.7	9.9

TABLE 4

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
A. SHORT TREATMENT

(Forty parts per million)

TABLE 4A

SERIES 96

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	110	52.6	41	19.6	58	27.8	209
1	125	58.4	72	33.6	17	7.9	214
2	106	48.8	93	43.3	17	7.9	215
3	114	49.6	51	22.2	65	28.3	230
4	149	74.1	10	5.0	42	20.9	201
5	48	69.6	10	14.5	11	15.9	69
6, 7, 8, 9 10, 11, 12	Insufficient Division Figures						
Total Treated	541	58.2	236	25.4	152	16.4	929

TABLE 4B

SERIES 120

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	126	51.0	48	19.4	73	29.6	247
1	112	53.6	73	34.9	24	11.5	209
2	109	52.9	65	31.6	32	15.5	206
3	130	53.9	33	13.7	78	32.4	241
4	108	67.1	13	8.1	40	24.8	161
5	21	75.0	4	14.3	3	10.7	28
6, 7, 8, 9 10, 11, 12	Insufficient Division Figures						
Total Treated	480	56.8	188	22.2	177	20.9	845

TABLE 4C

SERIES 96 AND 120 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	236	51.8	89	19.5	131	28.7	456
1	237	56.0	145	34.3	41	9.7	423
2	214	50.8	158	37.5	49	11.6	421
3	244	51.8	84	17.8	143	30.4	471
4	257	71.0	23	6.4	82	22.7	362
5	69	71.1	14	14.4	14	14.4	97
Total Treated	1021	57.6	424	23.9	329	18.5	1774

TABLE 4D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	51.8	50.0	19.5	50.0	28.7	50.0
1	56.0	54.3	34.3	87.8	9.7	16.9
2	50.8	49.3	37.5	96.0	11.6	20.2
3	51.8	50.2	17.8	45.6	30.4	52.9
4	71.0	68.9	6.4	16.4	22.7	39.5
5	71.1	69.0	14.4	36.9	14.4	25.1

TABLE 5

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
A. SHORT TREATMENT

(Eighty parts per million)

TABLE 5A

SERIES 118

	Prophase		Metaphase		Post-Meta.		
Hours	No.	%	No.	%	No.	%	Total
0	132	52.6	63	25.1	56	22.3	251
1	127	60.8	67	32.1	15	7.2	209
2	150	56.0	103	38.4	15	5.6	268
3	124	47.7	73	28.1	63	24.2	260
4	136	62.4	15	6.9	67	30.7	218
5	124	58.8	17	8.1	70	33.2	211
6	164	75.9	13	6.0	39	18.1	216
7, 8, 9, 10, 11, 12	Insufficient Division Figures						
Total Treated	825	59.7	288	20.8	269	19.5	1382

TABLE 5B

SERIES 122

	Prophase		Metaphase		Post-Meta.		
Hours	No.	%	No.	%	No.	%	Total
0	154	51.9	75	25.3	68	22.9	297
1	146	66.1	61	27.6	14	6.3	221
2	162	52.3	111	35.8	37	11.9	310
3	126	49.2	15	5.9	115	44.9	256
4	139	61.0	14	6.1	75	32.9	228
5	127	77.9	4	2.5	32	19.6	163
7, 8, 9, 10, 11, 12	Insufficient Division Figures						
Total Treated	700	59.4	205	17.4	273	23.2	1178

TABLE 5C

SERIES 118 AND 122 COMBINED

	Prophase		Metaphase		Post-Meta.		
Hours	No.	%	No.	%	No.	%	Total
0	286	52.2	138	25.2	124	22.6	548
1	273	63.5	128	29.8	29	6.7	430
2	312	54.0	214	37.0	52	9.0	578
3	250	48.4	88	17.0	178	34.5	516
4	275	61.7	29	6.5	142	31.8	446
5	251	67.1	21	5.6	102	27.3	374
6	164	75.9	13	6.0	39	18.1	216
Total Treated	1525	59.6	493	19.3	542	21.2	2560

TABLE 5D

PERCENTAGES CONVERTED USING BASE OF 50

	Prophase		Metaphase		Post-Meta.	
Hours	Real	New	Real	New	Real	New
0	52.2	50.0	25.2	50.0	22.6	50.0
1	63.5	61.0	29.8	59.0	6.7	14.8
2	54.0	51.8	37.0	73.3	9.0	19.9
3	48.4	46.5	17.0	33.7	34.5	76.2
4	61.7	59.2	6.5	12.9	31.8	70.3
5	67.1	64.4	5.6	11.1	27.3	60.3
6	75.9	72.9	6.0	11.9	18.1	40.0

TABLE 6

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES

B. CONTINUOUS TREATMENT

(One part per million)

TABLE 6A

SERIES 146

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	117	48.1	62	25.5	64	26.3	243
1	146	53.1	73	26.5	56	20.4	275
2	166	63.6	47	18.0	48	18.4	261
3	142	61.7	43	18.7	45	19.6	230
4	117	62.9	52	27.9	17	9.1	186
5	126	56.3	72	32.1	26	11.6	224
6	119	57.8	67	32.5	20	9.7	206
7							
8							
9, 10, 11, 12	Insufficient Division Figures						
Total Treated	816	59.0	354	25.6	212	15.3	1382

TABLE 6B

SERIES 151

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	106	39.4	82	30.5	81	30.1	269
1	113	46.7	69	28.5	60	24.8	242
2	159	52.3	70	23.0	75	24.7	304
3	135	59.7	37	16.4	54	23.9	226
4	161	58.8	66	24.1	47	17.1	274
5	205	67.2	55	18.2	44	14.6	302
6	123	58.8	57	27.3	29	13.9	209
7	135	65.5	42	20.4	29	14.1	206
8	105	66.5	23	14.5	30	19.0	158
9, 10, 11, 12	Insufficient Division Figures						
Total Treated	1134	59.0	419	21.8	368	19.1	1921

TABLE 6 (Continued)

(One part per million)

TABLE 6C

SERIES 146 AND 151 COMBINED

	Prophase		Metaphase		Post-Meta.		
Hours	No.	%	No.	%	No.	%	Total
0	223	43.6	144	28.1	145	28.4	512
1	259	50.0	142	27.5	116	22.4	517
2	325	57.5	117	20.7	123	21.8	565
3	277	60.7	80	17.5	99	21.7	456
4	278	60.4	118	25.7	64	13.9	460
5	329	62.5	127	24.1	70	13.3	526
6	242	58.3	124	29.9	49	11.8	415
7	135	65.5	42	20.4	29	14.1	206
8	105	66.4	23	14.6	30	19.0	158
TOTAL TREATED	1950	59.0	773	23.4	580	17.5	3303

TABLE 6 D

PERCENTAGES CONVERTED USING BASE OF 50

	Prophase		Metaphase		Post-Meta.	
Hours	Real	New	Real	New	Real	New
0	43.6	50.0	28.1	50.0	28.4	50.0
1	50.0	57.3	27.5	48.9	22.4	39.4
2	57.5	65.9	20.7	36.8	21.8	38.4
3	60.7	69.6	17.5	31.1	21.7	38.2
4	60.4	69.3	25.7	45.7	13.9	24.5
5	62.5	71.6	24.1	42.9	13.3	23.4
6	58.3	66.8	29.9	53.2	11.8	20.8
7	65.5	75.2	20.4	36.3	14.1	24.8
8	66.4	76.1	14.6	26.0	19.0	33.4

TABLE 7

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
B. CONTINUOUS TREATMENT

(Five parts per million)

TABLE 7A

SERIES 139

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	141	52.8	50	18.7	76	28.5	267
1	146	57.0	63	24.6	47	18.4	256
2	158	56.6	57	20.4	64	22.9	279
3	154	67.0	36	15.6	40	17.4	230
4	129	60.0	55	25.6	31	14.4	215
5	136	63.8	50	23.5	27	12.7	213
6							
7							
8							
9, 10 11, 12	Insufficient Division Figures						
Total Treated	723	60.6	261	21.9	209	17.5	1193

TABLE 7B

SERIES 152A

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	127	49.4	57	22.2	73	28.4	257
1	121	50.4	77	32.0	42	17.5	240
2	195	62.7	62	19.9	54	17.4	311
3	183	61.0	72	24.0	45	15.0	300
4	175	64.1	57	20.9	41	15.0	273
5	161	62.6	59	23.0	37	14.4	257
6	147	57.4	71	30.0	32	12.5	256
7	143	63.5	56	24.9	26	11.6	225
8	109	49.1	76	34.2	37	16.7	222
	Insufficient Division Figures						
Total Treated	1234	59.2	536	25.7	314	15.1	2084

TABLE 7 (Continued)

(Five parts per million)

TABLE 7C

SERIES 139 AND 152A COMBINED

	Prophase		Metaphase		Post-Meta.		
Hours	No.	%	No.	%	No.	%	Total
0	268	51.1	107	20.4	149	28.5	524
1	267	53.8	140	28.2	89	17.9	496
2	353	59.8	119	20.2	118	20.0	590
3	337	63.6	108	20.4	85	16.0	530
4	304	62.3	112	22.9	72	14.7	488
5	297	63.2	109	23.2	64	13.6	470
6	147	57.4	77	30.1	32	12.5	256
7	143	63.5	56	24.9	26	11.5	225
8	109	49.1	76	34.2	37	16.7	222
Total Treated	1957	59.7	797	24.3	423	15.9	3277

TABLE 7D

PERCENTAGES CONVERTED USING BASE OF 50

	Prophase		Metaphase		Post-Meta.	
Hours	Real	New	Real	New	Real	New
0	51.1	50.0	20.4	50.0	28.5	50.0
1	53.8	52.7	28.2	69.0	17.9	31.3
2	59.8	58.6	20.2	49.5	20.0	35.0
3	63.6	62.3	20.4	50.0	16.0	28.0
4	62.3	61.0	22.9	56.1	14.7	25.7
5	63.2	61.9	23.2	56.8	13.6	23.8
6	57.4	56.2	30.1	73.7	12.5	21.8
7	63.5	62.2	24.9	61.0	11.5	20.1
8	49.1	48.1	34.2	83.8	16.7	29.2

TABLE 8

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
B. CONTINUOUS TREATMENT

(Twenty parts per million)

TABLE 8A

SERIES 128

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	105	45.6	48	20.9	77	33.5	230
1	132	56.9	78	33.6	22	9.5	232
2	142	61.2	79	34.0	11	4.7	232
3	112	49.1	111	48.7	5	2.2	228
4	114	48.3	112	47.4	10	4.2	236
5	111	50.2	98	44.3	12	5.4	221
6	88	36.2	148	60.9	7	2.9	243
7	103	43.6	121	51.3	12	5.1	236
8	87	36.4	145	59.8	9	3.8	239
9	103	42.0	137	55.9	5	2.0	245
10	87	40.3	115	52.3	16	7.4	216
11	103	46.3	106	47.7	13	1.4	222
12	88	40.2	127	57.1	4	1.8	219
Total Treated	1270	45.9	1373	49.6	126	4.6	2769

TABLE 8B

SERIES 134

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	112	52.6	47	22.1	54	25.4	213
1	132	60.0	68	30.9	20	9.1	220
2	153	57.1	104	38.8	11	4.1	268
3	163	59.1	94	34.1	19	6.9	276
4	152	59.4	89	34.8	15	5.9	256
5	167	60.3	90	32.5	20	7.2	277
6	166	61.0	91	33.4	15	5.5	272
7	146	53.1	110	40.0	19	6.9	275
8	147	58.1	88	34.8	18	7.1	253
9	126	51.4	107	43.7	12	4.9	245
10	153	59.8	92	35.9	11	4.3	256
11	140	56.5	94	37.9	14	5.6	248
12	141	58.3	91	37.6	10	4.1	242
Total Treated	1786	57.8	1118	36.3	184	6.0	3088

TABLE 8 (Continued)

(Twenty parts per million)

TABLE 8C

SERIES 128 AND 134 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	217	49.0	95	21.4	131	29.6	443
1	264	58.4	146	32.3	42	9.3	452
2	295	59.0	183	36.6	22	4.4	500
3	275	54.5	205	40.7	24	4.8	504
4	266	54.1	201	40.8	25	5.1	492
5	278	55.8	188	37.8	32	6.4	498
6	254	49.3	239	46.4	22	4.3	515
7	249	48.7	231	45.2	31	6.1	511
8	234	47.6	231	47.0	27	5.5	492
9	229	46.7	244	49.8	17	3.5	490
10	240	50.8	205	43.4	27	5.7	472
11	243	51.7	200	42.5	27	5.7	470
12	229	49.7	218	47.3	14	3.0	461
Total Treated	3056	52.2	2491	42.5	310	5.3	5857

TABLE 8D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	49.0	50.0	21.4	50.0	29.6	50.0
1	58.4	59.6	32.3	75.6	9.3	15.7
2	59.0	60.2	36.6	85.6	4.4	7.4
3	54.5	55.6	40.7	95.2	4.8	8.1
4	54.1	55.2	40.8	95.5	5.1	8.6
5	55.8	56.9	37.8	88.4	6.4	10.8
6	49.3	50.3	46.4	108.6	4.3	7.3
7	48.7	49.7	45.2	105.8	6.1	10.3
8	47.6	48.6	47.0	110.0	5.5	9.3
9	46.7	47.6	49.8	116.5	3.5	5.9
10	50.8	51.8	43.4	108.6	5.7	9.6
11	51.7	52.7	42.5	99.4	5.7	9.6
12	49.7	50.7	47.3	110.7	3.0	5.1

TABLE 9

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
B. CONTINUOUS TREATMENT

(Forty parts per million)

TABLE 9A

SERIES 95

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	128	63.4	34	16.8	40	19.8	202
1	131	64.2	59	28.9	14	6.9	204
2	136	65.4	62	29.8	10	4.8	208
3	104	49.8	87	41.6	18	8.6	209
4	111	50.9	83	38.1	24	11.0	218
5	99	45.0	96	43.6	25	11.4	220
6	101	47.6	81	38.2	30	14.2	212
7	89	38.5	111	48.0	31	13.5	231
8	86	36.3	126	53.2	25	10.5	237
9	53	24.2	122	55.7	44	20.1	219
10	86	36.1	119	50.0	33	13.9	238
11	65	31.3	109	52.4	34	16.3	208
12							
Total Treated	1061	44.1	1055	43.9	288	12.0	2404

TABLE 9B

SERIES 125

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	137	51.7	59	22.3	69	26.0	265
1	127	52.0	96	39.3	21	8.6	244
2	110	50.9	87	40.3	19	8.8	216
3	107	48.4	105	47.5	9	4.1	221
4	100	41.7	128	53.3	12	5.0	240
5	108	46.0	108	46.0	19	8.1	235
6	96	41.2	114	48.9	23	9.9	233
7	105	45.5	90	39.0	36	15.6	231
8	115	51.6	77	34.5	31	13.9	223
9	109	49.3	110	49.8	2	0.9	221
10	98	39.5	133	53.6	17	6.9	248
11	122	50.6	107	44.4	12	5.0	241
12	92	39.8	129	55.8	10	4.3	231
Total Treated	1289	46.3	1284	46.1	211	7.6	2784

TABLE 9 (Continued)

(Forty parts per million)

TABLE 9C

SERIES 95 AND 125 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	265	56.7	93	19.9	109	23.3	467
1	258	57.6	155	34.6	35	7.8	448
2	246	58.0	149	35.1	29	6.8	424
3	211	49.1	192	44.6	27	6.3	430
4	211	46.0	211	46.0	36	7.9	458
5	207	45.5	204	44.8	44	9.7	455
6	197	44.3	195	43.8	53	11.9	445
7	194	42.0	201	43.5	67	14.5	462
8	201	43.7	203	44.1	56	12.2	460
9	162	36.8	232	52.7	46	10.4	440
10	184	37.9	252	51.8	50	10.3	486
11	187	41.6	216	48.1	46	10.2	449
12	92	39.8	129	55.8	10	4.3	231
Total Treated	2350	45.3	2339	45.1	499	9.6	5188

TABLE 9D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	56.7	50.0	19.9	50.0	23.3	50.0
1	57.6	50.7	34.6	86.8	7.8	16.8
2	58.0	51.0	35.1	88.1	6.8	14.6
3	49.1	43.2	44.6	111.9	6.3	13.5
4	46.0	40.5	46.0	115.5	7.9	17.0
5	45.5	40.0	44.8	112.4	9.7	20.9
6	44.3	39.0	43.8	109.9	11.9	25.6
7	42.0	37.0	43.5	109.2	14.5	31.2
8	43.7	38.5	44.1	110.7	12.2	26.2
9	36.8	32.4	52.7	132.3	10.4	22.4
10	37.9	33.4	51.8	130.0	10.3	22.1
11	41.6	36.6	48.1	120.7	10.2	21.9
12	39.8	35.0	55.8	140.0	4.3	9.2

TABLE 10

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
B. CONTINUOUS TREATMENT

(Eighty parts per million)

TABLE 10A

SERIES 102

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	93	45.1	51	24.8	62	30.1	206
1	127	59.9	68	32.1	17	8.0	212
2	156	72.9	48	22.4	10	4.7	214
3	117	50.6	110	47.6	4	1.7	231
4	127	54.3	100	42.7	7	3.0	234
5	110	51.4	94	43.9	10	4.7	214
6	-	-	-	-	-	-	-
7	95	44.8	112	52.8	5	2.4	212
8	66	40.5	93	57.0	4	2.5	163
9, 10, 11, 12	Insufficient Division Figures						
Total Treated	798	53.9	625	42.2	57	3.8	1480

TABLE 10B

SERIES 115

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	119	53.8	47	21.3	55	24.9	221
1	137	55.9	99	40.4	9	3.7	245
2	116	52.5	96	43.4	9	4.1	221
3	160	56.9	106	37.7	15	5.3	281
4	137	50.9	117	43.5	15	5.6	269
5	-	-	-	-	-	-	-
6	98	37.5	134	51.3	29	11.1	261
7	93	39.2	115	48.5	29	12.2	237
8	94	36.2	128	49.2	38	14.6	260
9, 10, 11, 12	Insufficient Division Figures						
Total Treated	835	47.1	795	44.8	144	8.1	1774

TABLE 10 (Continued)

(Eighty parts per million)

TABLE 10 C

SERIES 102 AND 115 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	212	49.6	98	23.0	117	27.4	427
1	264	57.8	167	36.5	26	5.7	457
2	272	62.5	144	33.1	19	4.4	435
3	277	54.1	216	42.2	19	3.7	512
4	264	52.5	217	43.1	22	4.4	503
5	110	51.4	94	43.9	10	4.7	214
6	98	37.5	134	51.3	29	11.1	261
7	188	41.9	227	50.6	34	7.6	449
8	160	37.8	221	52.2	42	9.9	423
Total Treated	1633	50.2	1420	43.6	201	8.5	3254

TABLE 10D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	49.6	50.0	23.0	50.0	27.4	50.0
1	57.8	58.3	36.5	79.2	5.7	10.4
2	62.5	63.1	33.1	71.8	4.4	8.0
3	54.1	54.6	42.2	91.6	3.7	6.7
4	52.5	53.4	43.1	93.5	4.4	8.0
5	51.4	51.9	43.9	95.3	4.7	8.6
6	37.5	37.8	51.3	111.3	11.1	20.2
7	41.9	42.3	50.6	109.8	7.6	13.8
8	37.8	38.2	52.2	113.3	9.9	18.0

TABLE 11

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
C. INTERMITTANT TREATMENT

(Forty parts per million for 15 minutes every two hours)

TABLE 11 A

SERIES 126

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	98	43.2	42	18.5	87	38.3	227
1	117	51.3	84	36.8	27	11.8	228
2	113	51.8	66	30.3	39	17.3	218
3	110	48.5	34	15.0	83	36.6	227
4	147	69.7	12	5.7	52	24.6	211
5	145	78.8	17	9.2	22	12.0	184
6	166	80.6	10	4.9	30	14.6	206
7	-	-	-	-	-	-	-
8	65	83.3	9	11.5	4	5.1	78
Total Treated	863	63.8	232	17.2	257	19.0	1352

TABLE 11 B

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	43.2	50.0	18.5	50.0	38.3	50.0
1	51.3	59.5	36.8	99.4	11.8	15.5
2	51.8	60.1	30.3	81.8	17.3	22.7
3	48.5	56.3	15.0	40.5	36.6	47.9
4	69.7	80.9	5.7	15.4	24.6	32.2
5	78.8	91.4	9.2	24.8	12.0	15.7
6	80.6	93.5	4.9	13.2	14.6	19.1
7	-	-	-	-	-	-
8	83.3	96.6	11.5	31.1	5.1	6.7

TABLE 12

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
C. INTERMITTANT TREATMENT

(Forty parts per million for 15 minutes every hour)

TABLE 12A

SERIES 135

Hours	Prophase Count	Prophase %	Metaphase Count	Metaphase %	Post-Meta. Count	Post-Meta. %	Total
0	136	59.6	50	21.9	42	18.4	228
1	154	58.3	89	33.7	21	8.0	264
2	147	58.1	73	28.9	33	13.0	253
3	149	58.0	73	28.4	35	13.6	257
4	145	56.4	82	31.9	30	11.7	257
5	139	50.9	81	29.7	53	19.4	273
6	126	53.8	88	37.6	20	8.5	234
7	129	52.8	88	35.6	30	12.1	247
8	125	53.6	84	36.1	24	10.3	233
9	126	49.2	100	39.1	30	11.7	256
10	116	45.3	118	46.1	22	8.6	256
11	131	53.5	99	40.4	15	6.1	245
12	151	61.9	81	33.2	12	4.9	244
Total Treated	1638	54.3	1056	35.0	325	10.8	3019

TABLE 12B

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	59.6	50.0	21.9	50.0	18.4	50.0
1	58.3	48.9	33.7	76.8	8.0	21.8
2	58.1	48.7	28.9	65.9	13.0	35.4
3	58.0	48.7	28.4	64.8	13.6	37.0
4	56.4	47.3	31.9	72.7	11.7	31.8
5	50.9	42.7	29.7	67.7	19.4	52.8
6	53.8	45.1	37.6	85.7	8.5	23.1
7	52.2	43.8	35.6	81.2	12.1	32.9
8	53.6	45.0	36.1	82.3	10.3	28.0
9	49.2	41.3	39.1	89.1	11.7	31.8
10	45.3	38.0	46.1	105.1	8.6	23.4
11	53.5	44.9	40.4	92.1	6.1	16.6
12	61.9	51.9	33.2	75.7	4.9	13.3

TABLE 13

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
D. UNTREATED

(Root tips collected at consecutive hours)

TABLE 13A

SERIES 127

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	108	50.5	49	22.9	57	26.7	214
1	113	48.1	49	20.8	73	31.1	235
2	125	55.7	56	25.5	48	21.0	229
3	141	55.1	45	17.6	70	27.3	256
4	102	44.1	54	23.4	75	32.5	231
5	98	41.3	44	18.6	95	40.1	237
6	123	47.1	66	25.3	72	27.6	261
7	110	49.8	62	28.0	49	22.2	221
8	117	52.5	52	23.3	54	24.2	223
9	115	47.9	68	28.3	57	23.8	240
10	107	46.7	51	22.3	71	31.0	229
11	101	44.5	57	25.1	69	30.4	227
12	118	48.5	53	21.8	72	29.6	243
Total	1476	48.5	708	23.2	862	28.3	3046

TABLE 13B

SERIES 132

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	122	48.6	64	25.5	65	25.9	251
1	112	41.2	70	25.8	90	33.1	272
2	109	45.4	61	25.4	70	29.2	240
3	120	52.1	58	25.2	52	22.7	230
4	112	43.5	84	32.6	62	24.0	258
5	98	46.2	59	27.8	55	25.9	212
6	125	52.1	63	26.3	52	21.7	240
7	123	51.0	63	26.1	55	22.8	241
8	116	38.9	85	28.5	97	32.5	298
9	106	45.7	59	25.4	67	28.9	232
10	91	39.6	69	30.0	70	30.4	230
11	-	-	-	-	-	-	-
12	71	45.2	51	32.5	35	22.3	157
Total	1305	45.6	786	27.5	770	26.9	2861

TABLE 13 (Continued)

(Root tips collected at consecutive hours)

TABLE 13C

SERIES 127 AND 132 COMBINED

Hours	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
0	230	49.9	113	24.3	122	26.2	465
1	225	44.4	119	23.5	163	32.1	507
2	232	49.5	119	25.4	118	25.2	469
3	261	53.7	103	21.2	122	25.1	486
4	214	43.8	138	28.2	137	28.0	489
5	196	43.7	103	22.9	150	33.4	449
6	248	49.5	129	25.7	124	24.7	501
7	233	50.4	125	27.1	104	22.5	462
8	235	44.7	137	26.3	151	29.0	521
9	221	46.8	127	26.9	124	26.3	472
10	198	43.1	120	26.1	141	30.8	459
11	101	44.5	57	25.1	69	30.4	227
12	189	47.3	104	26.0	107	26.8	400
Total	2781	47.1	1494	25.3	1632	27.6	5907

TABLE 13D

PERCENTAGES CONVERTED USING BASE OF 50

Hours	Prophase Real	Prophase New	Metaphase Real	Metaphase New	Post-Meta. Real	Post-Meta. New
0	49.9	50.0	24.3	50.0	26.2	50.0
1	44.4	44.4	23.5	48.4	32.1	61.3
2	49.5	49.5	25.4	52.3	25.2	48.1
3	53.7	53.7	21.2	43.7	25.1	47.9
4	43.8	43.8	22.8	58.1	28.0	55.5
5	43.7	43.7	22.9	47.2	33.4	64.0
6	49.5	49.5	25.7	52.9	24.7	47.2
7	50.4	50.4	27.1	55.8	22.5	43.0
8	44.7	44.7	26.3	54.2	29.0	55.4
9	46.8	46.8	26.9	55.4	26.3	50.2
10	43.1	43.1	26.1	53.8	30.8	58.8
11	44.5	44.5	25.1	51.7	30.4	58.1
12	47.3	47.3	26.0	53.6	26.8	51.2

TABLE 14

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
D. UNTREATED

(Root tips from same onion at same hour)

TABLE 14A

SERIES 157B

Root No.	Prophase Count	Prophase %	Metaphase Count	Metaphase %	Post-Meta. Count	Post-Meta. %	Total
2	236	54.4	94	21.7	104	24.0	434
3	238	47.8	128	25.7	132	26.5	498
4	211	51.1	124	30.0	78	18.9	413
6	231	54.7	99	23.5	92	21.8	422
7	237	52.9	111	24.8	100	22.3	448
8	229	50.9	105	23.3	116	25.8	450
10	232	54.1	109	25.4	88	20.5	429
11	254	59.2	81	18.9	94	21.9	429
12	195	44.1	111	25.1	136	30.8	442
13	220	47.9	125	27.3	114	24.8	459
15	220	49.9	105	23.8	116	26.3	441
21	213	43.4	163	33.2	115	23.4	491
Total	2716	50.7	1355	25.3	1285	23.5	5356

TABLE 14B

PERCENTAGES CONVERTED USING BASE OF 50

Root No.	Prophase Count	Prophase %	Metaphase Count	Metaphase %	Post-Meta. Count	Post-Meta. %
2	54.4	50.0	21.7	50.0	24.0	50.0
3	47.8	44.0	25.7	59.1	26.5	55.1
4	51.1	47.0	30.0	69.0	18.9	39.3
6	54.7	50.3	23.5	54.0	21.8	62.0
7	52.9	48.7	24.8	57.0	22.3	47.4
8	50.0	46.0	23.3	53.6	25.8	53.7
10	54.1	49.8	25.4	58.4	20.5	42.6
11	59.2	54.5	18.9	43.5	21.9	45.6
12	44.1	41.3	25.1	57.7	30.8	64.1
13	47.9	44.1	27.3	62.8	24.8	51.6
15	49.9	45.9	23.8	54.7	26.3	54.7
21	45.4	39.9	33.2	76.4	23.4	48.7

TABLE 15

PERCENTAGE OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES
D. UNTREATED

TABLE 15A

ZERO HOURS OF TREATED BULBS
SHORT TREATMENT

	Prophase		Metaphase		Post-Meta.		
Conc.	No.	%	No.	%	No.	%	Total
1 PPM	220	43.7	149	29.1	134	26.6	503
5 PPM	238	47.0	117	23.3	148	29.5	503
20 PPM	241	51.5	116	24.8	111	23.7	468
40 PPM	236	51.8	89	19.5	131	28.7	456
80 PPM	286	52.1	138	25.2	124	22.6	548
Total	1221	49.3	609	24.6	648	26.2	2478

TABLE 15B

ZERO HOURS OF TREATED BULBS
CONTINUOUS TREATMENT

	Prophase		Metaphase		Post-Meta.		
Conc.	No.	%	No.	%	No.	%	Total
1 PPM	223	43.6	144	28.6	145	28.4	512
5 PPM	268	51.2	107	20.4	149	28.5	524
20 PPM	217	49.0	95	21.4	131	29.6	443
40 PPM	265	56.7	93	19.9	109	23.3	467
80 PPM	212	49.6	98	22.9	117	27.4	427
Total	1185	49.9	537	22.6	651	27.4	2373

TABLE 15C

SUMMARY OF ALL UNTREATED MATERIAL

	Prophase		Metaphase		Post-Meta.		
	No.	%	No.	%	No.	%	Total
0-Hour Short	1221	49.3	609	24.6	648	26.2	2478
0-Hour Continuous	1185	49.9	537	22.6	651	27.4	2373
Consecutive hours	2781	47.1	1494	25.3	1632	27.6	5907
Same hour	2716	50.7	1355	25.3	1285	23.4	5356
Total	7903	49.0	3995	24.8	4216	26.2	16114

TABLE 16

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS[1]
A. SHORT TREATMENT

(One part per million)

	SERIES 143		SERIES 147A		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	267	13	236	27	503	40	12.6
1	280	14	214	28	494	42	11.8
2	254	7	212	17	466	24	19.4
3	248	32	220	17	468	49	9.6
4	251	12	244	19	495	31	16.0
5	230	31	269	15	499	46	10.9
6	253	20	201	19	454	39	11.6
7	269	12	260	20	529	32	16.5
8	234	9	230	8	464	17	27.3
9	281	7	245	16	526	23	22.9
10	254	6	235	18	489	24	20.4
11	273	9	222	23	495	32	15.5
12	265	8	220	26	485	34	14.3
Total Treated	3092	167	2772	226	5864	393	14.9

TABLE 17

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
A. SHORT TREATMENT

(Five parts per million)

	SERIES 136		SERIES 152B		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	273	17	230	11	503	28	18.0
1	264	14	270	10	535	24	22.3
2	263	12	236	20	499	32	15.6
3	255	24	256	14	511	38	13.5
4	223	36	229	33	452	69	6.6
5	129	41	295	19	424	60	7.1
6			249	22	249	22	11.3
7			267	29	267	29	9.2
Total Treated	1134	127	1803	147	2937	274	10.7

1. The term "axis" as used in Tables 16 through 28C refer to the consecutive series of high powered fields taken from edge to edge of the coverslip.

TABLE 18

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
A. SHORT TREATMENT

(Twenty parts per million)

	SERIES 92		SERIES 140		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	207	12	261	10	468	22	21.3
1	225	9	257	10	482	19	25.4
2	201	12	290	16	491	28	17.5
3	203	12	245	20	448	32	14.0
4	199	31	228	35	427	66	6.5
5	209	19	212	31	421	50	8.4
6	127	40	-	-	127	40	3.2
Total Treated	1164	123	1232	112	2396	235	10.2

TABLE 19

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
A. SHORT TREATMENT

(Forty parts per million)

	SERIES 96		SERIES 120		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	209	17	247	18	456	35	13.0
1	214	17	209	29	423	46	9.2
2	215	13	206	42	421	55	7.7
3	230	14	241	24	471	38	12.4
4	201	28	161	44	362	72	5.0
5	69	17	28	18	97	35	2.8
Total Treated	929	89	845	157	1774	246	7.2

TABLE 20

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
A. SHORT TREATMENT

(Eighty parts per million)

	SERIES 118		SERIES 122		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	251	21	297	17	548	38	14.4
1	209	14	221	19	430	33	13.0
2	268	13	310	13	578	26	22.2
3	260	17	256	14	516	31	16.6
4	218	25	228	19	446	44	10.1
5	211	29	163	24	374	53	7.1
6	216	43	-	-	216	43	5.0
Total Treated	1382	141	1178	89	2560	230	11.1

TABLE 21

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
B. CONTINUOUS TREATMENT

(One part per million)

	SERIES 146		SERIES 151		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	243	20	269	8	512	28	18.3
1	275	15	242	18	517	33	15.7
2	261	18	304	8	565	26	21.7
3	230	25	226	10	456	35	13.0
4	186	33	274	14	460	47	9.8
5	224	31	302	19	526	50	10.5
6	206	41	209	30	415	71	5.9
7	-	-	206	26	206	26	7.9
8	-	-	158	35	158	35	4.1
Total Treated	1139	163	1921	160	3503	385	10.2

TABLE 22

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
B. CONTINUOUS TREATMENT

(Five parts per million)

	SERIES 139		SERIES 152A		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	267	18	257	6	524	24	21.8
1	256	19	240	10	496	29	17.1
2	279	11	311	14	590	25	23.6
3	230	19	300	14	530	33	16.1
4	215	28	273	20	488	48	10.2
5	213	30	257	17	470	47	10.0
6	-	-	256	16	256	16	16.0
7	-	-	225	27	225	27	8.3
8	-	-	222	36	222	36	6.2
Total Treated	1193	107	2084	154	3277	261	12.6

TABLE 23

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
B. CONTINUOUS TREATMENT

(Twenty parts per million)

	SERIES 128		SERIES 134		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	230	16	213	8	443	24	18.5
1	232	10	220	6	452	16	28.3
2	232	8	268	8	500	16	31.3
3	228	13	276	6	504	19	26.5
4	236	13	256	10	492	23	21.4
5	221	17	277	9	498	26	19.2
6	243	16	272	11	515	27	19.1
7	236	20	275	9	511	29	17.6
8	239	18	253	11	492	29	17.0
9	245	20	245	8	490	28	17.5
10	216	12	256	10	472	22	21.5
11	222	16	248	8	470	24	19.6
12	219	16	242	11	461	27	17.1
Total Treated	2769	179	3088	107	5857	286	20.5

TABLE 24

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
B. CONTINUOUS TREATMENT

(Forty parts per million)

	SERIES 95		SERIES 125		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	202	18	265	19	467	37	12.6
1	204	17	244	23	448	40	11.2
2	208	11	216	13	424	24	17.7
3	209	9	221	17	430	26	16.5
4	218	17	240	17	458	34	13.5
5	220	15	235	16	455	31	14.7
6	212	14	233	21	445	35	12.7
7	231	22	231	19	462	41	11.3
8	237	16	223	16	460	32	14.4
9	219	23	221	23	440	46	9.6
10	238	21	248	19	486	40	12.2
11	208	30	241	26	449	56	8.0
12	-	-	231	18	231	18	12.8
Total Treated	2404	195	2784	228	5188	423	12.3

TABLE 25

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
B. CONTINUOUS TREATMENT

(Eighty parts per million)

	SERIES 102		SERIES 115		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	206	14	221	18	427	32	13.3
1	212	23	245	17	457	40	11.4
2	214	20	221	15	435	35	12.4
3	231	12	281	15	512	27	19.0
4	234	26	269	22	503	48	10.5
5	214	29	-	-	214	29	7.4
6	-	-	261	11	261	11	23.7
7	212	39	237	13	449	52	8.6
8	163	33	260	16	423	49	8.6
Total Treated	1480	182	1774	109	3254	291	11.2

TABLE 26

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
C. UNTREATED

(Root tips collected at consecutive hours)

	SERIES 127		SERIES 132		COMBINED		
Hours	Count	Axes	Count	Axes	Count	Axes	Avg.
0	214	13	251	20	465	33	14.1
1	235	15	272	13	507	28	18.1
2	229	13	240	9	469	22	21.3
3	256	13	230	11	486	24	20.3
4	231	12	258	15	489	27	18.1
5	237	13	212	5	449	18	24.9
6	261	14	240	11	501	25	20.0
7	221	12	241	13	462	25	18.5
8	223	18	298	8	521	26	20.0
9	240	19	232	10	472	29	16.3
10	229	16	230	18	459	34	13.5
11	227	14	-	-	227	14	16.2
12	243	19	157	32	400	51	7.8
Total	3046	191	2861	165	5907	356	16.6

TABLE 27

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS
C. UNTREATED

(Root tips collected from same onion at same hour)

Hours	Root No.	Count	Axes	Avg.
0	2	434	21	20.7
1	3	498	21	23.7
2	4	413	25	16.5
3	6	422	23	18.4
4	7	448	24	18.7
5	8	450	12	37.5
6	10	429	24	17.9
7	11	429	22	19.5
8	12	442	14	31.6
9	13	459	19	24.2
10	15	441	33	13.4
11	21	491	24	20.5
12	-	-	-	-
Total		5356	262	20.4

TABLE 28

AVERAGE NUMBERS OF DIVISION FIGURES PER AXIS[1]

TABLE 28A

ZERO HOURS OF TREATED BULBS
SHORT TREATMENT

Parts per million	Count	Axes	Avg.
1	503	40	12.6
5	505	28	18.0
20	468	22	21.3
40	456	35	13.0
80	546	38	14.4
Total	2478	163	15.2

TABLE 28B

ZERO HOURS OF TREATED BULBS
CONTINUOUS TREATMENT

Parts per million	Count	Axes	Avg.
1	512	28	18.3
5	524	24	21.8
20	445	24	18.5
40	467	37	12.6
80	427	32	13.3
Total	2373	145	16.4

TABLE 28C

SUMMARY OF ALL UNTREATED MATERIAL

	Count	Axes	Avg.
0-Hour Short	2478	163	15.2
0-Hour Continuous	2373	145	16.4
Consecutive Hours	5907	356	16.6
Same Hour	5356	262	20.4
Total	16114	926	17.4

1. The term "axis" as used in Tables 16 through 28C refer to the consecutive series of high powered fields taken from edge to edge of the coverslip.

TABLE 29

HOURLY CHANGES IN AVERAGE NUMBER OF DIVISION FIGURES PER AXIS
SHORT TREATMENT

	1 P.P.M.*		5 P.P.M.		20 P.P.M.		40 P.P.M.		80 P.P.M.	
Hrs.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.
0	12.6	0.0	18.0	0.0	21.3	0.0	13.0	0.0	14.4	0.0
1	11.8	-0.8	22.3	+ 4.3	25.4	+ 4.1	9.2	- 3.8	13.0	- 1.0
2	19.4	+ 6.8	15.6	- 2.4	17.5	- 3.8	7.7	- 5.3	22.2	+ 7.8
3	9.6	- 3.0	13.5	- 4.5	14.0	- 7.3	12.4	- 0.6	16.6	+ 2.2
4	16.0	+ 3.4	6.6	-11.4	6.5	-14.8	5.0	- 8.0	10.1	- 4.3
5	10.9	- 1.7	7.1	-10.9	8.4	-12.9	2.8	-10.2	7.1	- 7.3
6	11.6	- 1.0	11.3	- 6.7	3.2	-18.1			5.0	- 9.4
7	16.5	+ 3.9	9.2	- 8.8						
8	27.3	+14.7								
9	22.9	+10.3								
10	20.4	+ 7.8								
11	15.5	+ 2.9								
12	14.3	+ 1.7								

* Parts per million

TABLE 30

HOURLY CHANGES IN AVERAGE NUMBER OF DIVISION FIGURES PER AXIS
CONTINUOUS TREATMENT

	1 P.P.M.*		5 P.P.M.		20 P.P.M.		40 P.P.M.		80 P.P.M.	
Hrs.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.	Avg.	Dev.
0	18.3	0.0	21.8	0.0	18.5	0.0	12.6	0.0	13.3	0.0
1	15.7	- 2.6	17.1	- 4.7	28.3	+ 9.8	11.2	- 1.4	11.4	- 1.9
2	21.7	+ 3.4	23.6	+ 1.8	31.3	+12.8	17.7	+ 5.1	12.4	- 0.9
3	13.0	- 5.3	16.1	- 5.7	26.5	+ 8.0	16.5	+ 3.9	19.0	+ 5.7
4	9.8	- 8.5	10.2	-11.6	21.4	+ 2.9	13.5	+ 0.9	10.5	- 2.8
5	10.5	- 7.8	10.0	-11.8	19.2	+ 0.7	14.7	+ 2.1	7.4	- 5.9
6	5.9	-12.4	16.0	- 5.8	19.1	+ 0.6	12.7	+ 0.1	23.7	+10.4
7	7.9	-10.4	8.3	-13.5	17.6	- 0.9	11.3	- 1.3	8.6	- 4.7
8	4.1	-14.2	6.2	-15.6	17.0	- 1.5	14.4	+ 1.8	8.6	- 4.7
9					17.5	- 1.0	9.6	- 3.0		
10					21.5	+ 3.0	12.2	- 0.4		
11					19.6	+ 1.1	8.0	- 4.6		
12					17.1	- 1.4	12.8	+ 0.2		

* Parts per million

TABLE 31

HOURLY CHANGES IN AVERAGE NUMBER OF DIVISION FIGURES PER AXIS UNTREATED

(Root tips taken at consecutive hours)

Hours	Avg.	Dev.
0	14.8	0.0
1	18.9	+ 4.1
2	21.3	+ 6.5
3	20.3	+ 5.5
4	18.1	+ 3.3
5	24.9	+10.1
6	20.0	+ 5.2
7	18.5	+ 3.7
8	20.0	+ 5.8
9	16.3	+ 1.5
10	13.5	- 1.3
11	16.2	+ 1.4
12	7.8	- 7.0

TABLE 32

HOURLY CHANGES IN AVERAGE NUMBER OF DIVISION FIGURES PER AXIS UNTREATED

(Root tips taken from same onion at same hour)

Root No.	Avg.	Dev.
2	20.7	0.0
3	23.7	+ 3.0
4	16.5	- 4.2
6	18.4	- 2.3
7	18.7	- 2.0
8	37.5	+16.8
10	17.9	- 2.8
11	19.5	- 1.8
12	31.6	+10.9
13	24.2	+ 3.5
15	13.4	- 7.5
21	20.5	- 0.2

TABLE 33

PERCENTAGES OF ABERRANT PROPHASES
A. SHORT TREATMENT

(One part per million)

	SERIES 143			SERIES 147A			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	11	103	10.7	0	117	0.0	11	220	5.0
1	19	113	16.8	3	109	2.8	22	222	9.9
2	13	167	7.8	2	129	1.6	15	296	5.1
3	5	116	4.3	1	104	1.0	6	220	2.7
4	14	106	3.2	0	122	0.0	14	228	6.1
5	6	149	4.0	0	150	0.0	6	299	2.0
6	15	163	9.2	0	119	0.0	15	282	5.3
7	3	170	1.8	1	173	0.6	4	343	1.2
8	2	159	1.3	0	132	0.0	2	291	0.7
9	4	158	2.5	1	130	0.8	5	288	1.7
10	5	124	4.0	4	128	3.1	9	252	3.6
11	1	132	0.8	1	130	0.8	2	262	0.8
12	2	125	1.6	1	138	0.7	3	263	1.1
Total Treated	89	1682	5.3	14	1564	0.9	103	3246	3.2

TABLE 34

PERCENTAGES OF ABERRANT PROPHASES
A. SHORT TREATMENT

(Five parts per million)

	SERIES 136			SERIES 182B			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	0	125	0.0	6	113	5.3	6	238	2.5
1	2	141	1.4	4	113	3.5	6	254	2.4
2	73	203	36.0	7	168	4.2	80	371	21.6
3	116	177	65.5	94	185	50.8	210	362	58.0
4	103	120	85.8	106	160	66.3	209	280	74.6
5	56	60	93.3	74	152	48.7	130	212	61.3
6				79	137	57.7	79	137	57.7
7				72	140	51.4	72	140	51.4
Total Treated	350	701	49.9	436	1055	41.3	786	1756	44.8

TABLE 35

PERCENTAGES OF ABERRANT PROPHASES
A. SHORT TREATMENT

(Twenty parts per million)

	SERIES 92			SERIES 140			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	7	113	6.2	0	128	0.0	7	241	2.9
1	14	142	9.9	9	143	6.3	23	285	8.1
2	32	126	25.4	84	164	51.2	116	290	40.0
3	24	114	21.1	174	185	94.1	198	299	66.2
4	47	132	35.6	158	163	96.9	205	295	69.5
5	71	146	48.6	90	98	91.8	161	244	66.0
6	38	94	40.4	-	-	-	38	94	40.4
Total Treated	226	754	30.0	515	753	68.4	741	1507	49.2

TABLE 36

PERCENTAGES OF ABERRANT PROPHASES
A. SHORT TREATMENT

(Forty parts per million)

	SERIES 96			SERIES 120			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	15	110	13.6	24	126	19.0	39	236	16.5
1	13	125	10.4	77	112	68.8	90	237	38.0
2	41	105	39.0	90	109	82.6	131	214	61.2
3	61	114	53.5	117	130	90.0	178	244	73.0
4	75	149	50.3	101	108	93.5	176	257	68.5
5	26	48	54.2	21	21	100.0	47	69	68.1
Total Treated	216	541	39.9	406	480	84.6	622	1021	60.9

TABLE 37

PERCENTAGES OF ABERRANT PROPHASES
A. SHORT TREATMENT

(Eighty parts per million)

	SERIES 118			SERIES 122			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	8	132	6.1	31	154	20.1	39	286	13.6
1	29	127	22.8	84	146	57.5	113	273	41.4
2	64	150	42.7	125	162	77.2	189	312	60.6
3	85	124	68.5	112	126	88.9	197	250	78.8
4	134	136	98.5	127	139	91.4	261	275	94.9
5	118	124	95.2	117	127	92.1	235	251	93.6
6	164	164	100.0	-	-	-	164	164	100.0
Total Treated	594	825	72.0	565	700	80.7	1159	1525	76.0

TABLE 38

PERCENTAGES OF ABERRANT PROPHASES
B. CONTINUOUS

(One part per million)

	SERIES 146			SERIES 151			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	4	117	3.4	2	106	1.9	6	223	2.7
1	36	146	24.7		113	0.0	36	259	13.9
2	27	166	16.3	1	159	0.6	28	325	8.6
3	85	142	59.9	24	135	17.8	109	277	39.4
4	98	117	78.6	32	161	19.9	124	278	44.6
5	86	126	68.3	132	203	65.0	218	329	66.3
6	98	119	82.4	88	123	71.5	186	242	76.9
7	-	-	-	117	135	86.7	117	135	86.7
8	-	-	-	97	105	92.4	97	105	92.4
Total Treated	424	1123	37.8	491	827	59.4	915	1950	46.9

TABLE 39

PERCENTAGES OF ABERRANT PROPHASES
B. CONTINUOUS TREATMENT

(Five parts per million)

	SERIES 139			SERIES 152A			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	5	141	3.5	9	127	7.1	14	268	5.2
1	29	146	19.9	1	121	0.8	30	267	11.2
2	121	158	76.6	96	195	49.2	217	353	61.5
3	138	154	89.6	169	183	92.3	307	337	91.1
4	119	129	92.2	171	175	97.7	290	304	95.4
5	134	136	98.5	149	161	92.5	283	297	95.3
6	-	-	-	137	147	93.2	137	147	93.2
7	-	-	-	142	143	99.3	142	143	99.3
8	-	-	-	107	109	98.2	107	109	98.2
Total Treated	541	723	74.8	972	1234	78.8	1513	1957	77.3

TABLE 40

PERCENTAGES OF ABERRANT PROPHASES
B. CONTINUOUS TREATMENT

(Twenty parts per million)

	SERIES 128			SERIES 134			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	32	105	30.5	3	112	2.7	35	217	16.1
1	81	132	61.4	71	132	53.8	152	264	57.6
2	122	142	85.9	104	153	68.0	226	295	76.6
3	89	112	79.5	128	163	78.5	217	275	78.9
4	93	114	81.6	109	152	71.7	202	266	75.9
5	93	111	83.8	132	167	79.0	225	278	80.9
6	76	88	86.4	146	166	88.0	222	254	87.4
7	92	103	89.3	142	146	97.3	234	249	94.0
8	87	87	100.0	137	147	93.2	224	234	95.7
9	100	103	97.1	115	126	91.3	215	229	93.9
10	81	87	93.1	142	153	92.8	223	240	92.9
11	99	103	96.1	137	140	97.9	236	243	97.1
12	87	88	96.9	131	141	92.9	218	229	95.1
Total Treated	1100	1270	86.6	1494	1786	83.7	2594	3056	84.9

TABLE 41

PERCENTAGES OF ABERRANT PROPHASES
B. CONTINUOUS TREATMENT

(Forty parts per million)

	SERIES 95			SERIES 125			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	7	128	5.5	13	137	9.5	20	265	7.5
1	26	131	19.8	25	127	19.7	51	258	19.8
2	30	136	22.1	89	110	80.9	119	246	48.4
3	49	104	47.1	99	107	92.5	148	211	70.1
4	44	111	39.6	99	100	99.0	143	211	67.8
5	53	99	53.5	106	108	98.1	159	207	76.8
6	46	101	45.5	95	96	99.0	141	197	71.6
7	77	89	86.5	104	105	99.0	181	194	93.3
8	83	86	96.5	115	115	100.0	198	201	98.5
9	51	53	96.2	104	109	95.4	155	162	95.7
10	86	86	100.0	93	98	94.9	179	184	97.3
11	64	65	98.5	122	122	100.0	186	187	99.5
12	-	-	-	92	92	100.0	92	92	100.0
Total Treated	609	1061	57.4	1143	1289	88.7	1752	2350	74.6

TABLE 42

PERCENTAGES OF ABERRANT PROPHASES
B. CONTINUOUS TREATMENT

(Eighty parts per million)

	SERIES 102			SERIES 115			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	7	93	7.5	30	119	25.2	37	212	17.5
1	15	127	11.8	60	137	43.8	75	264	28.4
2	40	156	25.6	67	116	57.8	107	272	39.3
3	59	117	50.4	110	160	68.8	169	277	61.0
4	70	127	55.1	114	137	83.2	184	264	69.7
5	52	110	47.3	-	-	-	52	110	47.3
6	-	-	-	85	98	86.7	85	98	86.7
7	67	95	70.5	89	93	95.7	156	188	83.0
8	59	66	89.4	86	94	91.5	145	160	90.6
Total Treated	362	798	45.4	611	835	73.2	973	1633	59.6

TABLE 43

PERCENTAGES OF ABERRANT PROPHASES
C. INTERMITTANT TREATMENT

(Forty parts per million for 15 minutes every two hours)

	SERIES 126		
Hours	Aber.	Total	%
0	6	98	6.1
1	41	117	35.0
2	56	113	49.6
3	103	110	93.6
4	144	147	98.0
5	143	145	98.6
6	165	166	99.4
7	-	-	-
8	65	65	100.0
Total	717	863	93.7

TABLE 44

PERCENTAGES OF ABERRANT PROPHASES
C. INTERMITTANT TREATMENT

(Forty parts per million for 15 minutes every hour)

	SERIES 135		
Hours	Aber.	Total	%
0	1	136	0.7
1	47	154	30.5
2	126	147	85.7
3	132	149	88.6
4	131	145	90.3
5	126	139	90.6
6	121	126	96.0
7	127	129	98.4
8	125	125	100.0
9	124	126	98.4
10	113	116	97.4
11	129	131	98.5
12	151	151	100.0
Total	1452	1638	96.7

TABLE 45

PERCENTAGES OF ABERRANT PROPHASES
D. UNTREATED

(Root tips collected at consecutive hours)

	SERIES 127			SERIES 132			COMBINED		
Hrs.	Aber.	Total	%	Aber.	Total	%	Aber.	Total	%
0	3	108	2.8	14	122	11.5	17	230	7.4
1	1	113	0.9	11	112	9.8	12	225	5.3
2	4	123	3.6	5	109	4.6	9	232	3.9
3	10	141	7.1	10	120	8.3	20	261	7.7
4	8	102	7.8	6	112	5.4	14	214	6.5
5	6	98	6.1	9	98	9.2	15	196	7.7
6	4	123	3.3	11	125	8.8	15	248	6.0
7	4	110	3.6	7	123	5.7	11	233	4.7
8	5	117	4.3	4	116	3.4	9	233	3.9
9	6	115	5.2	7	106	6.6	13	221	5.9
10	5	107	4.7	9	91	9.9	14	198	7.1
11	6	101	5.9	-	-	-	6	101	5.9
12	3	118	2.5	7	71	9.9	10	189	5.3
Total	65	1476	4.4	100	1183	8.5	165	2781	5.9

TABLE 46

PERCENTAGES OF ABERRANT PROPHASES
D. UNTREATED

(Root tips collected from same onion at same hour)

Hrs.	Root No.	Aber.	Total	%
0	2	6	256	2.5
1	3	10	238	4.2
2	4	1	211	0.5
3	6	2	231	0.9
4	7	5	237	2.1
5	8	9	229	3.9
6	10	6	232	2.6
7	11	2	254	0.8
8	12	2	195	1.0
9	13	2	220	0.9
10	15	3	220	1.4
11	21	0	213	0.0
12	-	-	-	-
Total		48	2716	1.8

TABLE 47

PERCENTAGES OF ABERRANT PROPHASES

TABLE 47A

ZERO HOURS OF TREATED BULBS
SHORT TREATMENT

Parts per million	Aber.	Total	%
1	11	220	5.0
5	6	238	2.5
20	7	241	2.9
40	39	236	16.5
80	39	286	13.6
Total	102	1221	10.2

TABLE 47B

ZERO HOURS OF TREATED BULBS
CONTINUOUS TREATMENT

Parts per million	Aber.	Total	%
1	6	223	2.7
5	14	268	5.2
20	35	217	16.1
40	20	265	7.5
80	37	212	17.5
Total	112	1185	9.5

TABLE 47C

SUMMARY OF ALL UNTREATED MATERIAL

	Aber.	Total	%
0-Hour Short	102	1221	10.2
0-Hour Continuous	112	1185	9.5
Consecutive Hours	165	2781	5.9
Same Hour	48	2716	1.8
Total	427	7903	5.6

TABLE 48

PERCENTAGES OF SCATTERED METAPHASES
A. SHORT TREATMENT

(One part per million)

	SERIES 145			SERIES 147A			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	73	0.0	0	76	0.0	0	149	0.0
1	1	68	1.5	1	46	2.2	2	114	1.8
2	0	46	0.0	2	38	5.3	2	84	2.4
3	1	69	1.4	0	43	0.0	1	112	0.9
4	3	77	3.9	0	47	0.0	3	124	2.4
5	1	47	2.1	0	64	0.0	1	111	0.9
6	0	53	0.0	0	39	0.0	0	92	0.0
7	2	64	3.1	2	34	5.9	4	98	4.1
8	0	44	0.0	1	48	2.1	1	92	1.1
9	4	67	6.0	0	57	0.0	4	124	3.2
10	5	71	7.0	1	52	1.9	6	123	4.9
11	0	82	0.0	1	42	2.4	1	124	0.8
12	4	72	5.5	3	43	7.0	7	115	6.1
Total Treated	21	760	2.8	11	553	1.8	32	1313	2.2

TABLE 49

PERCENTAGES OF SCATTERED METAPHASES
A. SHORT TREATMENT

(Five parts per million)

	SERIES 138			SERIES 152B			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	80	0.0	0	37	0.0	0	117	0.0
1	4	58	6.9	0	60	0.0	4	118	3.4
2	7	25	28.0	1	30	3.3	8	55	14.6
3	21	57	36.8	0	38	0.0	21	95	22.1
4	33	63	52.4	8	22	36.4	41	85	48.2
5	22	40	55.0	5	63	7.9	27	103	26.2
6	-	-	-	1	35	2.9	1	35	2.9
7	-	-	-	4	66	6.1	4	66	6.1
Total Treated	87	243	35.8	19	314	6.0	106	557	19.0

TABLE 50

PERCENTAGES OF SCATTERED METAPHASES
A. SHORT TREATMENT

(Twenty parts per million)

	SERIES 92			SERIES 140			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	44	0.0	1	72	1.4	1	116	0.9
1	1	45	2.2	4	84	4.8	5	129	3.9
2	3	28	10.7	12	41	29.3	15	69	21.7
3	13	26	50.0	16	21	76.2	29	47	61.7
4	15	22	68.2	54	59	91.5	69	81	85.2
5	28	45	62.2	42	59	71.1	70	104	67.3
6	20	27	74.1	-	-	-	20	27	74.1
Total Treated	80	193	41.4	128	264	48.5	208	457	45.5

TABLE 51

PERCENTAGES OF SCATTERED METAPHASES
A. SHORT TREATMENT

(Forty parts per million)

	SERIES 96			SERIES 120			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	41	0.0	0	48	0.0	0	89	0.0
1	10	72	13.9	1	73	1.4	11	145	7.6
2	3	93	3.2	12	65	18.5	15	158	9.5
3	21	51	41.2	19	33	57.6	40	84	47.6
4	9	10	90.0	12	13	92.3	21	23	91.3
5	10	10	100.0	4	4	100.0	14	14	100.0
Total Treated	53	236	22.5	48	188	25.5	101	424	23.8

TABLE 52

PERCENTAGES OF SCATTERED METAPHASES
A. SHORT TREATMENT

(Eighty parts per million)

	SERIES 118			SERIES 122			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	63	0.0	0	75	0.0	0	136	0.0
1	4	67	6.0	9	61	14.7	13	128	10.2
2	13	103	12.6	18	111	16.2	31	214	14.5
3	28	73	38.4	13	15	86.7	41	88	46.6
4	15	15	100.0	12	14	85.7	27	29	93.1
5	16	17	94.0	4	4	100.0	20	21	95.2
6	13	13	100.0	-	-	-	13	13	100.0
Total Treated	89	288	30.9	56	205	27.3	145	493	29.4

TABLE 53

PERCENTAGES OF SCATTERED METAPHASES
B. CONTINUOUS TREATMENT

(One part per million)

	SERIES 146			SERIES 151			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	1	62	1.6	0	82	0.0	1	144	0.7
1	1	73	1.4	0	69	0.0	1	142	0.7
2	2	47	4.3	1	70	1.4	3	117	2.6
3	7	43	16.3	0	37	0.0	7	80	8.8
4	34	52	65.4	2	66	3.0	36	118	30.5
5	54	72	75.0	11	55	20.0	65	127	51.2
6	58	67	86.6	18	57	31.6	76	124	61.5
7	-	-	-	25	42	59.5	25	42	59.5
8	-	-	-	17	23	73.9	17	23	73.9
Total Treated	156	354	44.1	74	419	17.7	230	773	29.5

TABLE 54

PERCENTAGES OF SCATTERED METAPHASES
B. CONTINUOUS TREATMENT

(Five parts per million)

	SERIES 139			SERIES 152A			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	50	0.0	0	57	0.0	0	107	0.0
1	1	63	1.6	0	77	0.0	1	140	0.7
2	18	57	31.6	1	62	1.6	19	119	16.0
3	17	36	47.2	12	72	16.7	29	108	26.9
4	39	55	70.9	22	57	38.6	61	112	54.5
5	49	50	98.0	48	59	81.3	97	109	89.0
6	-	-	-	70	77	90.9	70	77	90.9
7	-	-	-	52	56	92.9	52	56	92.9
8	-	-	-	72	76	94.7	72	76	94.7
Total Treated	124	261	47.5	277	536	51.7	401	797	50.3

TABLE 55

PERCENTAGES OF SCATTERED METAPHASES
B. CONTINUOUS TREATMENT

(Twenty parts per million)

	SERIES 128			SERIES 134			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	48	0.0	0	47	0.0	0	95	0.0
1	3	78	3.8	2	68	2.9	5	146	3.4
2	10	79	12.7	19	104	18.3	29	183	15.9
3	37	111	33.3	51	94	54.3	88	205	40.9
4	41	112	36.6	62	89	69.7	103	201	51.2
5	73	98	74.5	76	90	84.4	149	188	79.3
6	117	148	79.0	87	91	95.6	204	239	85.4
7	100	121	82.6	105	110	95.5	205	231	88.7
8	132	143	92.3	88	88	100.0	220	231	95.2
9	114	137	83.2	105	107	98.1	219	244	89.8
10	105	113	91.1	91	92	98.9	194	205	94.6
11	106	106	100.0	92	94	97.9	198	200	99.0
12	126	127	99.2	89	91	97.8	215	218	98.6
Total Treated	962	1373	70.1	867	1118	77.5	1829	2491	73.4

TABLE 56

PERCENTAGES OF SCATTERED METAPHASES
B. CONTINUOUS TREATMENT

(Forty parts per million)

	SERIES 95			SERIES 125			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	1	34	2.9	0	59	0.0	1	93	1.1
1	1	59	1.7	4	96	4.2	5	155	3.3
2	15	62	24.2	25	87	28.7	40	149	26.9
3	18	87	20.7	49	105	46.7	67	192	34.9
4	49	83	59.0	94	128	73.4	143	211	67.8
5	77	96	80.2	101	108	93.5	178	204	87.3
6	44	81	54.3	106	114	93.0	150	195	76.9
7	101	111	91.0	87	90	96.7	188	201	93.5
8	121	126	96.0	72	77	93.5	193	203	95.1
9	121	122	99.2	109	110	99.1	230	232	99.1
10	116	119	97.5	127	133	95.5	243	252	96.4
11	105	109	96.3	105	107	98.1	210	216	97.2
12	-	-	-	125	129	96.9	125	129	96.9
Total Treated	768	1055	72.8	1004	1284	78.2	1772	2339	75.8

TABLE 57

PERCENTAGES OF SCATTERED METAPHASES
B. CONTINUOUS TREATMENT

(Eighty parts per million)

	SERIES 102			SERIES 115			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	51	0.0	0	47	0.0	0	98	0.0
1	3	68	4.4	2	99	2.0	5	167	3.0
2	6	48	12.5	5	96	5.2	11	144	7.6
3	23	110	20.9	27	106	25.4	50	216	23.2
4	72	100	72.0	53	117	45.3	125	217	57.6
5	66	94	70.2	-	-	-	66	94	70.2
6	-	-	-	88	134	65.7	88	134	65.7
7	88	112	78.6	100	115	87.0	188	227	82.8
8	88	93	94.6	117	128	91.5	205	221	92.8
Total Treated	346	625	55.4	392	795	49.3	738	1420	52.0

TABLE 58

PERCENTAGES OF SCATTERED METAPHASES
C. INTERMITTENT TREATMENT

(Forty parts per million for 15 minutes every two hours)

		SERIES 126	
Hours	Scatt.	Total	%
0	0	42	0.0
1	5	84	6.0
2	27	66	40.9
3	23	34	67.6
4	12	12	100.0
5	17	17	100.0
6	10	10	100.0
7	-	-	-
8	9	9	100.0
Total	103	252	54.2

TABLE 59

PERCENTAGES OF SCATTERED METAPHASES
C. INTERMITTENT TREATMENT

(Forty parts per million for 15 minutes every hour)

		SERIES 135	
Hours	Scatt.	Total	%
0	0	80	0.0
1	11	89	12.4
2	42	73	57.5
3	55	73	75.3
4	76	82	92.7
5	80	81	98.8
6	88	88	100.0
7	88	88	100.0
8	83	84	98.8
9	99	100	99.0
10	118	118	100.0
11	99	99	100.0
12	81	81	100.0
Total	920	1056	87.1

TABLE 60

PERCENTAGES OF SCATTERED METAPHASES
D. UNTREATED

(Root tips collected at consecutive hours)

	SERIES 127			SERIES 132			COMBINED		
Hrs.	Scatt.	Total	%	Scatt.	Total	%	Scatt.	Total	%
0	0	49	0.0	1	64	1.6	1	113	0.9
1	0	49	0.0	7	70	10.0	7	119	5.9
2	0	58	0.0	2	61	32.8	2	119	1.7
3	0	45	0.0	1	58	1.7	1	103	1.0
4	0	54	0.0	7	84	8.3	7	138	5.1
5	0	44	0.0	4	59	6.8	4	103	3.9
6	0	66	0.0	3	63	4.8	3	129	2.3
7	0	62	0.0	15	63	23.8	15	125	12.0
8	0	52	0.0	7	85	8.2	7	137	5.1
9	0	68	0.0	0	59	0.0	0	127	0.0
10	0	51	0.0	8	69	11.6	8	120	6.7
11	0	57	0.0	-	-	-	0	57	0.0
12	0	53	0.0	5	51	9.8	5	104	4.8
Total	0	708	0.0	60	786	7.6	60	1494	4.0

TABLE 61

PERCENTAGES OF SCATTERED METAPHASES
D. UNTREATED

(Root tips collected from same onion at same hour)

Hrs.	Root No.	Scatt.	Total	%
0	2	1	94	10.6
1	3	5	128	3.9
2	4	0	124	0.0
3	6	1	99	10.1
4	7	3	111	2.7
5	8	2	105	1.9
6	10	4	109	3.7
7	11	2	81	2.5
8	12	1	111	0.9
9	13	3	125	2.4
10	15	10	105	9.5
11	21	5	163	3.1
12	-	-	-	-
Total		37	1355	2.7

TABLE 62

PERCENTAGES OF SCATTERED METAPHASES

TABLE 62A

ZERO HOUR OF TREATED BULBS
SHORT TREATMENT

Parts per million	Scatt.	Total	%
1	0	149	0.0
5	0	117	0.0
20	1	116	0.9
40	0	89	0.0
80	0	138	0.0
Total	1	609	0.2

TABLE 62B

ZERO HOUR OF TREATED BULBS
CONTINUOUS TREATMENT

Parts per million	Scatt.	Total	%
1	1	144	0.7
5	0	107	0.0
20	0	95	0.0
40	1	93	10.7
80	0	98	0.0
Total	2	537	0.4

TABLE 62C

SUMMARY OF ALL UNTREATED MATERIAL

	Scatt.	Total	%
0-Hour Short	1	609	0.2
0-Hour Continuous	2	537	0.4
Consecutive Hours	60	1494	4.0
Same Hour	37	1355	2.7
Total	100	3995	2.5

TABLE 63

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
A. SHORT TREATMENT

(One part per million)

	SERIES 145			SERIES 147A			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	29	267	0	18	236	0	47	0.0	503	9.3
1	1	40	280	0	13	214	1	53	1.9	494	10.7
2	1	17	254	1	14	212	2	31	6.5	466	6.7
3	1	34	248	1	28	220	2	62	3.2	468	13.2
4	0	32	251	1	19	244	1	51	2.0	495	10.3
5	0	13	230	1	21	269	1	34	2.9	499	6.8
6	0	16	253	0	11	201	0	27	0.0	454	5.9
7	1	11	269	4	14	260	5	25	20.0	529	4.7
8	1	16	234	0	12	230	1	28	3.6	464	6.0
9	2	32	281	0	31	245	2	63	3.2	526	12.0
10	0	27	254	0	14	235	0	41	0.0	489	8.4
11	0	26	273	0	17	222	0	43	0.0	495	8.7
12	2	16	265	1	16	220	3	32	9.4	485	6.6
Total Treated	9	280	3092	9	210	2772	18	490	3.7	5864	8.4

TABLE 64

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
A. SHORT TREATMENT

(Five parts per million)

	SERIES 138			SERIES 152B			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	27	273	0	30	230	0	57	0.0	503	11.1
1	8	41	264	0	47	271	8	88	9.1	535	16.4
2	2	13	263	1	18	236	3	31	9.7	499	6.2
3	2	9	255	1	13	256	3	22	13.6	511	4.3
4	5	20	223	2	13	229	7	33	21.2	452	7.3
5	3	13	129	1	21	295	4	34	11.8	424	8.0
6	-	-	-	0	26	249	0	26	0.0	249	10.4
7	-	-	-	1	22	267	1	22	4.6	267	8.2
Total Treated	20	96	1134	6	160	1065	26	256	10.1	2937	8.7

TABLE 65

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
A. SHORT TREATMENT

(Twenty parts per million)

	SERIES 92			SERIES 140			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	6	24	207	1	24	261	7	48	14.6	468	10.3
1	3	19	225	0	21	257	3	40	7.5	482	8.3
2	3	32	201	4	51	290	7	83	8.4	491	16.9
3	5	44	205	1	17	245	6	61	9.8	448	13.6
4	14	28	199	2	5	228	16	33	48.5	427	7.7
5	1	10	209	3	29	212	4	39	10.3	421	9.3
6	1	2	127	-	-	-	1	2	50.0	127	1.6
Total Treated	27	135	1164	10	123	1232	37	258	14.3	2396	10.8

TABLE 66

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
A. SHORT TREATMENT

(Forty parts per million)

	SERIES 96			SERIES 120			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	4	22	209	1	31	247	5	53	9.4	456	11.6
1	0	8	214	0	7	209	0	15	0.0	423	3.5
2	1	9	215	13	24	206	14	33	42.4	421	7.8
3	6	45	230	9	54	241	15	99	15.2	471	21.0
4	5	12	201	5	12	161	10	24	41.7	362	6.6
5	4	5	69	0	1	28	4	6	66.7	97	6.2
Total Treated	16	79	929	27	98	845	43	177	24.3	1774	10.0

TABLE 67

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
A. SHORT TREATMENT

(Eighty parts per million)

	SERIES 118			SERIES 122			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	22	251	1	29	297	1	51	2.0	548	9.3
1	0	9	209	2	10	221	2	19	20.5	430	4.4
2	1	10	268	3	33	310	4	43	9.3	578	7.4
3	5	57	260	9	60	256	14	117	12.0	516	4.5
4	5	23	218	1	12	228	6	35	17.1	446	7.8
5	5	10	211	1	9	163	6	19	31.6	374	5.1
6	1	2	216	-	-	-	1	2	50.0	216	0.9
Total											

TABLE 68

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
B. CONTINUOUS TREATMENT

(One part per million)

	SERIES 146			SERIES 151			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	1	18	245	0	32	269	1	50	2.0	512	9.8
1	2	28	275	0	25	242	2	53	3.8	517	10.3
2	0	24	261	1	30	304	1	54	1.9	565	9.6
3	2	15	230	1	14	226	3	27	11.1	456	5.9
4	4	4	186	0	20	274	4	24	16.7	460	5.2
5	2	9	224	0	21	302	2	30	6.7	526	5.7
6	0	10	206	3	18	209	3	28	10.7	415	6.7
7	-	-	-	6	14	206	6	14	42.9	206	6.8
8	-	-	-	0	5	158	0	5	0.0	158	0.3
Total Treated	10	88	1382	11	147	1921	21	235	8.9	3303	7.1

TABLE 69

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
B. CONTINUOUS TREATMENT

(Five parts per million)

	SERIES 159			SERIES 158A			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	1	31	267	0	41	257	1	72	1.4	524	13.7
1	1	14	256	0	27	240	1	41	2.4	496	8.3
2	4	43	279	0	34	311	4	77	5.2	590	13.0
3	3	22	230	0	19	300	3	41	7.3	530	7.7
4	3	19	215	2	21	273	5	40	8.0	488	8.2
5	1	16	213	3	28	257	4	44	9.1	470	9.4
6	-	-	-	5	21	256	5	21	23.8	256	8.2
7	-	-	-	3	15	225	3	15	20.0	225	6.7
8	-	-	-	6	20	222	6	20	30.0	222	9.0
Total Treated	12	114	1193	19	185	2084	31	299	10.4	3277	9.1

TABLE 70

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
B. CONTINUOUS TREATMENT

(Twenty parts per million)

	SERIES 128			SERIES 134			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	28	230	0	21	213	0	49	0.0	443	11.1
1	0	14	232	1	12	220	1	26	3.9	452	5.8
2	0	8	232	0	8	268	0	16	0.0	500	3.2
3	0	3	228	2	14	276	2	17	11.8	504	3.4
4	4	9	236	4	12	256	8	21	38.1	492	4.3
5	2	10	221	9	16	277	11	26	42.3	498	5.2
6	4	6	243	5	10	272	9	16	56.3	515	3.1
7	8	10	236	4	11	275	12	21	57.1	511	4.1
8	6	6	239	6	6	253	12	12	100.0	492	2.4
9	2	3	245	8	10	245	10	13	76.9	490	2.7
10	12	14	216	5	10	256	17	24	70.8	472	5.1
11	8	9	222	4	9	248	12	18	66.7	470	3.8
12	0	0	219	3	6	242	3	6	50.0	461	1.3
Total Treated	46	92	2769	51	124	3088	97	216	49.9	5857	3.7

TABLE 71

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
B. CONTINUOUS TREATMENT

(Forty parts per million)

	SERIES 95			SERIES 125			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	4	20	202	1	31	265	5	51	9.8	467	10.9
1	0	4	204	1	11	244	1	15	6.7	448	3.3
2	0	6	208	3	15	216	3	21	14.3	424	5.0
3	2	11	209	2	7	221	4	18	22.2	430	4.2
4	5	17	218	7	11	240	12	28	42.9	458	6.1
5	15	21	220	9	18	235	24	39	61.5	455	8.6
6	7	24	212	11	22	233	18	46	39.1	445	10.3
7	13	25	231	30	35	231	43	60	71.7	462	13.0
8	12	20	237	26	28	223	38	48	79.2	460	10.4
9	29	38	219	1	1	221	30	39	76.9	440	8.9
10	16	29	238	10	11	248	26	40	65.0	486	8.2
11	18	21	208	6	8	241	24	29	82.8	449	6.5
12	-	-	-	7	8	231	7	8	87.5	231	3.5
Total Treated	117	216	2404	113	175	2784	230	391	58.8	5188	7.5

TABLE 72

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
B. CONTINUOUS TREATMENT

(Eighty parts per million)

	SERIES 102			SERIES 115			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	1	26	206	1	24	221	2	50	4.0	427	10.7
1	0	7	212	0	4	245	0	11	0.0	457	2.4
2	0	7	214	0	8	221	0	15	0.0	435	3.4
3	1	3	231	2	13	281	3	16	18.8	512	3.1
4	0	5	234	2	10	269	2	15	13.3	503	3.0
5	4	5	214	-	-	-	4	5	80.0	214	2.3
6	-	-	-	17	24	261	17	24	70.8	261	9.2
7	1	1	212	23	27	237	24	28	85.7	449	6.2
8	1	2	163	32	34	260	33	36	91.7	423	8.5
Total Treated	7	30	1480	76	120	1774	83	150	55.3	3254	4.6

TABLE 73

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
C. INTERMITTENT TREATMENT

(Forty parts per million for 15 minutes every two hours)

SERIES 126

Hours	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	1	31	3.2	227	13.7
1	0	15	0.0	228	6.6
2	10	35	28.6	218	16.1
3	3	49	6.1	227	21.6
4	6	20	30.0	211	9.5
5	5	7	71.4	184	3.8
6	5	5	100.0	206	2.4
7	-	-	-	-	-
8	2	2	100.0	78	2.6
Total Treated	31	133	23.3	1352	9.8

TABLE 74

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
C. INTERMITTENT TREATMENT

(Forty parts per million for 15 minutes every hour)

SERIES 135

Hours	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	16	0.0	228	7.0
1	3	12	25.0	264	4.5
2	6	31	19.4	253	12.3
3	5	30	16.7	257	11.7
4	8	24	33.3	257	9.3
5	12	39	30.8	273	14.3
6	6	9	66.7	254	2.8
7	16	20	80.0	247	8.1
8	15	18	83.3	233	7.7
9	12	14	85.7	256	5.5
10	8	8	100.0	256	3.1
11	3	3	100.0	245	1.2
12	6	6	100.0	244	2.4
Total Treated	100	214	46.7	3019	7.1

TABLE 75

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
D. UNTREATED MATERIAL

(Root tips collected at consecutive hours)

	SERIES 127			SERIES 132			COMBINED				
Hours	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	Total Div.	Disorg.	Total Ana.	% Disorg.	Total Div.	% Ana.
0	0	22	214	2	24	251	2	46	4.4	465	9.9
1	1	36	235	10	49	272	11	85	12.9	507	16.8
2	0	22	229	4	29	240	4	51	7.8	469	10.9
3	0	33	256	11	24	230	11	57	19.3	486	11.7
4	0	26	231	3	40	258	3	66	4.5	489	13.5
5	0	46	237	1	26	212	1	72	1.4	449	16.0
6	0	28	261	3	21	240	3	49	6.1	501	9.8
7	0	24	221	6	25	241	6	49	12.2	462	10.6
8	0	28	223	1	39	298	1	67	1.5	521	12.9
9	0	25	240	0	28	232	0	53	0.0	472	11.2
10	0	31	229	3	27	230	3	58	5.2	459	12.6
11	0	21	227	-	-	-	0	21	0.0	227	9.3
12	0	34	243	0	13	157	0	47	0.0	400	11.8
Total	1	376	3046	44	345	2861	45	721	6.2	5907	12.2

TABLE 76

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES
D. UNTREATED MATERIAL

(Root tips collected from same onion at same hour)

Root No.	Disorg. Ana.	Total Ana.	% Disorg.	Total Div.	% Ana.
2	0	34	0.0	434	7.8
3	8	63	12.7	498	12.7
4	1	37	2.7	413	9.0
6	1	31	3.2	422	7.3
7	2	48	4.2	448	10.7
8	2	48	4.2	450	10.7
10	0	29	0.0	429	6.8
11	0	26	0.0	429	6.1
12	0	51	0.0	442	11.5
13	1	43	2.3	459	9.4
15	9	48	18.7	441	10.9
21	0	47	0.0	491	9.6
Total	24	505	4.7	5356	9.4

TABLE 77

PERCENTAGES OF DISORGANIZED AND TOTAL ANAPHASES

TABLE 77A

ZERO HOURS OF TREATED BULBS
SHORT TREATMENT

Parts per million	Disorg. Ana.	Total Ana.	% Disorg.
1	0	47	0.0
5	0	57	0.0
20	7	48	14.6
40	5	53	9.4
80	1	51	2.0
Total	13	256	5.1

TABLE 77B

ZERO HOURS OF TREATED BULBS
CONTINUOUS TREATMENT

Parts per million	Disorg. Ana.	Total Ana.	% Disorg.
1	1	50	2.0
5	1	72	1.4
20	0	49	0.0
40	5	51	9.8
80	2	50	4.0
Total	9	272	3.3

TABLE 77C

SUMMARY OF ALL UNTREATED MATERIAL

	Total Disorg.	Total Ana.	% Disorg.
0-Hour Short	13	256	5.1
0-Hour Continuous	9	272	3.3
Consecutive Hours	45	721	6.2
Same Hour	24	505	4.7
Total	91	1754	5.2

TABLE 78

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
A. SHORT TREATMENT

(One part per million)

	SERIES 143		SERIES 147A		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	3	176	1	193	4	369	1.1
1	2	181	0	155	2	336	0.6
2	3	213	2	167	5	380	1.3
3	3	185	0	147	3	332	0.9
4	3	183	1	169	4	352	1.1
5	1	196	2	214	3	410	0.7
6	3	216	0	158	3	374	0.8
7	6	234	3	207	9	441	2.0
8	5	203	0	180	5	383	1.3
9	4	225	1	187	5	412	1.2
10	8	195	1	180	9	375	2.4
11	2	214	1	172	3	386	0.8
12	2	197	4	181	6	378	1.6
Total Treated	42	2442	15	2117	57	4559	1.3

TABLE 79

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
A. SHORT TREATMENT

(Five parts per million)

	SERIES 138		SERIES 152B		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	1	205	2	150	3	355	0.8
1	5	199	1	173	6	372	1.6
2	7	228	9	198	16	426	3.8
3	1	234	4	223	5	457	1.1
4	6	183	6	182	12	365	3.3
5	1	100	3	215	4	315	1.3
6	-	-	3	172	3	172	1.7
7	-	-	1	206	1	206	0.5
8	-	-	2	41	2	41	4.9
Total Treated	20	944	29	1410	49	2354	2.1

TABLE 80

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
A. SHORT TREATMENT

(Twenty parts per million)

	SERIES 92		SERIES 140		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	7	157	8	200	15	357	4.2
1	8	187	7	227	15	414	3.6
2	5	154	9	205	14	359	3.9
3	4	140	1	206	5	346	1.4
4	6	154	3	222	9	376	2.4
5	3	191	0	157	3	348	0.9
6	4	121	-	-	4	121	3.3
Total Treated	30	947	20	1017	50	1964	2.6

TABLE 81

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
A. SHORT TREATMENT

(Forty parts per million)

	SERIES 96		SERIES 120		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	4	151	4	174	8	325	2.5
1	18	197	8	185	26	382	6.8
2	4	198	16	174	20	372	5.4
3	16	165	6	163	22	328	6.7
4	8	159	1	121	9	280	3.2
5	5	58	0	25	5	83	6.0
Total Treated	51	777	31	668	82	1445	5.7

TABLE 82

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
A. SHORT TREATMENT

(Eighty parts per million)

	SERIES 118		SERIES 122		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	9	195	10	229	19	424	4.5
1	9	194	5	207	14	401	3.5
2	10	253	7	273	17	526	3.2
3	11	197	2	141	13	338	3.9
4	2	151	1	153	3	304	1.0
5	0	141	2	131	2	272	0.7
6	4	177	-	-	4	177	2.3
Total Treated	36	1113	17	905	53	2018	2.6

TABLE 83

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
B. CONTINUOUS TREATMENT

(One part per million)

	SERIES 146		SERIES 151		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	1	179	3	188	4	367	1.1
1	2	219	1	182	3	401	0.8
2	2	213	3	229	5	442	1.1
3	4	185	0	172	4	357	1.1
4	0	169	11	227	11	396	2.8
5	0	198	15	258	15	456	3.3
6	2	186	3	180	5	366	1.4
7	-	-	1	177	1	177	0.6
8	-	-	2	128	2	128	1.6
Total Treated	10	1170	36	1553	46	2723	1.7

TABLE 84

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
B. CONTINUOUS TREATMENT

(Five parts per million)

	SERIES 139		SERIES 152A		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	3	191	3	184	6	375	1.6
1	4	209	5	198	9	407	2.2
2	9	215	3	257	12	472	2.5
3	2	190	6	255	8	445	1.8
4	7	184	4	232	11	416	2.6
5	3	186	20	220	23	406	5.7
6	-	-	6	224	6	224	2.7
7	-	-	2	199	2	199	1.0
8	-	-	12	185	12	185	6.5
Total Treated	25	984	58	1770	83	2754	3.0

TABLE 85

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
B. CONTINUOUS TREATMENT

(Twenty parts per million)

	SERIES 128		SERIES 134		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	2	153	0	159	2	312	0.6
1	8	210	4	200	12	410	2.9
2	0	221	19	257	19	478	4.0
3	4	223	16	257	20	480	4.2
4	4	226	14	241	18	467	3.9
5	4	209	4	257	8	466	1.7
6	2	236	2	257	4	493	0.8
7	1	224	8	256	9	480	1.9
8	1	230	10	235	11	465	2.4
9	2	240	8	233	10	473	2.1
10	6	200	7	245	13	445	2.9
11	5	209	2	234	7	443	1.6
12	5	215	3	232	8	447	1.8
Total Treated	42	2643	97	2904	139	5547	2.6

TABLE 86

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
B. CONTINUOUS TREATMENT

(Forty parts per million)

	SERIES 95		SERIES 125		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	5	162	0	196	5	358	1.4
1	25	190	5	223	30	413	7.3
2	21	198	4	197	25	395	6.3
3	11	191	4	212	15	403	3.7
4	20	194	11	228	31	422	7.4
5	24	195	5	216	29	411	7.1
6	9	182	9	210	18	392	4.6
7	14	200	4	195	18	395	4.6
8	7	212	6	192	13	404	3.2
9	18	175	10	219	28	394	7.1
10	1	205	7	231	8	436	1.8
11	1	174	7	229	8	403	2.0
12	-	-	9	221	9	221	4.1
Total Treated	151	2116	81	2573	232	4331	5.4

TABLE 87

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
B. CONTINUOUS TREATMENT

(Eighty parts per million)

	SERIES 102		SERIES 115		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	5	144	1	166	6	310	1.9
1	4	195	5	236	9	431	2.1
2	6	204	2	212	8	416	1.9
3	10	227	21	266	31	493	6.3
4	30	227	10	254	40	481	8.3
5	18	204	-	-	18	204	8.8
6	-	-	25	232	25	232	10.8
7	19	207	15	208	34	415	8.2
8	17	159	16	222	33	381	8.7
Total Treated	104	1423	94	1630	198	3054	6.4

TABLE 88

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
C. UNTREATED

(Root tips collected at consecutive hours)

	SERIES 127		SERIES 132		COMBINED		
Hours	Seg.	Total	Seg.	Total	Seg.	Total	%
0	1	157	1	186	2	343	0.6
1	2	162	7	182	9	344	0.3
2	1	181	2	170	3	351	0.9
3	3	186	5	178	8	364	2.2
4	0	156	1	196	1	352	0.3
5	4	142	3	157	7	299	2.3
6	0	189	2	188	2	377	0.5
7	0	172	3	186	3	358	0.8
8	0	169	0	201	0	370	0.0
9	1	183	1	165	2	348	0.6
10	0	158	1	160	1	318	0.3
11	1	158	-	-	1	158	0.6
12	0	171	0	122	0	293	0.0
Total	13	2184	26	2091	39	4275	0.9

TABLE 89

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS
C. UNTREATED

(Root tips collected from same onion at same hour)

		SERIES 137B		
Hours	Root No.	Seg.	Total	%
0	2	3	330	0.9
1	3	6	366	1.6
2	4	6	335	1.8
3	6	4	330	1.2
4	7	4	348	1.2
5	8	0	334	0.0
6	10	4	341	1.2
7	11	2	335	0.7
8	12	0	306	0.0
9	13	10	345	2.9
10	15	12	325	3.7
11	21	1	376	0.3
12		-	-	-
Total		52	4071	1.3

TABLE 90

PERCENTAGES OF CELLS CONTAINING "REDUCTIONAL" OR "SEGREGATIONAL" GROUPINGS

TABLE 90A

ZERO HOURS OF TREATED BULBS
SHORT TREATMENT

Parts per million	Seg.	Total	%
1	4	369	1.1
5	3	355	0.8
20	15	357	4.2
40	8	325	2.5
80	19	424	4.5
Total	49	1830	2.7

TABLE 90B

ZERO HOURS OF TREATED BULBS
CONTINUOUS TREATMENT

Parts per million	Seg.	Total	%
1	4	367	1.1
5	6	375	1.6
20	2	312	0.6
40	5	358	1.4
80	6	310	1.9
Total	23	1722	1.3

TABLE 90C

SUMMARY OF ALL UNTREATED MATERIAL

	Seg.	Total	%
0-Hour Short	49	1830	2.7
0-Hour Continuous	23	1722	1.3
Consecutive Hours	39	4275	0.9
Same Hour	52	4071	1.3
Total	163	11898	1.4