A COMPARATIVE STUDY OF THE EFFECTS OF SEVERAL ANTIMITOTICS

By

Charles Clark Bowen

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

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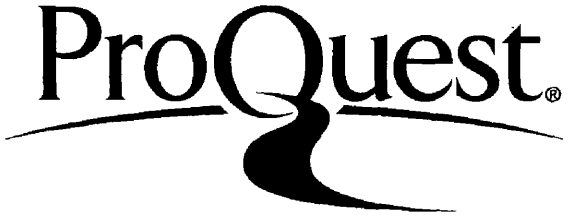

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Plant meristems are readily made polyploid by exposure to colchicine. The characteristic pattern of mitosis under the influence of this drug has been termed "C-mitosis". Many drugs which do not readily produce polyploidy, including certain antibiotics, cause cytological effects which at least superficially resemble those produced by colchicine. Such drugs have usually been called "C-mitotic" in the literature. The purpose of this investigation was to study the cytological effects of three antibiotics--Acti-dione, streptomycin and chloromycetin--on a qualitative and quantitative basis, and to compare the results with similar data obtained from a study of the effects of colchicine.

The root tips of pea seedlings were immersed in known concentrations of these drugs dissolved in a weak balanced mineral salt solution and material collected for examination at known intervals up to eight hours. Recovery was checked by rinsing seedlings thoroughly after eight hours of treatment and leaving them in moist paper toweling for forty-eight hours.

It was found that the effects of colchicine and Acti-dione differed in almost every respect. Acti-dione markedly decreased divisions by causing preprophase inhibition of divisions, while colchicine caused an increase in divisions which appeared in part to be due to a true stimulation of mitosis. Acti-dione primarily affected prophases, blocking their transition to metaphases and thus resulting in an accumulation of highly overcontracted prometaphases. Reversion to interphase occurred in abundance at all prophase stages. Acti-dione had little effect on postprophase stages. Colchicine primarily

affected postprophase stages by specific and complete impairment of spindle control and showed no effect on prophases. Streptomycin effects were qualitatively similar to those of Acti-dione, but streptomycin was shown to have a very narrow margin between its threshold of cytological effectiveness and its lethal threshold. Chloromycetin carried this tendency of streptomycin to the extreme, and except for rather erratically expressed preprophase inhibition of divisions, its level of cytological effectiveness was not separable from its necrotic dosage level.

With the exception of colchicine, the cytological activity of the drugs tested was antagonized by dissolved mineral salts. This "salt effect" was very marked with streptomycin and only moderate with Actidione and chloromycetin. Evidence of spindle impairment was not seen in significant proportions in material treated with the antibiotics except at dosages high enough to prevent recovery. These and other facts are used to support the hypothesis that the spindle derangements caused by the three antibiotics have a very different basis than the spindle disturbances caused by colchicine. A basis is shown to exist for the notion that spindle disturbance caused by treatment with Actidione, streptomycin and chloromycetin are actually early stages in pyknotic degeneration.

The term "akinetic mitosis" is proposed as a substitute for "C-mitosis" to describe any mitotic process where spindle control of postprophase chromosomes is impaired. "C-mitosis" would be reserved to describe the colchicine type of "akinetic" mitoses where such spindle impairment is a) a highly specific reaction, b) accompanied by little or no prophase disturbance, and c) productive of substantial amounts of polyploidy.

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INTRODUCTION

The importance of the cell as a basic unit of organization and control in living entities scarcely needs emphasis today. Accordingly, every scrap of information that can be gained concerning the nature and mechanics of the nucleus represents an advance in biological knowledge at the most fundamental level.

The study of chemically induced mitotic aberrations as a tool in this quest gained its first big impetus with the discovery of the polyploidizing action of colchicine (Blakeslee and Avery, 1937; Dustin, Havas and Lits, 1937; Nebel and Ruttle, 1938). After the war when security bars were lifted on certain phases of biological research, it became known that workers on both sides had succeeded in inducing mutations by chemical means (Auerbach and Robson, 1946; Oehlkers, 1943). Since that time literally hundreds of chemicals have been used to treat a wide variety of living tissues, and results have been checked cytologically as well as genetically. Comparison of results is complicated by the variety of experimental conditions and, in contrast to the standardized tests used to detect mutations, cytological analysis has been largely subjective and non-quantitative.

Despite the problems of comparison and lack of standardization of terminology, certain general tendencies have been observed and some general conclusions made. Levan in a recent review (1952) has divided these reactions into three main headings

and points out that some chemicals may produce one, two or even all three of these reactions depending on the conditions of treatment. Most of Levan's conclusions were based on the reactions of Allium root meristems, sprouted in water, to immersion in a solution of the drug for a definite period of time--the "Allium cepa test".

The first category is that of "mutagenic" reactions i.e. reactions where the principle result is that of genetic change. Levan further divides these into the subheadings "genetical mutagens" i.e. substances that produce gene mutations and "cytological mutagens" which cause genetic changes by producing structural alterations of the chromosomes. Generally speaking genetical mutagens also cause chromosome breakage and vice versa, but the two effects do not appear to be parallel in all chemicals.

The last two categories are those which primarily produce cytological effects of one sort or another and Levan designates these as 1) reversible physiological reactions and 2) lethal and toxic reactions. Under the first heading the effects of colchicine, so called C-mitosis, is the main type of reaction. This Levan describes as the "reversible inactivation of the mechanism of movement of the chromosomes". He recognizes C-tumour formation and excessive chromosome contraction as reactions belonging to this general complex.

Substances of widely varying chemical constitution have been classed as C-mitotic agents. In the case of a number of lipoid soluble substances of the narcotic type, it has been shown that

increased C-mitotic activity is correlated with increased fat solubility and decreased water solubility. The materials with the lowest known C-mitotic thresholds are the inorganic salts of heavy metals, particularly mercury, the activity of which can be increased even more by alkylation (Levan, 1945). Colchicine is unique among these compounds in that any change within the colchicine molecule destroys its activity (Steinegger and Levan, 1945). Levan (1952) suggests that the so called somatic meiosis of Huskins (1948) consists mainly of deviating C-mitoses due to incomplete impairment of the spindle mechanism.

Lethal and toxic reactions form the final category according to Levan. Effects in this category ranged from reversion of prophases with dissolution of matrix and relaxation of spirals to pyknosis. Levan describes this process as induction of an "artificial interphase". Substances causing such effects may also cause C-mitosis, but this will seldom be noted as the toxic thresholds are close to or below the C-mitotic threshold. D'Amato (1948, 1949) describes a large class of materials he calls "typical cell poisons" (as opposed to "C-mitotic poisons"). He describes two principle types of effects as typical of the cell poisons.

a) "Prophase poisoning" in that prophases are "unable" to reach metaphase. These arrested prophases may revert to the resting condition.

b) "Preprophase inhibition of mitosis" in that no new divisions are initiated once treatment has started to take effect.

Only occasionally could material with intense reversion be recovered and then only normal divisions could be found, no polyploidy or diplo-chromosomes were noted. D'Amato further points out that the occasional C-mitotic effects produced at some concentrations of these "poisons" are the result of a "shock action" or "massive toxic effect" and do not represent the typical cytological effect of these drugs.

During the past few years the cytology group at Michigan State College has screened a large number of compounds for cytological effects (Wilson, 1950; Wilson and Bowen, 1951; Powell, 1951; Huston, 1952; and others unpublished). These included, among many other materials, a long list of antibiotics and insecticides. While much of the work has been on the qualitative level, it appears to be definite that, of the long list of drugs tested, only two--colchicine and cyclochlorohexane (Lindane)--belong to the group of true "C-mitotic" agents. All other materials fall into one or another of the following categories:

A) Without effect, at least until fantastic concentrations were attained. An example is penicillin (Wilson, 1950).

B) Caused pyknosis and/or stickiness at a level of concentration low enough to mask any other cytological effects. An example in this category was the antifungal antibiotic, Rimocidin (Huston, 1952).

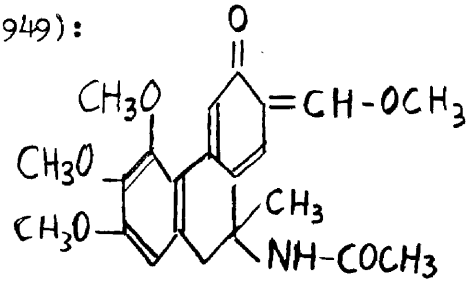
C) Substantially answered the requirements of a typical "cell poison" in the D'Amato sense with apparent failure of prophases to reach metaphase and

preprophase inhibition of divisions. Reversion of prophases and metaphases to interphase commonly but not invariably accompanied the other symptoms. C-mitoses were occasionally present. The bulk of materials tested fell into this class and Acti-dione, an antifungal antibiotic, may be cited as representative of this type of compound (Wilson, 1950; Hawthorne and Wilson, 1952).

During the course of this group's work a number of compounds with interesting properties have turned up. It was the purpose of this study to investigate the effects of several of these drugs on a comparative basis, namely colchicine, Acti-dione, streptomycin, and chloromycetin. A second purpose was to devise a cytological test method that would eliminate the principal problems encountered in the Allium cepa test i.e. the difficulty of obtaining onion bulbs that can be sprouted at will throughout the year, the frequent variability in division frequency and finally the occasional appearance of toxicity symptoms without apparent cause.

Colchicine

The alkaloid colchicine is obtained from the corm of Colchicum autumnale and has the following probable structural formula (Gortner and Gortner, 1949):



Colchicine ($C_{22}H_{25}O_6N$)

As previously mentioned, colchicine is the type substance for the cytological reaction labled "C-mitosis" by Levan (1938). In the original sense this term was used to describe the reaction as caused by colchicine, and thus implied a process which, under correct conditions, will result in polyploidy. Today, however, the term is used to describe any cytological reaction where postprophase chromosomes are subject to deficiency of spindle control, even though this may be a symptom of a completely different sort of cellular disturbance than that caused by colchicine. In this respect several workers have pointed out that the C-mitosis caused by the majority of antimitotics is probably a very different reaction than that produced by colchicine and a few other true polyploidizing agents (D'Amato, 1949; Allen, Wilson and Powell, 1950).

Guttman (1952) using Allium made a statistical analysis of the numbers of division figures in each stage of division per random field of view as observed under high dry power. Approximately two hundred cells were included in the average such field. She combined the data from three different treatments:

a) 200 ppm colchicine for twenty-four hours,

b) 500 ppm colchicine for twenty-four hours,

c) Saturated solution of acenaphthene for twenty-four hours.

Using a Poisson distribution she reported that colchicine and acenaphthene 1) Did not prevent any cells from entering prophase

2) Doubled the mean length of the total division cycle while quadrupling the mean duration of metaphase

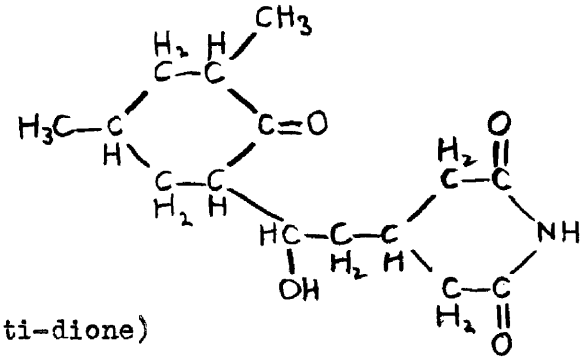
3) Caused 8.5 % of metaphases to revert to interphase

4) Afforded every cell reverting the same chance as any other cell of going through a new cycle of mitotic division.

In order to demonstrate the nature of such differences, colchicine was selected as one of the drugs, the cytological effects of which were to be studied in this investigation.

Acti-dione

In 1947, Whiffen, Bohones and Emerson reported the presence of an antifungal antibiotic in media fermented by streptomycin-producing strains of Streptomyces griseus. Isolated and crystallized by Ford and Leach (1948) it has the following structure:



Cyclohexamide (Acti-dione)

Acti-dione is the registered trademark of the Upjohn Company for its brand of this antibiotic. Possessing no marked antibacterial properties it has, however, proved useful in plant pathology in controlling certain fungus infections (Vaughn et al, 1949).

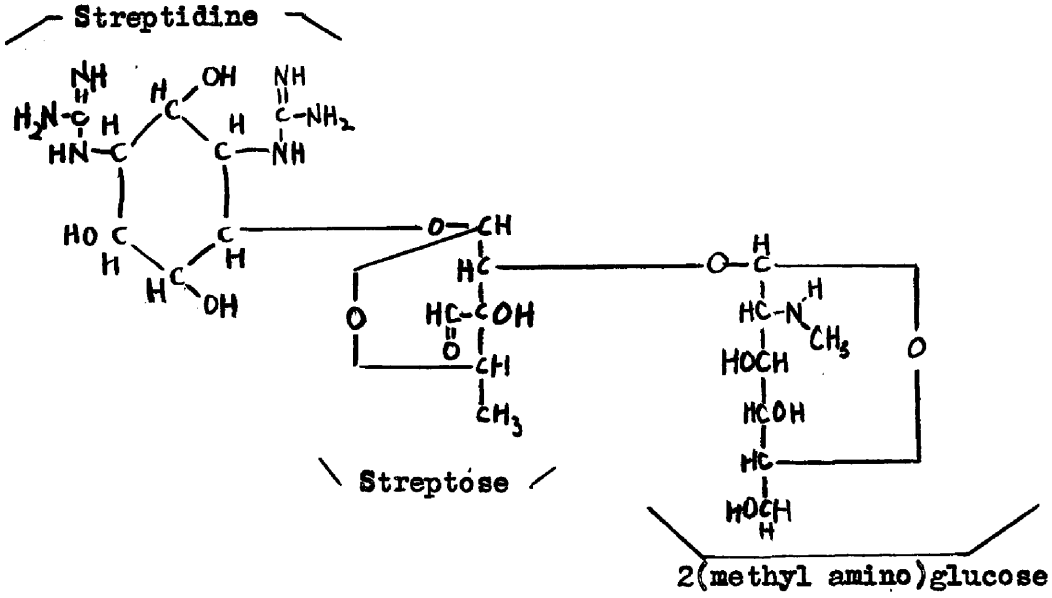
The cytology group at Michigan State College has investigated the cytological effects of Acti-dione on Allium root tips (Wilson, 1950; Hawthorne and Wilson, 1952). Generally speaking

the effects of this drug seemed quite unlike colchicine, and in general conformed with those set forth by D'Amato as belonging to his group of "cell poisons". No recovery of Allium root tips was obtained after treatment at dosages and times sufficient to produce obvious cytological effects.

Because of the reliability of the Acti-dione reaction, and the fact that its effects are as striking as colchicine effects, but apparently of a very different nature, it was selected for this study.

Streptomycin

Streptomycin, the first antibiotic of clinical importance to be isolated from an actinomycete was found by Waksman and co-workers (Schatz, Bugie and Waksman, 1944) in cultures of Streptomyces griseus. The structure of streptomycin is as follows (Pratt and Dufrenoy, 1949):



Streptomycin

An important modification of the drug is dihydrostreptomycin which is produced by reduction of the carbonyl group of the streptose moiety to an alcohol. Both streptomycin and dihydrostreptomycin are bases and are used clinically, as well as in this study, as the sulfates. Considerably lower toxicity has been shown for the reduced form than for streptomycin in clinical use, with no change in antibiotic activity reported (Waksman, 1949).

A phenomenon of interest is that the presence of certain anions and cations alter the effectiveness of streptomycin. This has been called "salt effect" (Pratt and Dufrenoy, 1949). There have been conflicting reports in the literature, but generally nitrate, chloride, lactate, phosphate, tartrate, citrate and sulfate among the anions; and bivalent magnesium, calcium and barium among the cations have been found to cause the greatest interference with antibacterial activity.

Streptomycin has several special properties of interest to the geneticist. Provasoli, Hutner and Pintner (1952) have reviewed the work that has been done on the induction of permanent chlorosis in seed plants and some algal flagellates by streptomycin. To date determinations as to whether or not such plastid mutations may be inherited has been held up by the numerous technical problems involved.

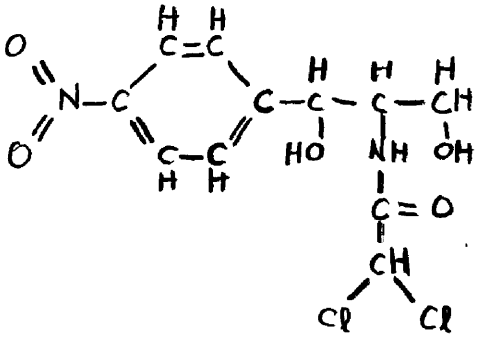
A second property worthy of mention is the use of streptomycin dependent strains of bacteria (developed by long exposure to low concentrations of the drug) in genetic studies. The loss of dependence upon streptomycin is easily determined quantitatively in such a

culture after exposure to chemical or physical mutagens (Bertani, 1950).

The cytological effects of streptomycin in Allium have been investigated by several workers (Wilson, 1950; Wilson and Bowen, 1951). I n general these effects have included overcontraction and some spindle impairment accompanied by reversion of later stages to the energic condition. Pyknosis is reported to be present in varying degrees at dosages only slightly over those necessary to produce cytological effects. No reports have been made of recovery after dosage at levels cytologically effective. These observations were confirmed in Tradescantia and chromosome fragmentation in that species by streptomycin was reported by Tanaka and Sato (1952).

Chloromycetin

The isolation of chloromycetin from Streptomyces venezuelae (Erlich et al, 1947) gave man a most useful antibiotic despite some recent adverse publicity concerning several fatal cases of aplastic anemia following extended treatment with the drug. Its structural formula is as follows:



Chloromycetin (Chloroamphenicol) $C_{11}H_{12}Cl_2N_2O_5$

Chloromycetin was subjected to the Allium test in our laboratory (Wilson and Bowen, 1951) and was found to cause almost complete cessation of divisions at concentrations of 1000 ppm. The occasional figures seen after twelve hours of treatment were strongly affected, overcontraction and C-mitosis both being present. Root tips after such treatment were recovered and no abnormalities noted. Because of the marked suppression of mitosis and ease of recovery shown by chloromycetin in these preliminary Allium runs, it was selected for testing in this investigation.

MATERIALS AND METHODS

A. Experimental Procedure

The root tips of young seedlings of Pisum sativum var. Alaska provided the meristematic material for these studies. Furnished through the courtesy of Ferry-Morse Seed Company, the seeds were reported to be fresh stock from a disease-free strain of relative genetic uniformity. They had not been subjected to any form of antifungal treatment.

Seeds were germinated in moist paper toweling and 72 hours after starting produced a root approximately three to four centimeters long. Germination and all subsequent operations were carried out at room temperature (about 24° C). At this time the seedlings were removed from the paper, and their roots were submersed in a mineral salt solution. This was accomplished by stretching wide mesh cheesecloth over the tops of one liter beakers filled to within one centimeter of the brim with the solution. The roots were inserted into the solution through the mesh of the cheesecloth, and the plumule, seed coat and cotyledons were thus suspended out of contact with the solution.

The mineral salt solution used was half-strength of a modified Hoagland solution as developed by Huskins and Steinitz (1948) with minor elements omitted and pH adjusted to 5.6. The composition of one liter of full strength solution was as follows:

.095 gm.	$Ca(NO_3)_2 \cdot 4H_2O$	.134 gm.	KH_2PO_4
.129 gm.	NH_4NO_3	.007 gm.	$K_2HPO_4 \cdot 3H_2O$
.180 gm.	$MgSO_4 \cdot 7H_2O$		

Constant aeration and agitation was obtained by bubbling air at a rate of approximately 10 cc/minute through capillary openings in the ends of glass tubes inserted into the solution through the cheesecloth. The air was obtained from the laboratory service outlets but was first rendered free of dust and oil particles by being passed through a filter consisting essentially of one liter of eighty mesh charcoal.

By use of this technique it was a relatively simple procedure to obtain any desired quantity of vigorously growing seedlings with roots well acclimated to an aqueous environment. After twelve hours in half strength Hoagland solution, cytological examination demonstrated that root tips rather uniformly possessed a high rate of meristematic activity with only rare abnormalities of division and relatively constant proportions of cells in the several stages of mitosis (Column A, Table I). This procedure was uniformly carried out to this point, and such root tips will henceforth be referred to as "zero hour controls".

Subsequent treatment varied with the chemical to be tested, but in all cases the seedlings were removed after twelve hours from the large beakers, the root tips rinsed in several changes of distilled water and then inserted through cheesecloth stretched over 250 ml beakers which were filled with a solution of the drug the cytological effects of which were to be tested. Aeration and agitation were maintained during treatment by bubbling air as before. Zero hour controls were always collected and fixed at this time.

Up to forty root tips could be treated in one vessel at one time and each collection included a minimum of four root tips from one vessel. Fixation was for at least thirty minutes in a three to one absolute alcohol - acetic acid mixture. Hydrolysis in one normal hydrochloric acid for twelve minutes at 60° C was followed by staining by means of the Feulgen reaction. Squash preparations were then dehydrated overnight in 95% ethanol containing a small amount of fast geen as a counterstain, and made permanent with Diaphane.

Control runs were carried out simultaneously with the testing of a particular drug. The control vessels contained only the particular concentration of Hoagland solution or distilled water being used as the solvent in the run in question.

Collections were made at intervals up to eight hours, and at the end of that time a minimum of six seedlings were rinsed in several changes of distilled water and placed in moist paper toweling in order to check recovery from the effects of the drug. These seedlings were inspected visually for gross changes and the root tips collected and fixed for subsequent cytological examination forty-eight hours after removal from the treatment vessel.

B. Drugs

Six different drug preparations were checked for cytological activity. Acti-dione (Control No. 247B-JHF-5), dihydrostreptomycin sulfate (Control No. T4520), and streptomycin sulfate (Control No. 113-JA-12) were manufactured by the Upjohn Company and furnished through the courtesy of that company's research division.

Similarly, preparations of synthetic chloromycetin (Control No. 164562) and fermentation chloromycetin (Control No. 168220) were furnished through the courtesy of Parke-Davis Company research division. The colchicine used was a preparation of the Mallinkrodt Chemical Works.

Stock solutions of relatively high concentration of each of the drugs were made up and stored under refrigeration until use. All concentrations were expressed in parts per million as a convenient measure of concentration.

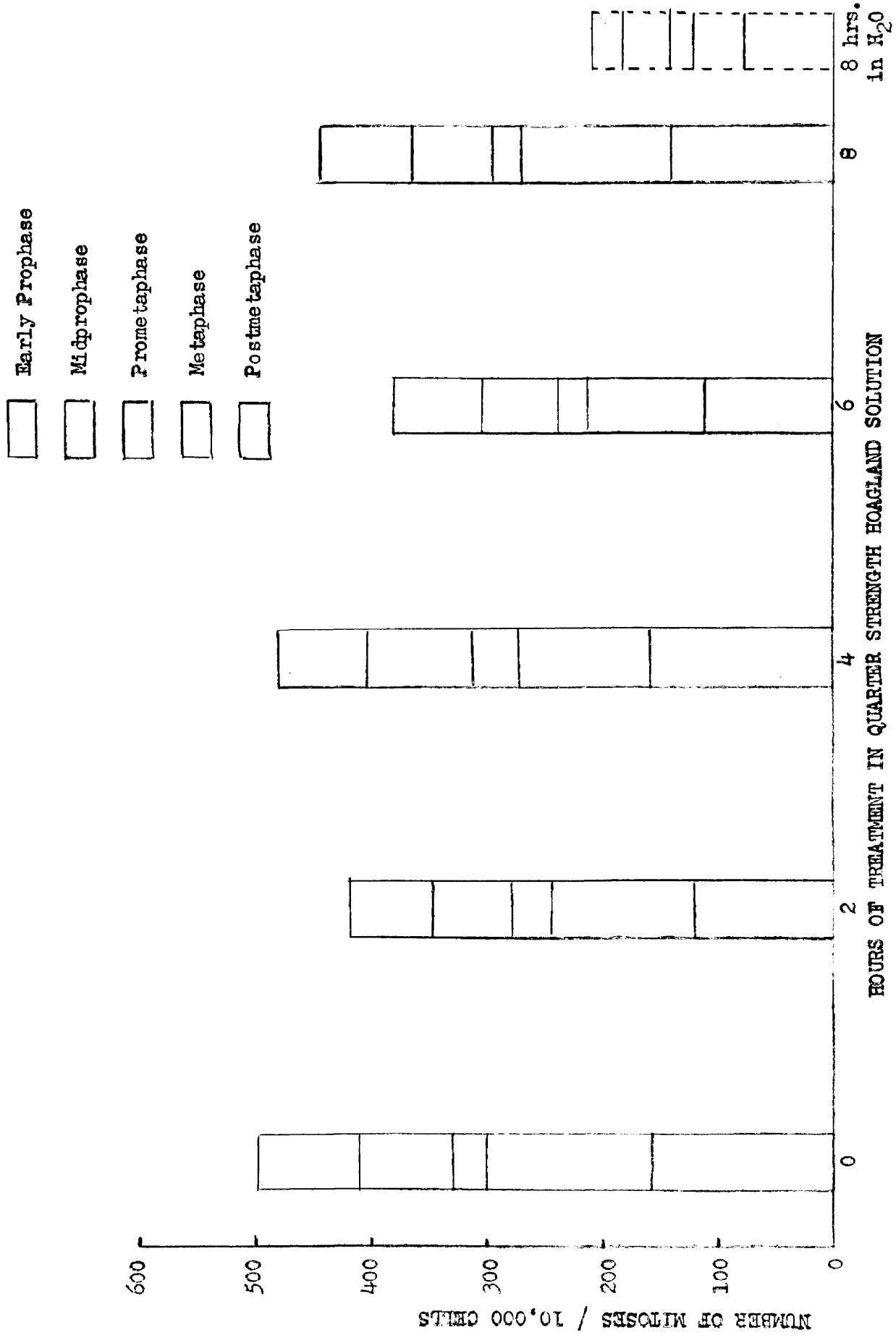
The problem of solvent for the drugs was investigated at some length in a series of preliminary experiments. It was found that a significant drop in frequency of divisions over zero hour controls occurred in root tips exposed to distilled water for eight hours (Column C in Table I). On the other hand, Galinsky (1948, 1949) reported cytological aberrations caused by very small amounts of the phosphate ion, which ruled out as suspect the use of full strength balanced mineral salt solutions. Investigation showed that eight hour treatment in a quarter-strength Hoagland solution, identical in composition with that used at half-strength in the preliminary preparation of seedlings, gave no significant decreases in division frequency, changes in relative frequency of stages, or increases in incidence of abnormalities over initial controls (Column B in Table I; Text fig. 1).

In general quarter-strength modified Hoagland solution was used as solvent, and all quantitative data were taken from such runs. Each drug was also tested utilizing distilled water from

TABLE I

CYTOLOGICAL ANALYSIS OF CONTROL RUNS

	(A) Zero Hour Control	(B) 8 Hours in ¼ Hoag.	(C) 8 Hours in Dist. H_2O
Number of root tips	30	6	6
Number of Division Figures/10,000 cells	493 ±18	475 ± 50	210 ±39
% of Total Divisions in Early Prophase	31.1 ± 0.7	31.7 ± 2.0	37.5 ±1.6
% of Total Divisions in Midprophase	29.4 ± 0.7	29.3 ±2.7	20.3 ±1.1
% of Total Divisions in Prometaphase	5.9 ± 0.4	6.4 ± 0.6	8.7 ±1.3
% of Total Divisions in Metaphase	16.3 ± 0.7	16.1 ±2.1	19.7 ±1.0
% of Total Divisions in Postmetaphase	17.3 ±0.7	16.6 ±0.9	13.8 ±1.2
% of Divisions Abnormal	1.2 ±0.2	1.3 ± 0.4	4.0 ±0.6



Text Figure 1A. Control Runs - Variations of Absolute Numbers of Mitoses with Time.

an ordinary laboratory metal still as solvent in order to determine if effective dosage thresholds varied from those found using quarter-strength Hoagland solution. In the case of streptomycin, where a wide discrepancy was found to exist, a run was made with half-strength Hoagland to further explore this discrepancy.

Runs were planned with the goal of testing each drug over a range of concentrations wide enough that, after eight hours of treatment, the effects would range from complete necrosis at one extreme to insignificant deviation from eight hour control slides at the other extreme. This proved impractical in several cases. The lethal threshold of colchicine was never ascertained, as it was decided that the information to be gained was not worth the expenditure of relatively large quantities of this expensive drug. In the case of Acti-dione the precise lower limit of cytological effectiveness was not determined. Runs made at concentrations as low as 0.2 ppm showed well defined reactions. A further problem was encountered with the latter drug in that, even at the lowest dosages tested, its action in reducing frequency of divisions was so effective that by eight hours cytological analysis was extremely difficult due to the scarcity of division figures. For this reason the effects of different concentrations of Acti-dione were studied after four hours of treatment rather than after eight hours.

After the effects of varying the concentration of a drug had been studied, runs were made to investigate changes occurring with time when a typically effective dose of the drug was applied. For this purpose a dose was selected which exhibited a high degree of

cytological effectiveness, but which was below the level of complete lethality after eight hours of treatment. In such runs collections were made at the end of 1, 2, 3, 4, 6, and 8 hours of treatment.

C. Cytological Examination

All examination of slides was done with a 90X oil immersion objective and 12.5X oculars. Critical illumination of the slide was provided by a ribbon filament lamp with a type B green filter between the lamp and the microscope mirror.

Where only qualitative information concerning effects was desired several widely separate strips of the slide were carefully examined by tracking across the entire slide using the horizontal adjustment of the mechanical stage only. Careful notes were taken on apparent frequency of division figures, types and prevalence of abnormalities and finally evidences of necrotic changes in both division figures and resting nuclei.

Where quantitative data were desired, this procedure was rigidly standardized. The slide was examined systematically in horizontal strips as before, but extra care was used to insure that an unbiased sample was examined, despite the fact that with good slide preparation one can expect a relatively random distribution of the different portions of the root tip over the entire slide. Never less than three complete tracks across the slide were examined, distributed approximately one quarter, one half, and three quarters of the way down the 22 x 22 mm cover glass.

This examination consisted of keeping accurate count of all division figures seen. Division figures were scored by first deciding to which of the five general stages of mitosis they corresponded. These were somewhat arbitrarily defined as prophase, midprophase, prometaphase, metaphase and postmetaphase. Following this decision, it was then determined whether or not the figure in question was normal. If abnormal, a further decision had to be made as to which of several predetermined types of abnormalities the figure corresponded. This procedure was continued until a minimum of one hundred division figures had been examined per slide, except in the case of 6 and 8 hour Acti-dione slides, where fifty and thirty-three figures respectively were examined as a minimum due to the extreme scarcity of divisions.

At the same time as the examination of division figures was being carried out, a count was kept of resting nuclei until approximately 1500 had been counted. At this time the ratio of division figures to total cells examined was calculated. A count of all abnormalities seen in the resting nuclei such as micronuclei, unusually shaped or obviously polyploid cells was also kept.

Data taken in these studies are summarized in Appendix Tables I to VII and form the basis for all quantitative analysis of the cytological effects.

D. Scope of Investigation

The effects of colchicine, Acti-dione and streptomycin sulfate were studied quantitatively on both a concentration and a time basis. The several runs with dihydrostreptomycin sulfate

bore so striking a resemblance to the streptomycin sulfate runs that only qualitative studies were carried out. Results with both synthetic and fermentation chloromycetin, while interesting, proved, as will be subsequently discussed, impractical to score on the basis used with the other drugs and only qualitative data were obtained.

OBSERVATIONS

The first portion of the following observations consists of a comparative study of the quantitative data obtained from colchicine, streptomycin sulfate and Actidione treatments. Following this the qualitative information obtained from the chloromycetin and dihydrostreptomycin treatments will be presented separately.

A. Frequency of Divisions

Colchicine

Colchicine dosages of 30 ppm and above for eight hours caused significant increases in frequency of division figures over zero hour controls, and it is likely that this effect occurred at dosages as low as 15 ppm (Text figs. 2A, 3A). The effect of increasing dosage on increase in division figures appeared to be a geometric one within the range of 10 ppm to at least 50 ppm, the data indicating that doubling the dose increased divisions by a relatively constant amount (roughly 120 divisions per 10,000 cells).

By three hours a dose of 50 ppm resulted in a significant increase of division figures (Text figs. 5A, 6A). While the bulk of this increase is accounted for by an increase in the number of metaphases, there is good reason to suspect that all stages share in the increase. In this respect it was found that prophases in some eighteen slides representing treatment with 50 ppm for three to eight hours showed an increase in frequency significant at the three per cent level over zero hour controls (Table II).

TABLE II

FREQUENCY OF DIVISIONS IN COLCHICINE TREATED ROOT TIPS COMPARED WITH CONTROLS

	No. of Slides	Divisions in Prophase per 10,000 Cells		Divisions Later than Prophase per 10,000 Cells	
Zero Hour Controls	30	331.5	13.4	161.5	7.5
50 ppm Colchicine 3 to 8 hrs.	18	407.3	3.1(1)	338.4	19.3(2)

(1) Difference from controls probably significant

(2) Difference from controls highly significant

Acti-dione

Acti-dione, in contrast to colchicine resulted in very significant decreases in division frequency by four hours at all concentrations tested (Text figs. 2B, 3B). Maximum effectiveness at four hours was exhibited by concentrations of 1 and 4 ppm. It further appears that 16 ppm was significantly less effective in this respect than all lower concentrations tested.

By one hour a dosage of 6 ppm resulted in a striking decrease in frequency and by six hours the number of division figures per slide dropped to such a low value that cytological analysis became difficult (Text figs. 5B, 6B).

Streptomycin

Streptomycin sulfate in general effected a reduction in number

of divisions, but not nearly as severely as did Acti-dione. Concentrations of 100 ppm and above showed significant decreases by eight hours (Text figs. 2C, 3C). It was not until six hours of treatment with 75 ppm that a significant decrease was observed (Text figs. 5C, 6C).

B. Classification of Aberrations

Prophase

Generally speaking, prophase figures in colchicine treated material showed no more deviations from a normal appearance than did control material. Prophases in Acti-dione material on the other hand showed very marked deviations from normal; in fact, at moderate dosages the bulk of Acti-dione aberrations occurred in this stage. Streptomycin effects in prophase in general resembled those produced by Acti-dione, but as a rule did not approach the same intensity of expression or high frequency.

Prophase aberrations observed in these treatments can be placed in three general categories. The most striking abnormality is overcontraction. Typically chromosomes in such a cell are oriented to form a hollow sphere as if contained in an intact nuclear membrane in normal midprophase arrangement. In the type of overcontraction designated as "ball prometaphase" (Plate IV, Figs. 7, 8), chromosomes are contracted to normal prometaphase or metaphase length and in the type designated as "overcontracted prometaphase" (Plate IV, Figs. 9, 10) chromosomes are definitely shorter than those of a normal metaphase. Such figures show no evidence of the disappearance of nuclear membrane and the clumping of chromosomes

in the center of the cell characteristic of the normal prometaphase (Plate I, Figs. 7, 8, 9).

In control and colchicine affected material, such overcontraction was noted but rarely and then only in the mildest degree but both types were abundant in Acti-dione treatments. Ball prometaphases were moderately frequent in streptomycin affected slides, but contraction apparently rarely proceeded to the point of producing significant numbers of overcontracted prometaphases.

The second important category of prophase abnormality will be designated as "reverting prophase" (Plate IV, Figs. 4, 5, 11, 12). While by definition overcontracted prophases of either type can only occur in prometaphase, reversion appears to occur at any time from early prophase to prometaphase. Typically, chromosomes in such figures, while retaining good prophase orientation, become diffuse and tend to lose precise outline. Relaxing of coiling occurs and relationally coiled double chromonemata frequently become clearly visible. When severe reversion occurs, it is seen in a complete series of expression from near normal prophases to cells which have what appear to be resting nuclei with just a suggestion of prophase chromosome arrangement (Plate IV, Fig. 6).

Reverting prophases were seldom encountered in control and colchicine material, but were moderately frequent in streptomycin slides and, except for overcontracted prophases, were the most frequent aberration found in Acti-dione affected cells.

The final prophase anomaly of significance is designated as "pyknosis". Pyknotic prophases (Plate VI, Figs. 11, 12) assume a

variety of forms, some of which when mildly expressed, are difficult to separate from reversion prophases. In general, the picture is one of nuclear disintegration and chromosomal deformation, stickiness, melting or fusing. Often coiling becomes clearly visible (Plate VI, Fig. 11). Pyknosis in prophase is invariably accompanied by similar phenomena in all other stages as well as in adjacent resting nuclei.

At the dosages used for the runs from which quantitative data were drawn pyknosis in prophase was almost non-existent except for streptomycin treatments where it was moderately frequent at the highest dosages.

Metaphase

In contrast to its effect on prophase Acti-dione produced relatively few metaphase figures of extreme abnormality. On the other hand, metaphases affected by colchicine bore little or no resemblance to control metaphases. Streptomycin, like Acti-dione, produced a much lower proportion of aberrant metaphases than did colchicine.

Aberrant metaphase figures seen in these studies fall into three general categories. The first are designated "overcontracted metaphases" (Plate IV, Figs. 13, 14). In such figures the chromosomes have normal orientation on an equatorial plate, but are markedly overcontracted in comparison to normal metaphase chromosomes (Plate I, Figs. 10, 11).

The second and most striking sort of abnormal metaphase is designated as "akinetic metaphase". In such figures chromosome

length may be the same or shorter than that of normal metaphase chromosomes. No arrangement on an equatorial plate has taken place despite the fact that chromatid arms show some separation with only the kinetochore holding the chromatids together. Actual location of the chromosomes in the cell may vary from scattered (Plate V, Fig. 2) to clumped (Plate II, Figs. 6, 7, 8).

The final category of metaphases falls under the heading of pkynosis. "Pyknotic metaphases" like pyknotic prophases are rarely seen except in company with pyknotic resting nuclei and other stages. These figures represent nuclear dissolution and degeneration and the picture is one of fusing, amorphous chromosomes usually clumped in a highly alveolar mass (Plate VII, Fig. 6).

Generally speaking, metaphases strongly affected by colchicine were close to 100% akinetic with the other anomalies appearing rarely if at all. Streptomycin affected tissue showed occasional akinetic metaphases and occasional overcontraction. Acti-dione affected metaphases were rarely akinetic but overcontraction was common. Pyknosis was rarely caused by any of the drugs at the concentrations used for the quantitative runs except occasional pyknotic metaphases in the streptomycin tested material.

Postmetaphase

As with metaphases, the great majority of postmetaphases in colchicine affected material were very different in appearance from those in controls. Acti-dione and streptomycin treated material presented fewer and far less striking aberrations at this stage than were produced by colchicine treatments.

At this stage aberrations were divided into four more or less distinct groups. On occasion the first two groups will be referred to as a single broad class--"akinetic postmetaphases". In unipolar figures the entire 4N complement of chromosomes forms a single more or less clumped group rather centrally located in the cell. In such cells chromosomes range from nearly normal in length to considerably overcontracted (Plate II, Figs. 11, 12, 15, 16). It was often difficult on analysis to distinguish such a cell from a clumped akinetic metaphase.

The second class, designated "disorganized postmetaphases", includes all deviations from normal anaphase or telophase separation and grouping except those figures which can be strictly classified as unipolar. Thus unequal or multipolar groupings, bridges, lagging chromosomes and random scattering fall into this class (Plate II, Figs. 9, 10, 13, 14).

The third class "overcontracted postmetaphases" includes those figures in which chromosomes have separated into two equal well oriented groups, but where chromosomes are strongly overcontracted compared to normal postmetaphase chromosomes (Plate IV, Figs. 15, 16).

The final category, almost non-existent at the dosages employed for the quantitative studies, is that of "pyknotic postmetaphases". Such postmetaphases, like pyknotic figures in prophase and metaphase, consist of a variety of deteriorating necrotic forms. (Plate V, Figs. 15, 16).

In general colchicine treatment resulted in an abundance of unipolar and to a less extent disorganized postmetaphases. On the

other hand, overcontracted postmetaphases with their normal grouping and orientation of chromosomes were never found in colchicine affected material, but formed the principle aberration in Acti-dione treated material. Except at the highest concentrations, unipolar and disorganized postmetaphases resulted only infrequently from Acti-dione treatment. Streptomycin showed only infrequent abnormalities in this stage at the dosages used, overcontracted, unipolar and disorganized figures being seen at the longer times and higher dosages.

Resting Nuclei

Deviations of resting nuclei can be separated into two principle classes. The most interesting of these are those where the chromatin has become distributed in micronuclei. Except in recovered material cytokinesis rarely occurs to form microcytes. Binucleate cells with or without equality in the size of nuclei and cells with grotesquely shaped, bilobed or multilobed nuclei were frequently observed after some treatments and appear to belong to this same category (Plate III, Figs. 1, 2). All the preceeding types were seen in colchicine treated material, but were not found after any of the other treatments in any significant frequency.

The second class of aberrant resting nuclei are designated as pyknotic. Here the appearance ranges from resting nuclei which are smaller than any found in controls and are very heavily stained (Plate VII, Fig. 3), to nuclei in which the chromatic material has condensed into a vast number of tiny droplets which may be arranged

more or less discontinuously into strands like strings of beads (Plate VI, Fig. 12). At degrees of necrosis between these extremes the coiling of the chromonemata is often clearly visible in portions of the degenerating nuclei.

Pyknosis was not encountered in resting nuclei of colchicine treated material, and only rarely did Acti-dione cause it at doses below 16 ppm. It was encountered fairly frequently in most streptomycin treated material.

Polyploidy

Except for some obviously polyploid resting nuclei seen after colchicine treatments, none of the drugs at the end of eight hours had produced any significant visible evidence of polyploidy. In recovered material only colchicine treatments produced significant amounts of polyploid division (Plate III, Figs. 3, 4, 5, 6). However, inasmuch as quantitative analysis was not made of recovered material, no classification of the expression of this phenomenon was attempted.

C. Range of Toxicity

Results of the investigation of the dosage levels required to produce cytological deviations from normal and to permanently kill the tissues are summarized in Table III. Several items of interest were brought out by this phase of the investigation.

Considerably higher doses of all drugs except colchicine were required to produce the same level of effects when Hoagland solution instead of distilled water was used as the drug solvent. This effect was most marked by far in the streptomycin treatments,

TABLE III

SUMMARY OF DOSAGE LEVEL (PARTS PER MILLION) NECESSARY TO PRODUCE CYTOLOGICAL EFFECTS AND NON-RECOVERY AFTER EIGHT HOURS OF TREATMENT

Drug	COLCHICINE		ACTI-DIONE		STREPTOMYCIN			DIHYDRO-(1) STREPTOMYCIN		SYNTHETIC CHLOROMYCETIN		FERMENTATION CHLOROMYCETIN
Solvent	Dist. H_2O	¼ Hoag.	Dist. H_2O	¼ Hoag.	Dist. H_2O	¼ Hoag.	½ Hoag.	Dist. H_2O	¼ Hoag.	Dist. H_2O	¼ Hoag.	¼ Hoag.
Cytological Threshold	20(2)	15(2)	0.4	0.5	10	175	400	10	200	500	750	Ca 750
Maximum Dosage with 100% recovery			2	8	5	150	400	5	100	250	750	500
LD_{50}			Ca 3	Ca 12	7.5	175	600	Ca 7.5	Ca 175	Ca 375	Ca 850	Ca 750
No Recovery			8	16	10	200	800	10	200	750	1000	1000
Dosage range investigated	5 to 50	5 to 50	0.1 to 12.0	0.2 to 16.0	5 to 100	50 to 400	50 to 800	5 to 25	100 to 400	50 to 750	100 to 1500	500 to 1500

(1) Dosage of dihydrostreptomycin converted to streptomycin sulfate equivalent.

(2) 20 ppm was minimum dosage to produce significant amounts of polyploidy.

where from forty to eighty times the dosage was required in half-strength Hoagland solution as in distilled water. For Acti-dione and chloromycetin approximately twice the dosage was required in quarter-strength Hoagland solution as in distilled water, whereas for streptomycin approximately fifteen times the dosage was required. The possibility that this effect was due to pH differences was ruled out by the fact that in quarter-strength Hoagland solution no effects were observed after eighteen hours of treatment with 100 ppm streptomycin even though by eight hours, due to the limited buffer capacity of the quarter strength Hoagland solution, the pH had dropped to 5.0, the identical pH found in the 10 ppm streptomycin-distilled water solution which was extremely toxic by eight hours.

It is further to be noted that there was no significant differences in effective dosage levels of dihydrostreptomycin compared to streptomycin. Similarly synthetic and fermentation chloromycetin did not differ from one another significantly in this respect.

While quantitative cytological examination was not made of slides scored as recovered, they were examined thoroughly enough to draw the conclusion that, except for root tips treated with 20 ppm and stronger colchicine solutions, significant numbers of aberrations were not present at the end of forty-eight hours of recovery. Polyploidy in colchicine material by this time was often extreme. In some root tips giant cells that had undergone three and perhaps four divisions without cytokinesis were not rare (Plate III, Figs. 5, 6).

D. Changes in Effects with Variation in Dosage

Eight hours treatment with colchicine at concentrations from 5 to 50 ppm, four hours treatment with Acti-dione at concentrations from 0.2 to 16.0 ppm and eight hours treatment with streptomycin sulfate at concentrations from 50 to 200 ppm form the basis of this phase of the investigation.

Appendix Tables II, III, and IV set forth the consolidated data obtained from these runs. Text figs. 2A to 2C give the absolute number of figures for each stage for the several concentrations tested of each drug. Text figs. 4A through 4C show the variation with dosage of the relative proportions of each stage and the relative proportions of each type of abnormality.

Colchicine (Plates II and III)

It is doubtful that any effects of consequence can be demonstrated for colchicine at concentrations below 15 ppm. Except for a small number of akinetic metaphases, of perhaps questionable significance, at both 5 and 10 ppm total frequency of division, relative proportions of the several stages and incidence of aberrations were not appreciably altered from controls.

At 15 ppm and above several well-marked tendencies can be discerned. The increase in division frequency from this point on, for the most part, can be attributed to an increased proportion of metaphases, but there is also reason to suspect that absolute prophase numbers increased significantly as previously pointed out. Metaphase and postmetaphase exhibited marked spindle disturbances which increased with concentration up to 30 ppm at which point

substantially all metaphases were akinetic and all postmetaphases were unipolar.

Colchicine doses of 15 and 20 ppm did not affect all metaphases and postmetaphases. In addition to both normal and unipolar figures at these dosages a sizeable proportion of postmetaphases were multipolar. Furthermore, it was noted qualitatively that many of the akinetic metaphases were more or less scattered as well as clumped in arrangement. It is perhaps of interest in this connection that, of the six concentrations tested, 15 and 20 ppm produced by far the highest frequency of micronuclei.

Except for an increase in total numbers, there was no evidence of significant disturbance of prophases in any way at any colchicine dosage level.

Acti-dione (Plates IV and V)

The smallest dosage of Acti-dione, 0.2 ppm, tested gave considerable evidence of physiological activity in that it reduced the number of divisions almost by half compared with controls. Cytologically this concentration apparently caused a small but significant amount of overcontraction. As dosage was increased several rather definite trends could be observed. Early prophases decreased in relative proportion to the other stages until they practically disappeared at 4 to 8 ppm. The amount of prophase reversion increased throughout the range of concentrations. Prometaphases showed the most interesting changes at all stages. By the time a concentration of 1 ppm was reached, they represented nearly forty per cent of all figures in contrast to their share of

less than ten per cent in controls. This level was maintained throughout the rest of the concentration range. These large numbers consisted substantially of ball prometaphases with significant quantities of overcontracted and reverting nuclei.

Metaphase and postmetaphase were apparently much less disturbed than prophase by Acti-dione. The relative frequency of these figures dropped steadily as dosage was increased to 4 ppm; above this concentration this value appeared to return to that of the controls. Overcontraction in these stages became increasingly frequent as dosage increased and little spindle disturbance could be detected until a dosage of 16 ppm was reached. At this, the highest dose tested, a significant number of akinetic metaphases was found. Also, at this dosage, an increase in the proportion and absolute number of metaphases as well as a decrease in proportion and absolute number of postmetaphases occurred. An interesting qualitative difference noted in metaphases scored as "normal" and as "overcontracted" in material treated at 16 ppm was that the chromosomes in at least half of these, though oriented normally on a plate, showed wide separation of chromatid arms and were held together only at the kinetochore (Plate V, Fig. 1). Such a condition was never encountered in control material and only occasionally at lower dosages. Metaphases scored as "akinetic" were for the most part of the scattered type rather than clumped (Plate V, Fig. 2).

It should be re-emphasized at this point that pea root meristems treated with 4 ppm Acti-dione have been recovered and showed

no striking abnormalities forty-eight hours after removal from the drug. Material treated with 16 ppm showed occasional patches of pyknotic cells at the end of the four hours of treatment and recovery was not obtained.

Streptomycin (Plate VI)

Except for the increasingly lower frequency of divisions and the slightly lower ratio that early prophase bore to the rest of the stages in the 100 and 150 ppm treatments, no significant effects were noted in the range of dosage from 50 to 150 ppm.

At 175 and 200 ppm the same general pattern occurred as in the Acti-dione treated material. Early prophases decreased and prometaphases showed some overcontraction. Reversion occurred frequently. At the highest dosage, 200 ppm, a marked decrease in the proportion of postmetaphases was noted. Pyknotic resting nuclei were noted at both concentrations and were abundant at 200 ppm. Pyknotic metaphases were present in significant proportions by 200 ppm.

With streptomycin the LD_{50} dosage was found to be about 175 ppm, whereas no recovery was obtained after treatment for eight hours with 200 ppm. It should be pointed out that with Acti-dione there was a very wide range of concentrations causing marked cytological effects below lethal dosage levels and that this situation was not found to exist in the case of streptomycin.

General Summary

Text figs. 3A, 3B and 3C summarize in a general way the differences between colchicine, Acti-dione and streptomycin with

respect to the effect of changes in dosage concentrations on

(a) the frequency of division figures,

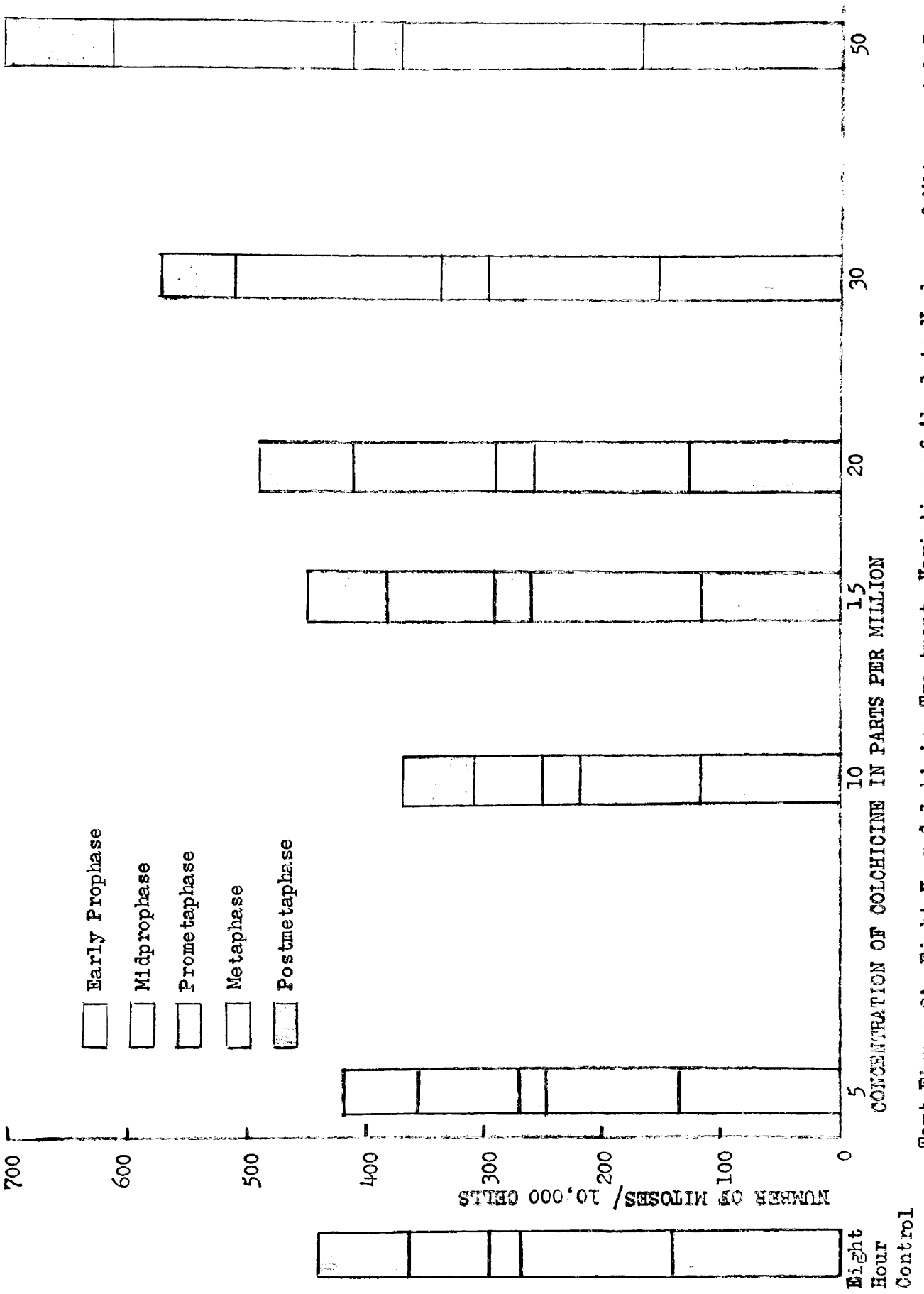
(b) the proportion of abnormalities in total prophase and

(c) the proportion of abnormalities in total postprophase.

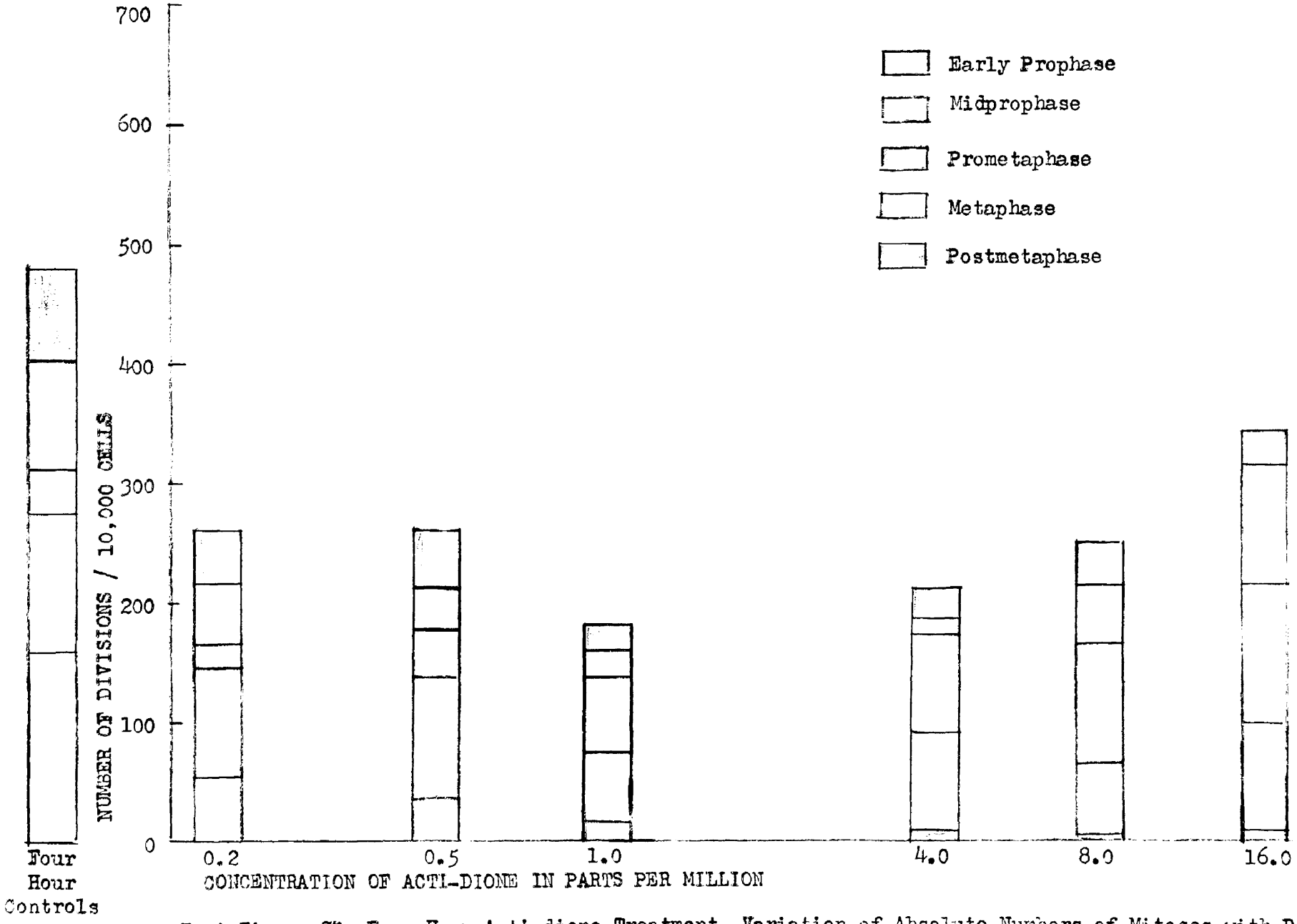
To supplement these graphs, it may be said that colchicine increased the number of divisions, principally but not entirely by increasing proportion of metaphases, affected prophases little if any, and modified metaphases and postmetaphases strikingly in that spindle control of chromosome arrangement was removed.

Acti-dione, at doses below the lethal level, decreased number of divisions and affected metaphases and postmetaphases only in that a degree of overcontraction could be noted. Prophase was rather severely modified in that early prophases disappeared, the proportion of prometaphases increased tremendously, reversion was frequent and both ball and overcontracted prometaphases appeared in quantity. Acti-dione could effect its typical cytological changes over a very wide range of concentrations below the lethal dosage level.

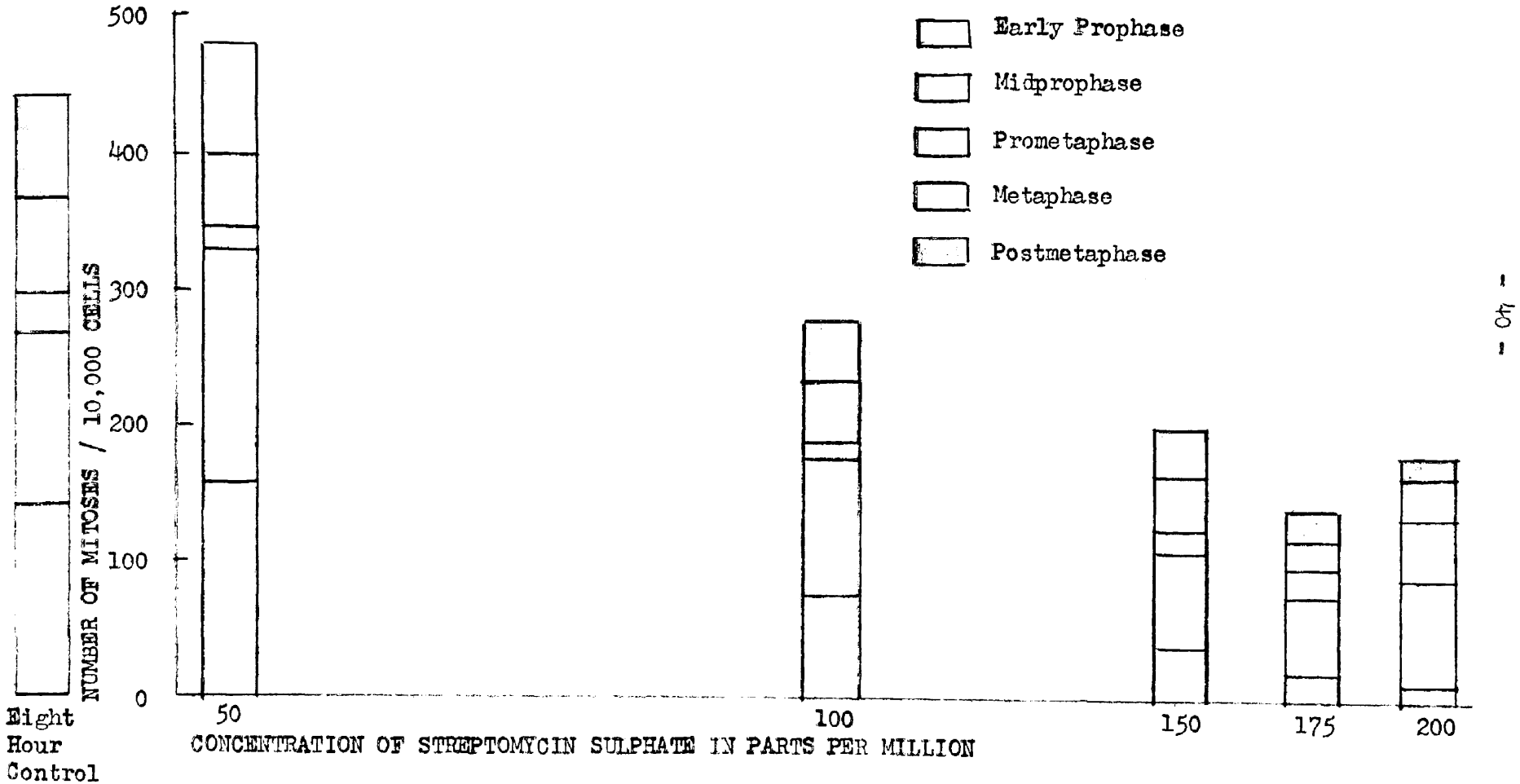
Streptomycin caused all of the same effects as Acti-dione, but expression was weaker. Unlike Acti-dione little margin existed between the minimum dosage effective cytologically and the lethal dosage. Pyknotic effects were often noted particularly in resting nuclei at all dosages causing cytological effects.



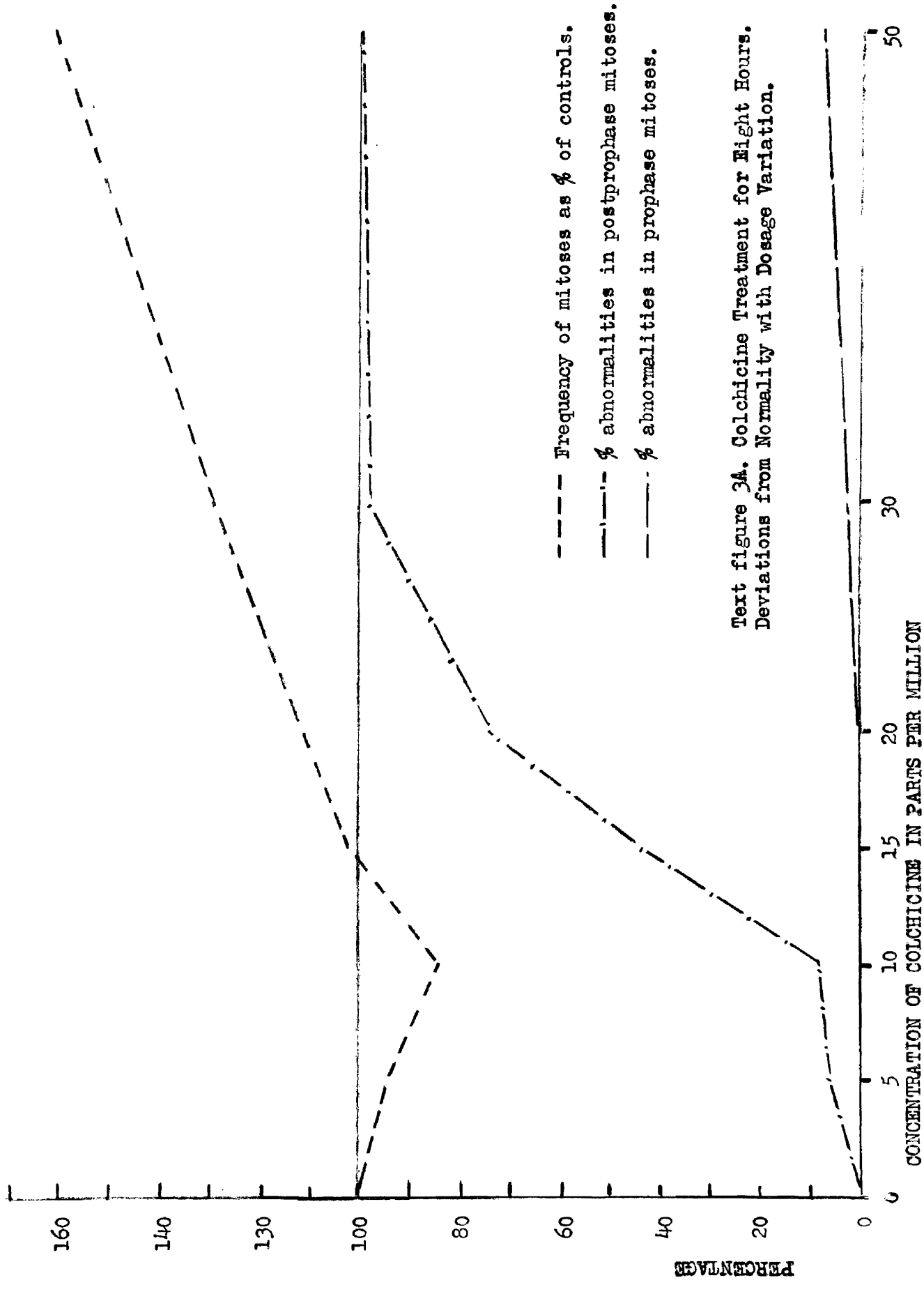
Text Figure 2A. Eight Hour Colchicine Treatment. Variation of Absolute Numbers of Mitoses with Dose.



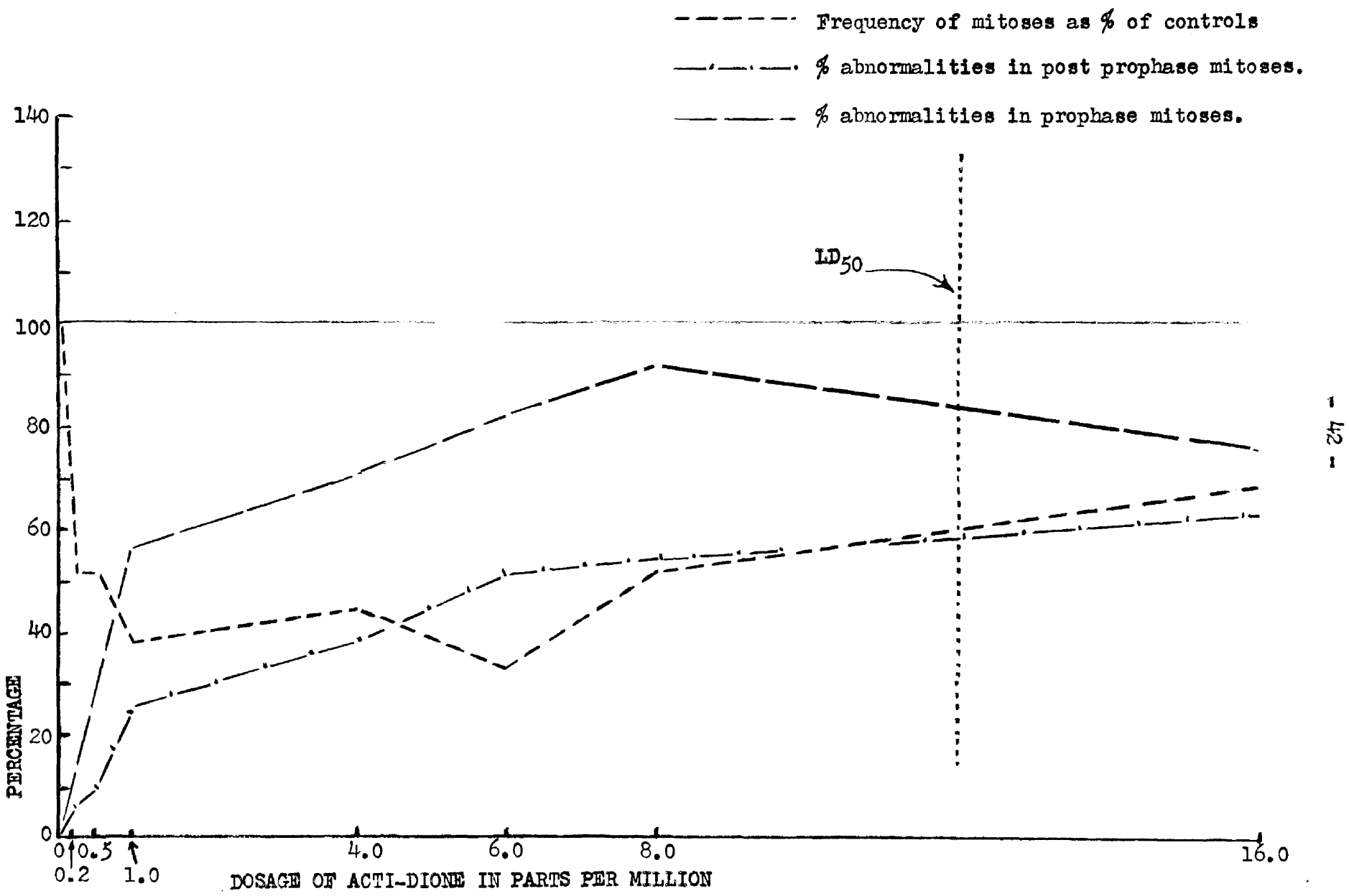
Text Figure 2B. Four Hour Acti-dione Treatment. Variation of Absolute Numbers of Mitoses with Dose.



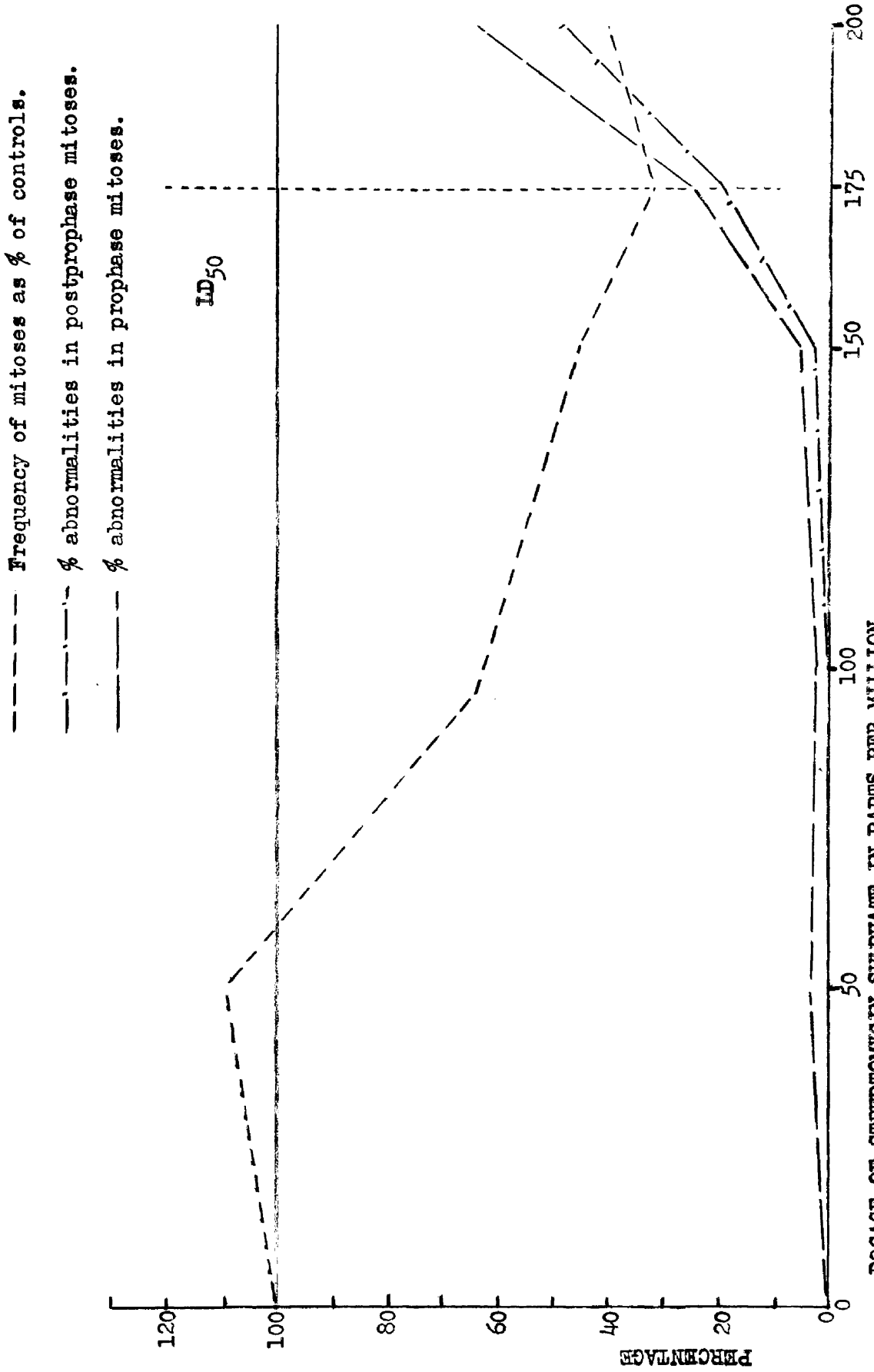
Text Figure 20. Eight Hour Streptomycin Treatment. Variation of Absolute Numbers of Mitoses with Dose.



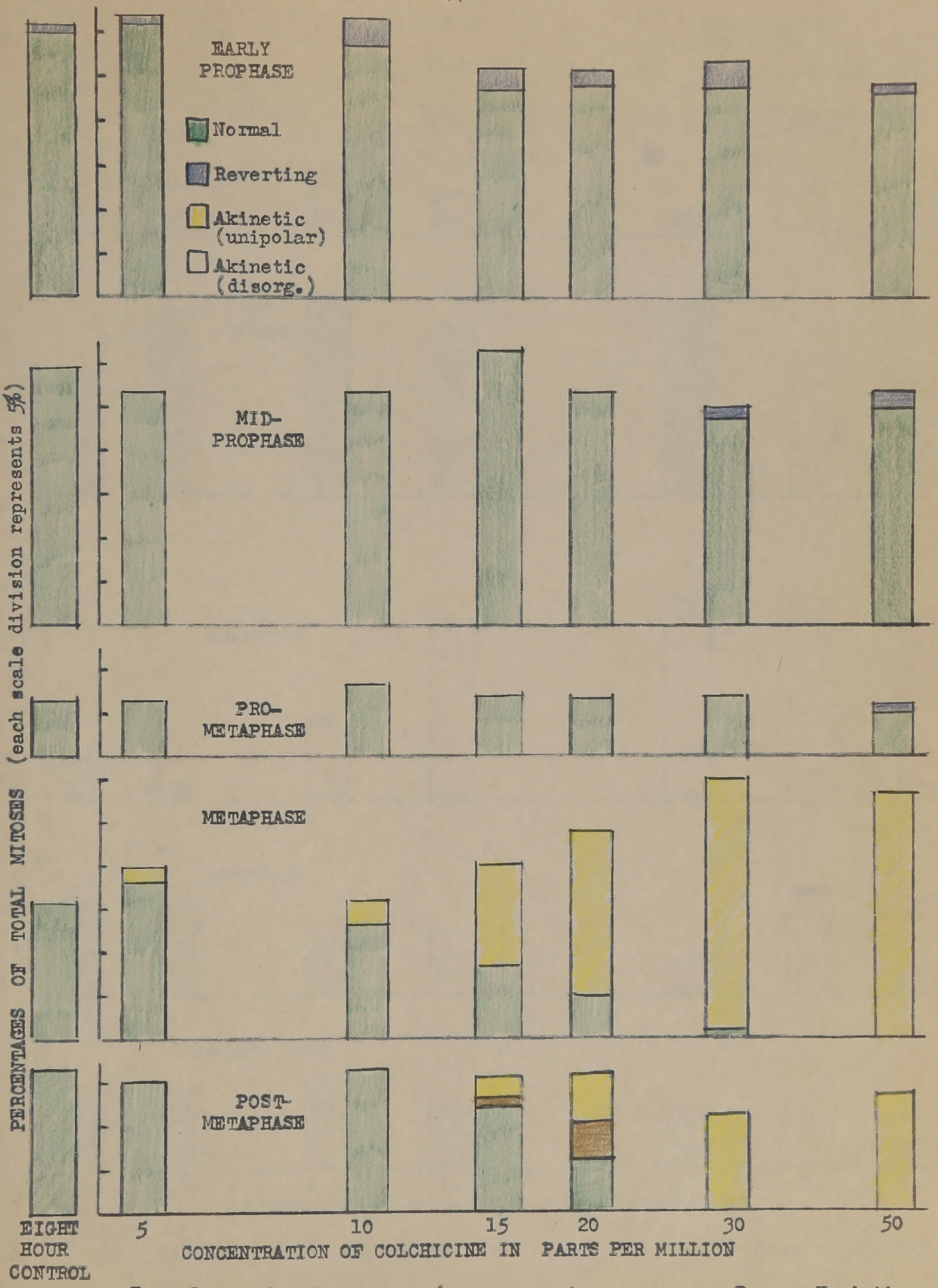
Text figure 34. Colchicine Treatment for Eight Hours. Deviations from Normality with Dosage Variation.



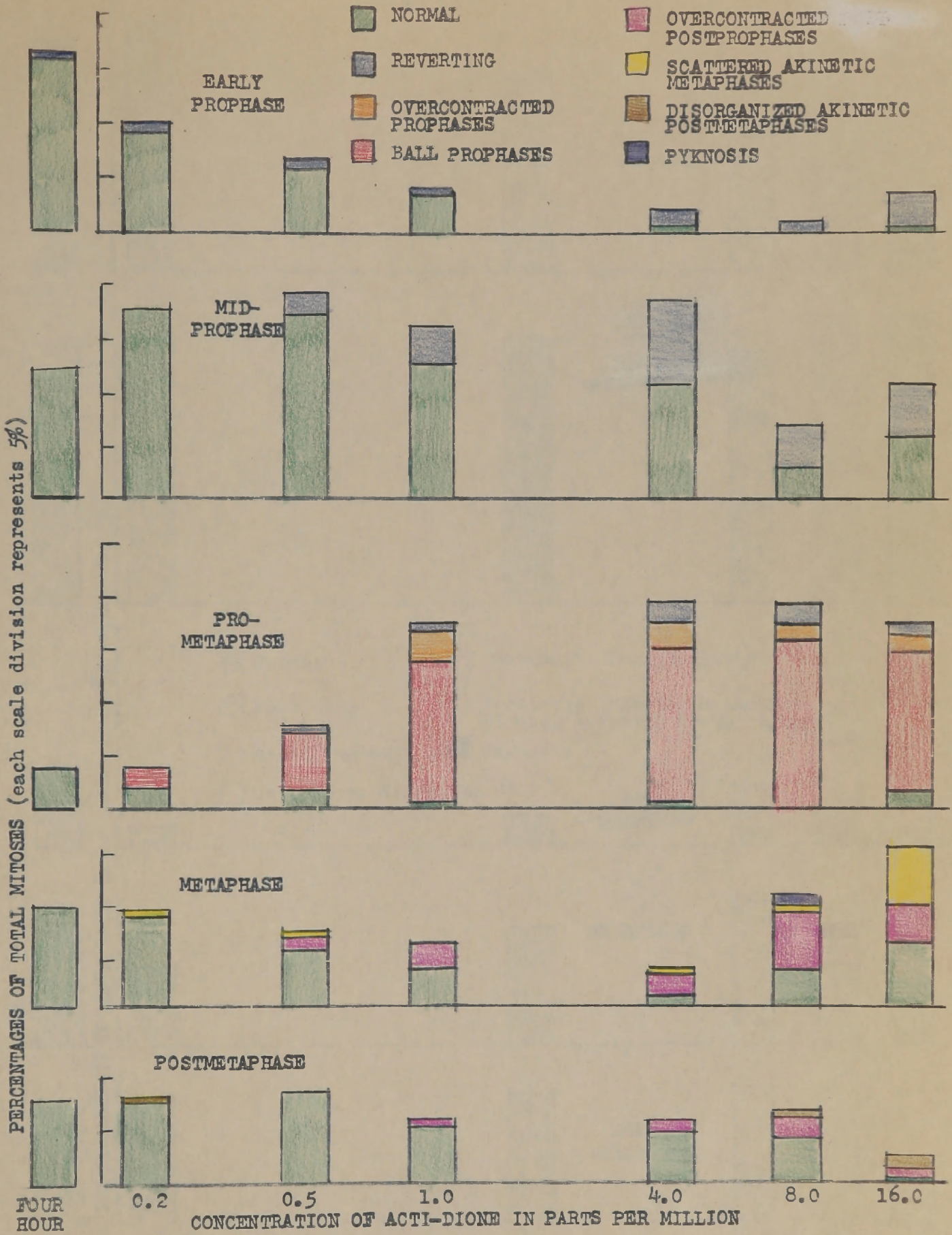
Text figure 3B. Acti-dione Treatment for Four Hours. Deviations from Normal with Dosage Variation.



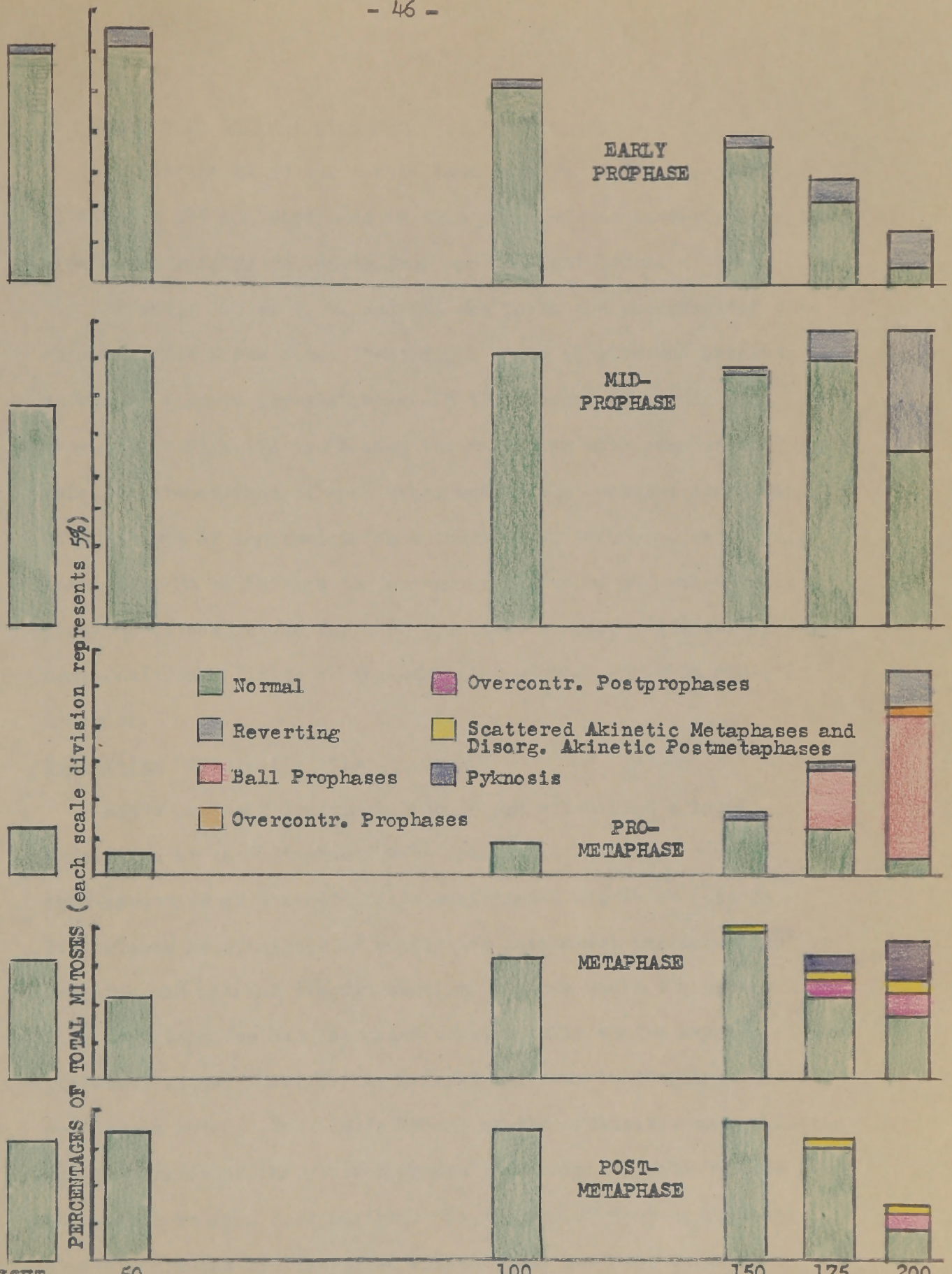
Text figure 30. Streptomycin Treatment for Eight Hours. Deviations from Normal with Dosage Variation.



Text Figure 4A. Colchicine (eight hours) Effects with Dosage Variation.



Text Figure 4B. Acti-dione (four hours) Effects with Dosage Variation



Text Figure 4C. Streptomycin (eight hour) Effects with Dosage Variation

E. Changes in Effects with Duration of Treatment

Colchicine at 50 ppm, Acti-dione at 6 ppm and streptomycin sulfate at 175 ppm were used in this phase of the investigation in treatments ranging in length from one to eight hours.

Appendix Tables V, VI and VII set forth the consolidated data obtained from these runs. Text figs. 5A to 5C give the absolute number of figures for each stage at the several times for each drug. Text figs. 7A to 7E show the variation with time of the relative proportions of each stage and of the relative proportions of each type of abnormality on a comparative basis for each drug. Text figs. 8A to 8E show the change with time of the relative proportions of each stage for each treatment compared with the 95 per cent confidence limits of the variation of each stage in control material.

Colchicine (Plate II, III)

After one hour treatment with 50 ppm colchicine a large proportion of the metaphases were seen to be akinetic and more than one-third of postmetaphases exhibited a degree of spindle disturbance as evidenced by multipolar anaphases, unequal groupings, laggards and general disorganization. By two hours the proportion of metaphases had increased substantially at the expense of the other stages, however, no general increase in division frequency was noted. Substantially all of the metaphases were akinetic and two-thirds of the postmetaphases were unipolar, the balance being more or less disorganized. At the end of four hours total division figures had increased almost fifty per cent and the

proportion of metaphases was double that in controls, accounting for most but probably not all of the increase in number of divisions. All metaphases were akinetic, mostly of the clumped type, whereas all postmetaphases were seen to be unipolar. At this point an equilibrium was apparently established and an additional four hours treatment brought about little change in the overall picture. No significant numbers of abnormalities or important shifts in relative proportions were observed in the prophase stages at any time during this treatment except for a moderate overall drop in proportions as metaphase increased its relative share of division figures.

Acti-dione (Plate IV)

The effect of 6 ppm of Acti-dione was immediate and drastic. Total division frequency dropped to less than two-thirds of control values by one hour, this drop continuing steadily with time until by eight hours there were about one-tenth as many divisions as in controls. Early prophases dropped similarly until by four hours all normal early prophases had disappeared. Ball prometaphases were much in evidence after one hour, and by two hours prometaphases had started to increase in proportion to all other stages which continued until, by eight hours, aberrant prometaphases of one sort or another accounted for more than seventy per cent of all division figures. The impression was one of constantly increasing numbers of prophases showing increasing degrees of overcontraction and disappearance of all other stages. Actually by the end of eight hours, in spite of the tenfold decrease in total numbers of divisions seen, more figures were classed as prometaphases

than in controls. Reversion of prophases was significant by two hours and reverting figures increased rapidly, becoming second only to various degrees of overcontraction as a major abnormality. Metaphases and postmetaphases, showing little if any spindle disturbance, became overcontracted and decreased in proportions even more than was necessary to account for the great increase in proportion of prometaphases. It will be noted that after eight hours treatment with this dosage no division figures were scored as normal, despite the fact that such root tips were noted to be recovered forty-eight hours after removal from treatment.

Streptomycin

As previously noted, streptomycin sulfate affected material shows the same overall frequency changes, kinds of aberrations and changes in relative proportions of atages as does material treated with Acti-dione, but generally with considerably less intensity of expression. This was substantiated in this phase of the investigation. Little of consequence was observed in material treated with 175 ppm streptomycin until four hours of treatment. During the last four hours of treatment total division frequency dropped, early prophase decreased and prometaphase increased in relative proportions. In prophases reversions became very frequent as did slight overcontraction as evidenced by ball prometaphases which increased with time. Metaphases and postmetaphases were little altered from controls except for moderate overcontraction, occasional spindle failures and a few pyknotic metaphases by eight hours of treatment.

General Summary

Text figs. 6A, 6B and 6C summarize in a general way the differences between colchicine, Acti-dione and streptomycin with respect to the effect of duration of treatment on

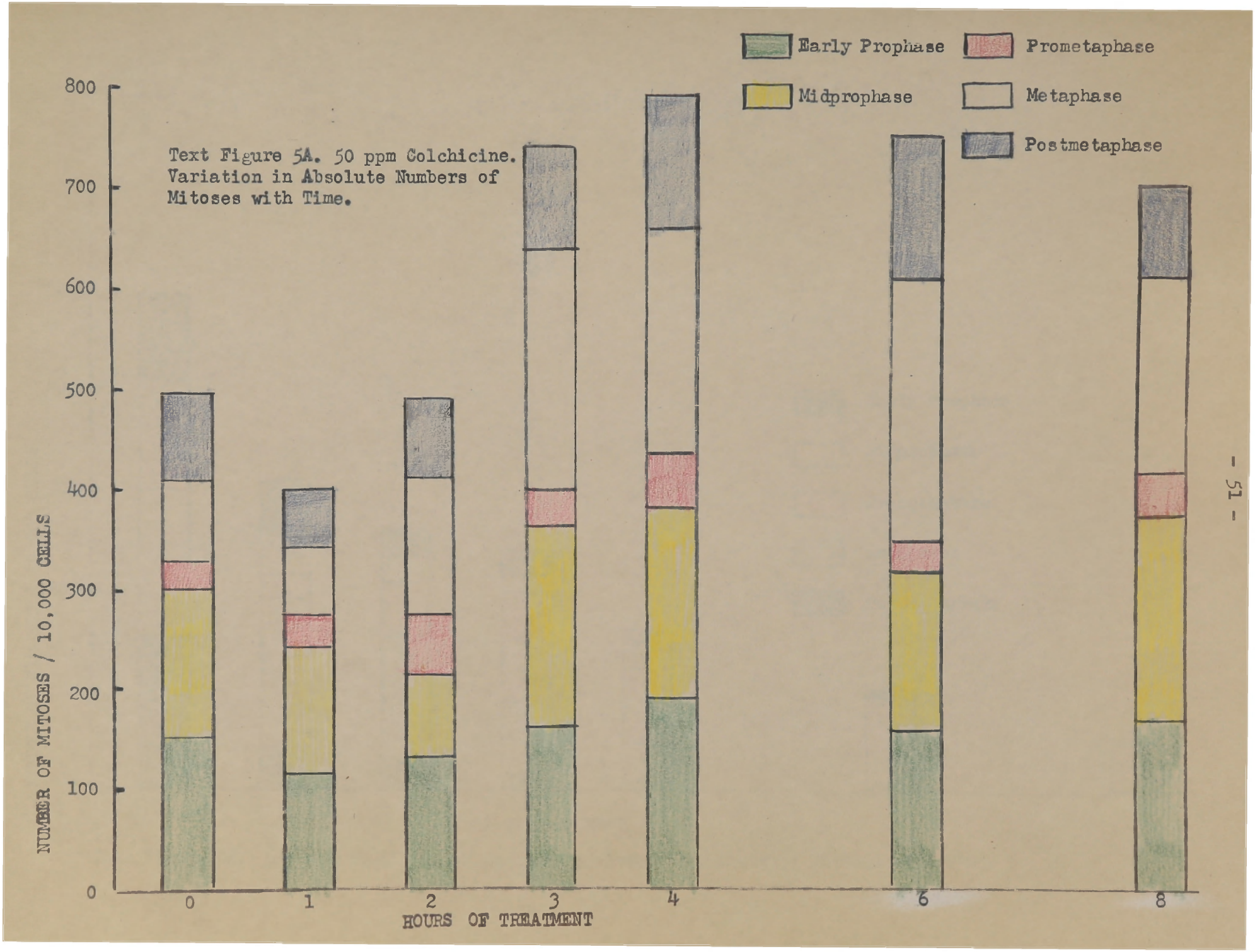
(a) the frequency of division figures,

(b) the proportion of abnormalities in total prophase and

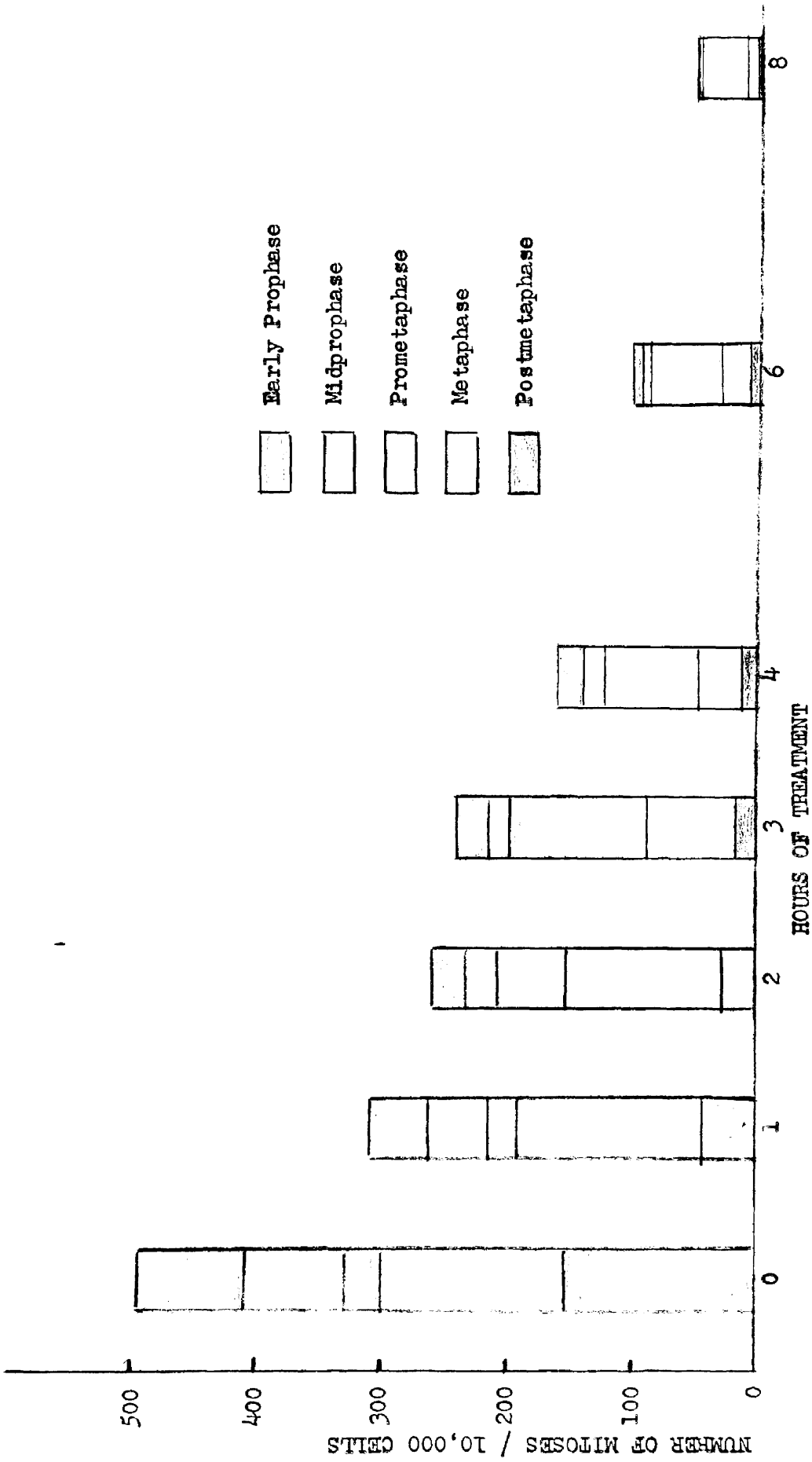
(c) the proportion of abnormalities in total postprophase.

Little can be said at this point to supplement these graphs and the information given in the preceeding sections, except to re-emphasize some fundamental differences between the action of these drugs at dosages below the lethal level.

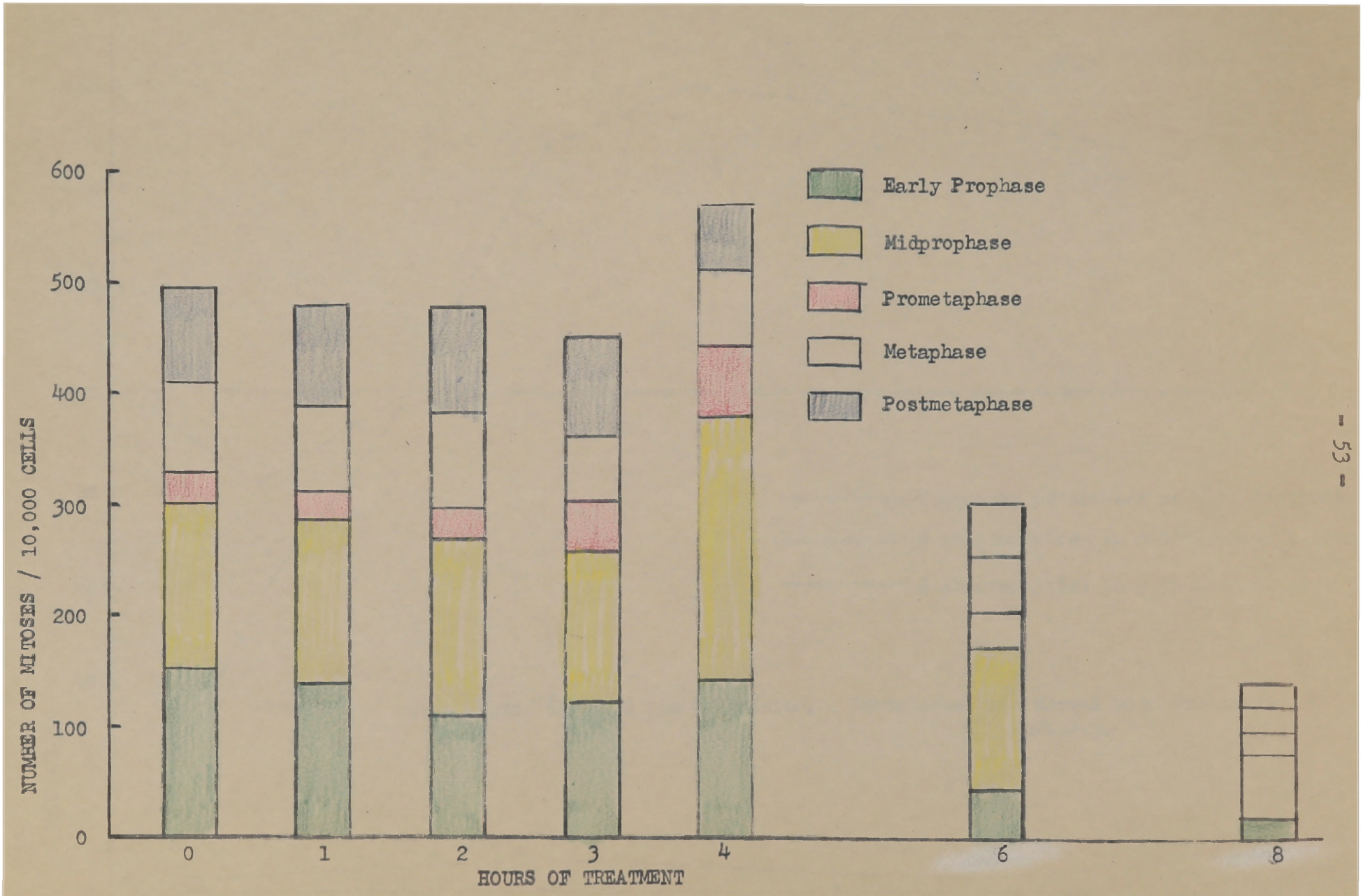
The effects of colchicine were found to be very different from those of Acti-dione, the former moderately increasing numbers of divisions and primarily affecting postprophase stages by causing deviants which can benerally be classified as spindle derangements. Acti-dione, on the other hand, causes extreme reduction in number of divisions and primarily affects prophase by causing effects that can generally be classed as reversions and various degrees of overcontraction. Streptomycin can be said to cause effects similar, but less in degree, to those caused by Acti-dione.



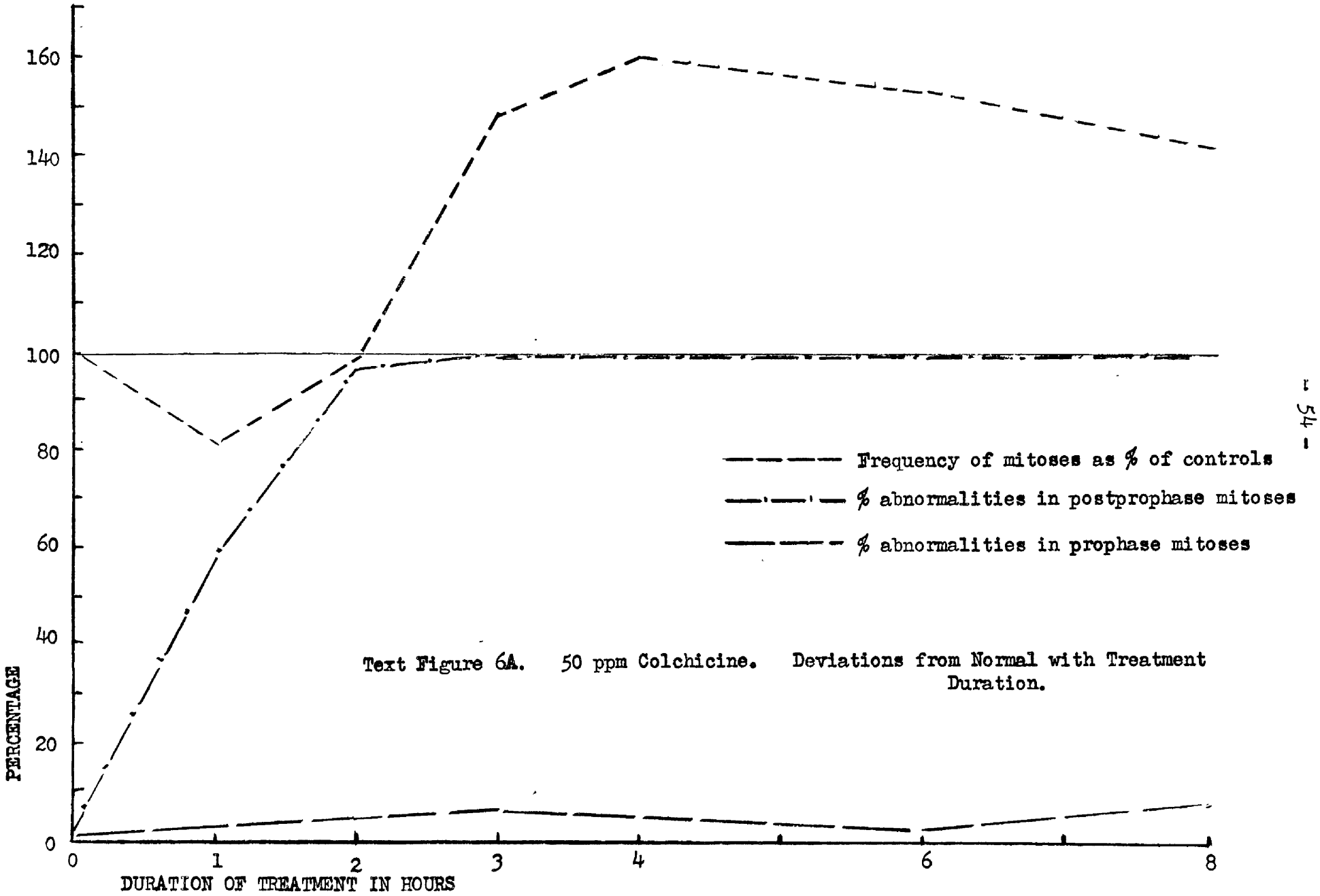
Text Figure 5A. 50 ppm Colchicine. Variation in Absolute Numbers of Mitoses with Time.



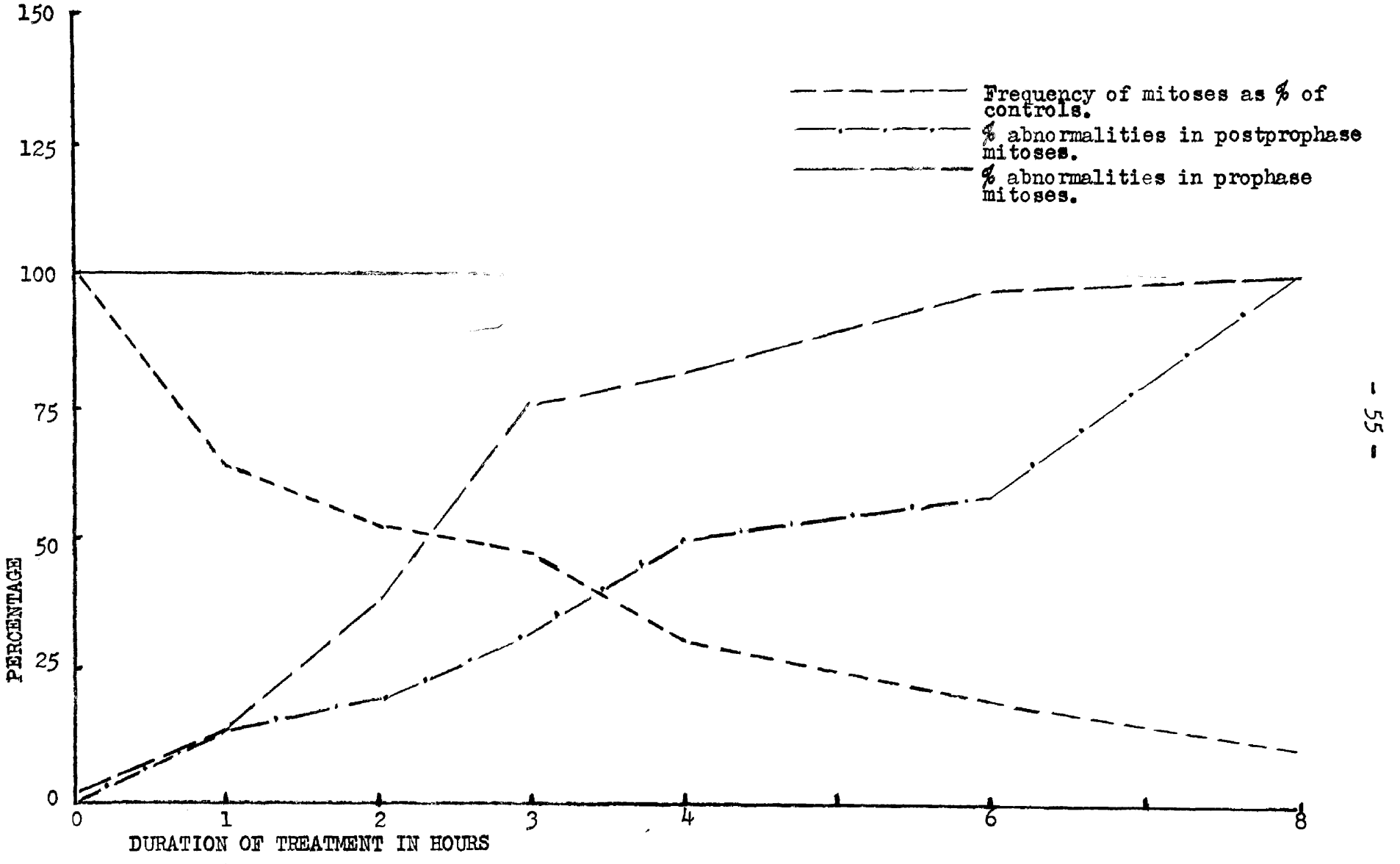
Text Figure 58. 6 ppm Actidione - Variations of Absolute Numbers of Mitoses with Time.



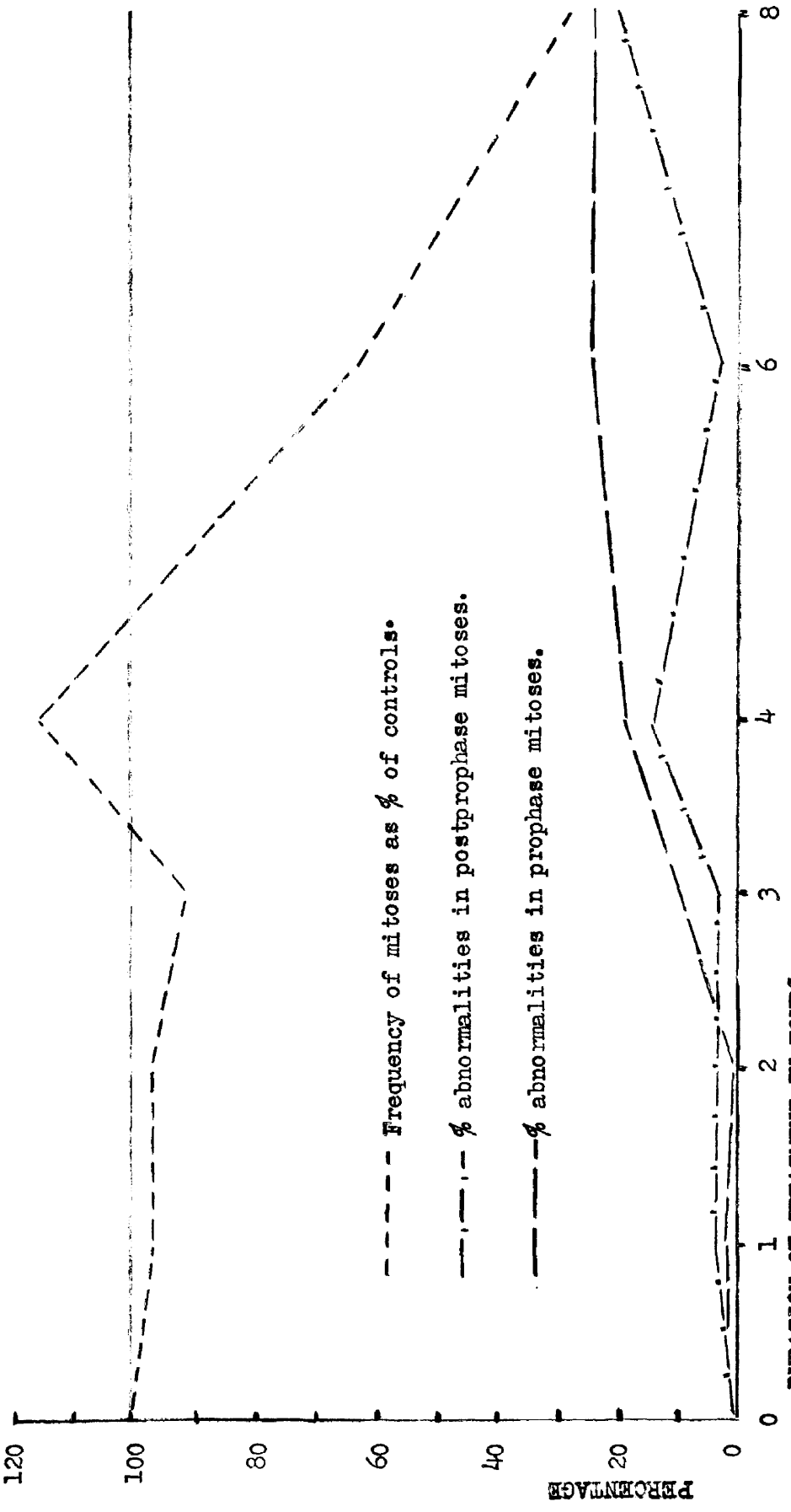
Text Figure 5C. 175 ppm Streptomycin Sulphate. Variations of Absolute Numbers of Mitoses.



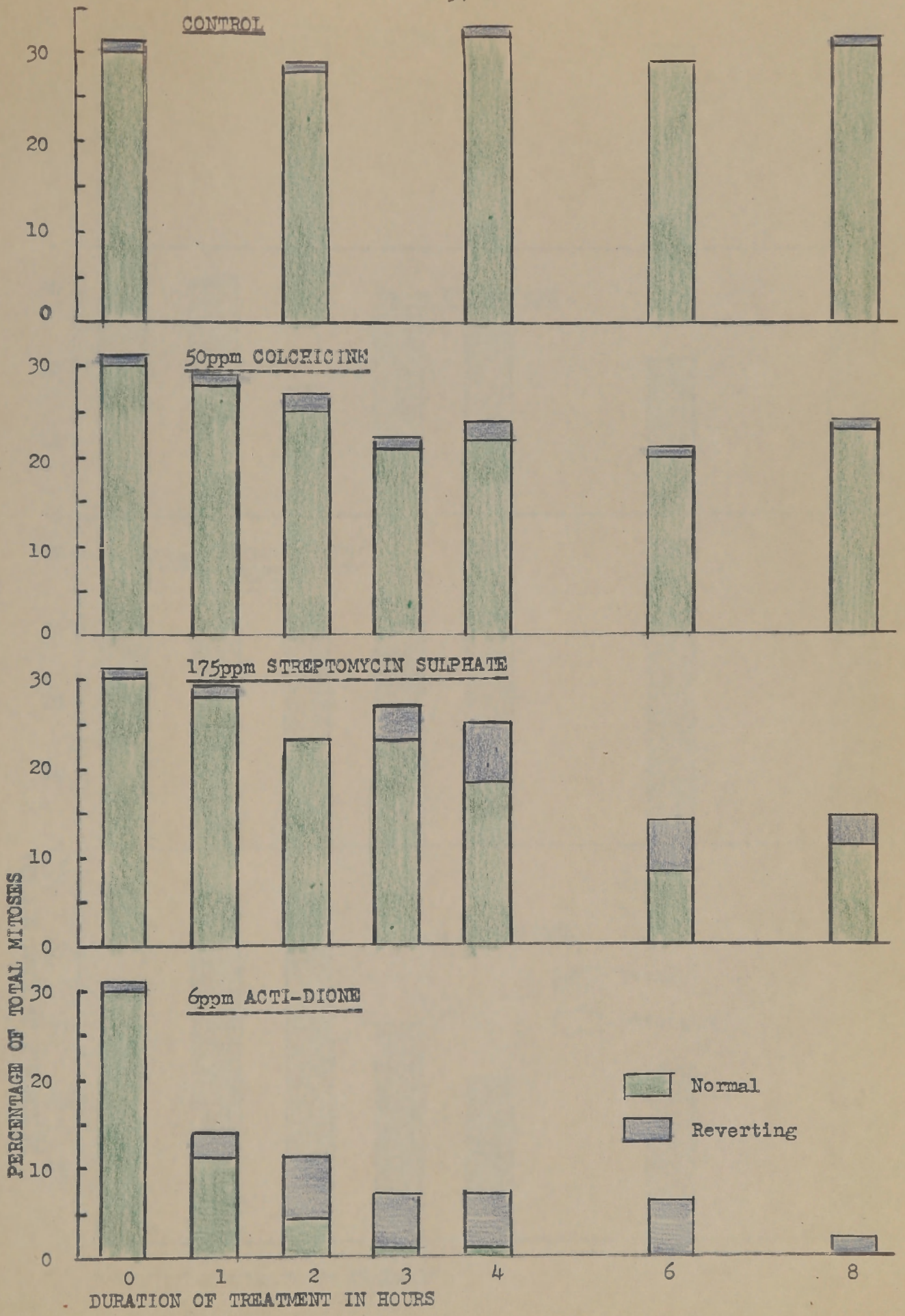
Text Figure 6A. 50 ppm Colchicine. Deviations from Normal with Treatment Duration.



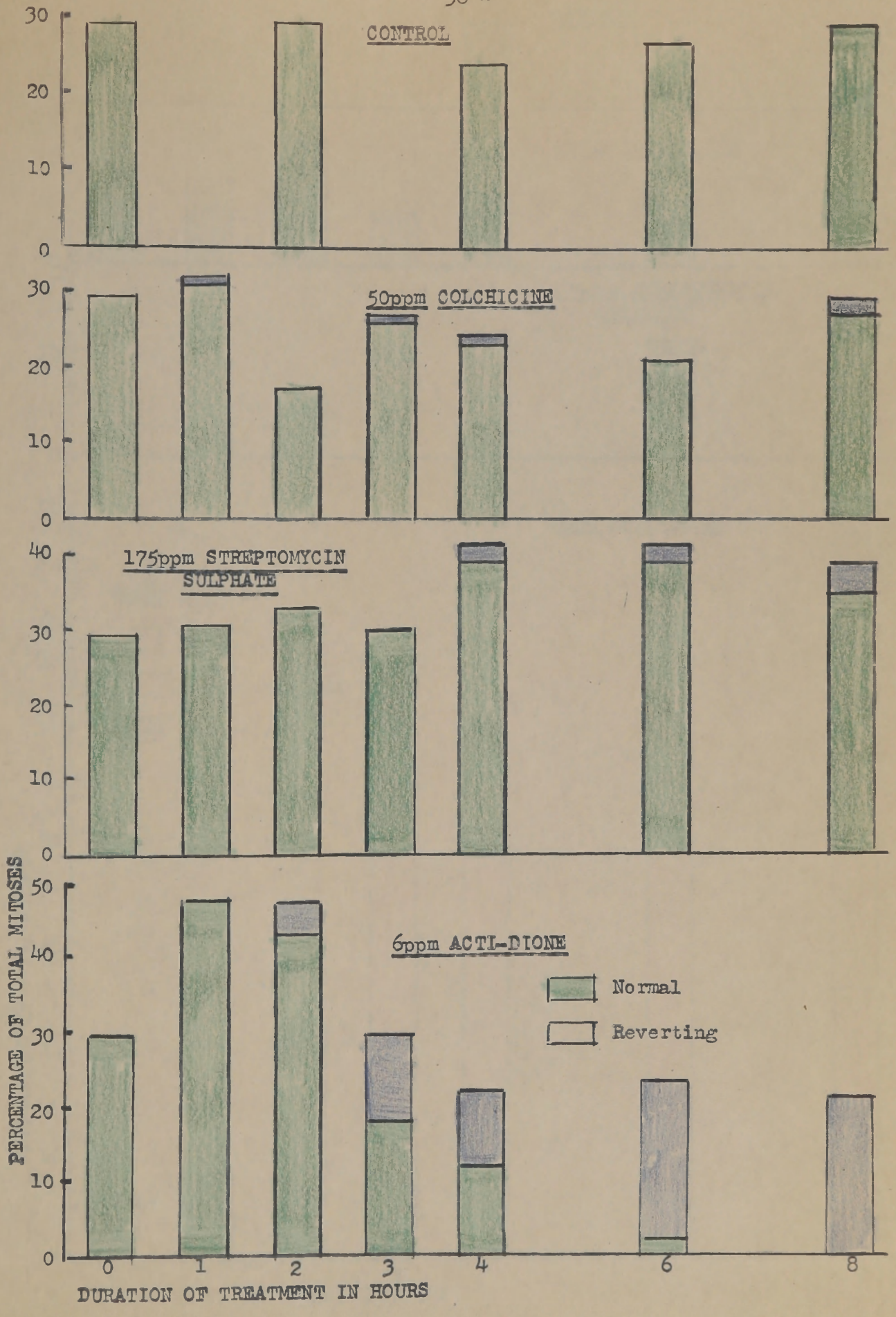
Text Figure 6B. 6 ppm Acti-dione. Deviations from normal with treatment duration.



Text Figure 6C. 175 ppm Streptomycin Sulphate. Deviations from Normal with Treatment Duration

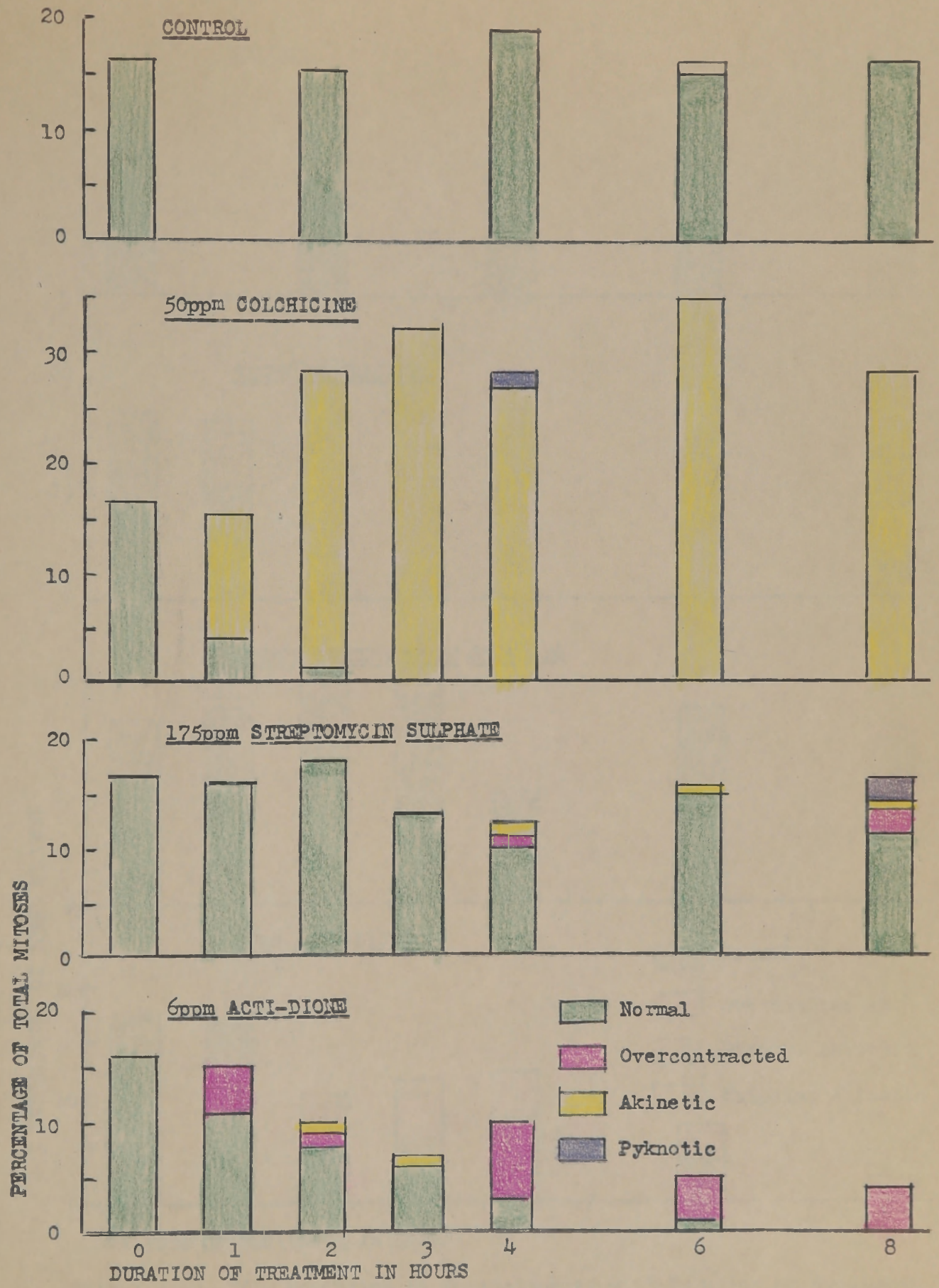


Text Figure 7A. Variations in Early Prophase with Duration of Treatment

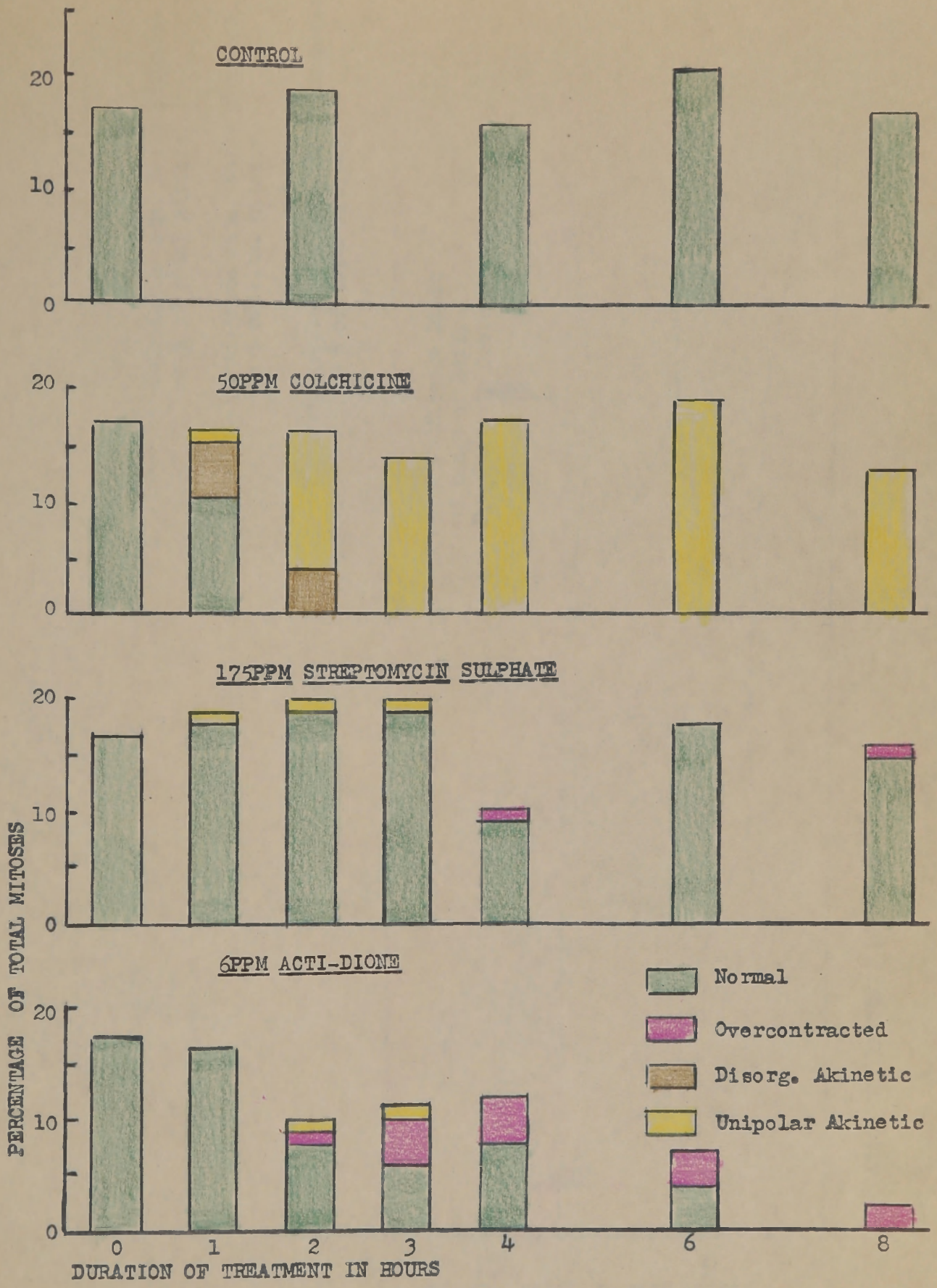


Text Figure 7B. Variations in Midprophase with Duration of Treatment.

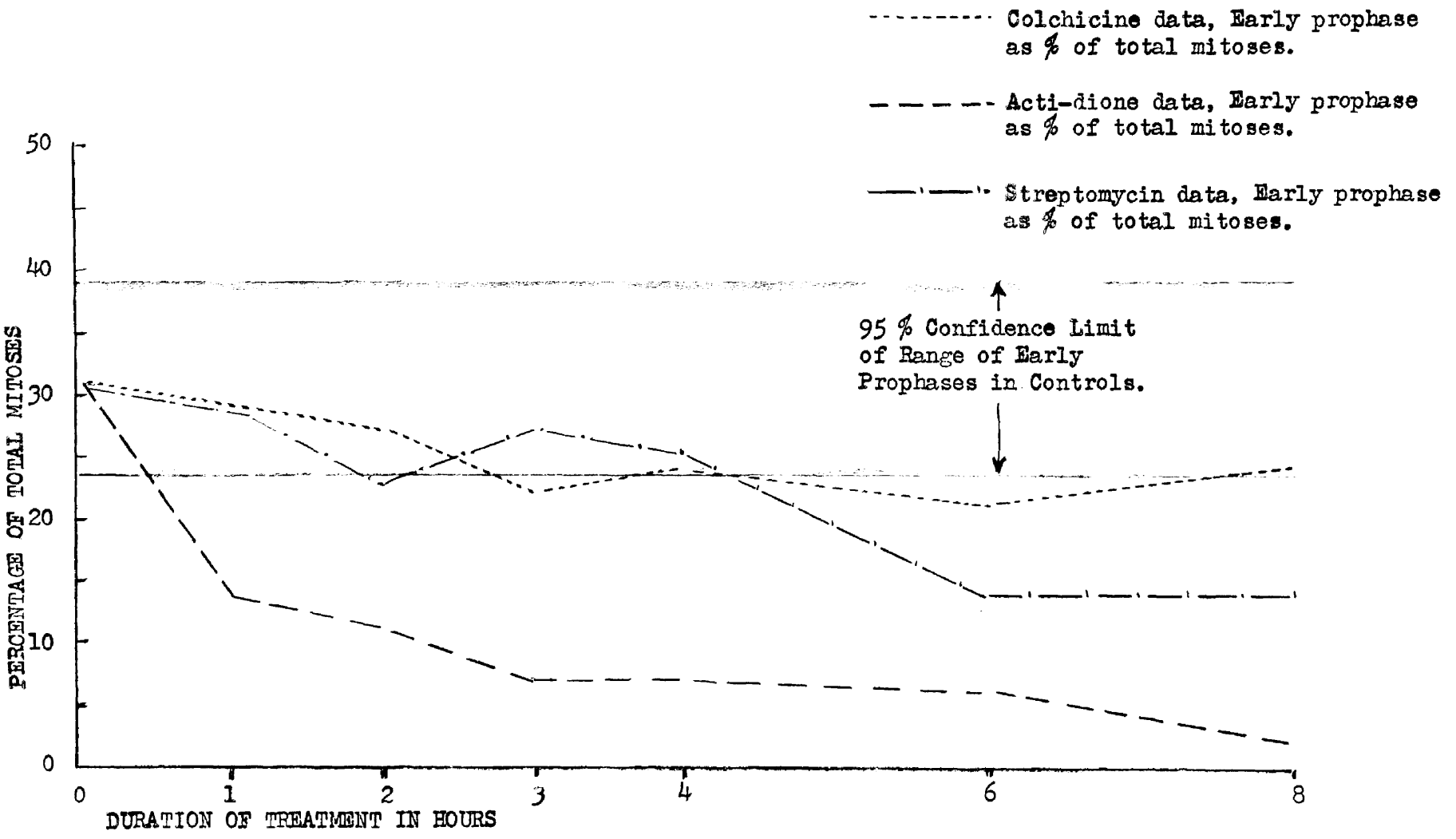
Text Figure 7C. Variations in Prometaphase with Duration of Treatment.



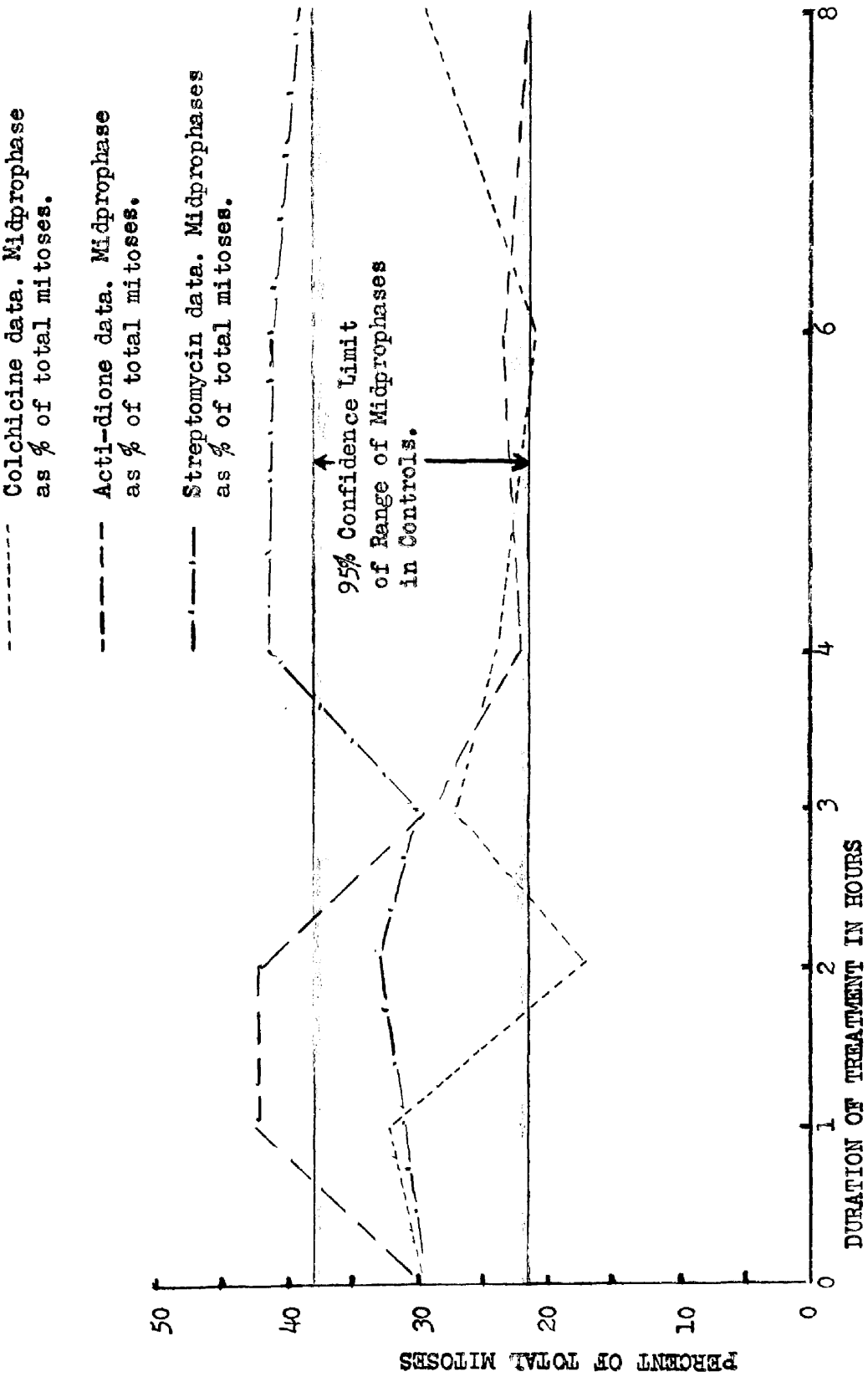
Text Figure 7D. Variations in Metaphase with Duration of Treatment.



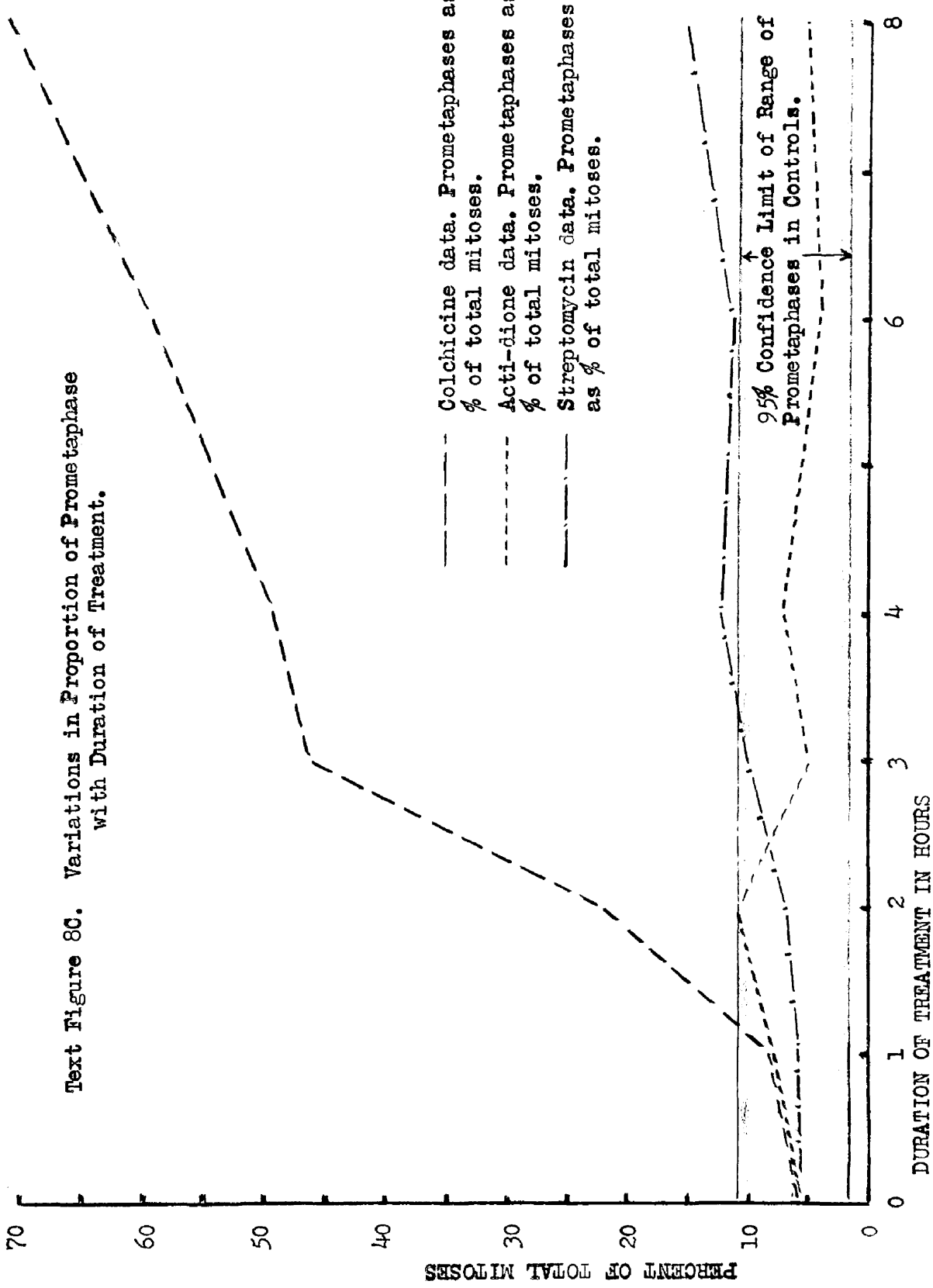
Text Figure 7E. Variation in Postmetaphase with Duration of Treatment.



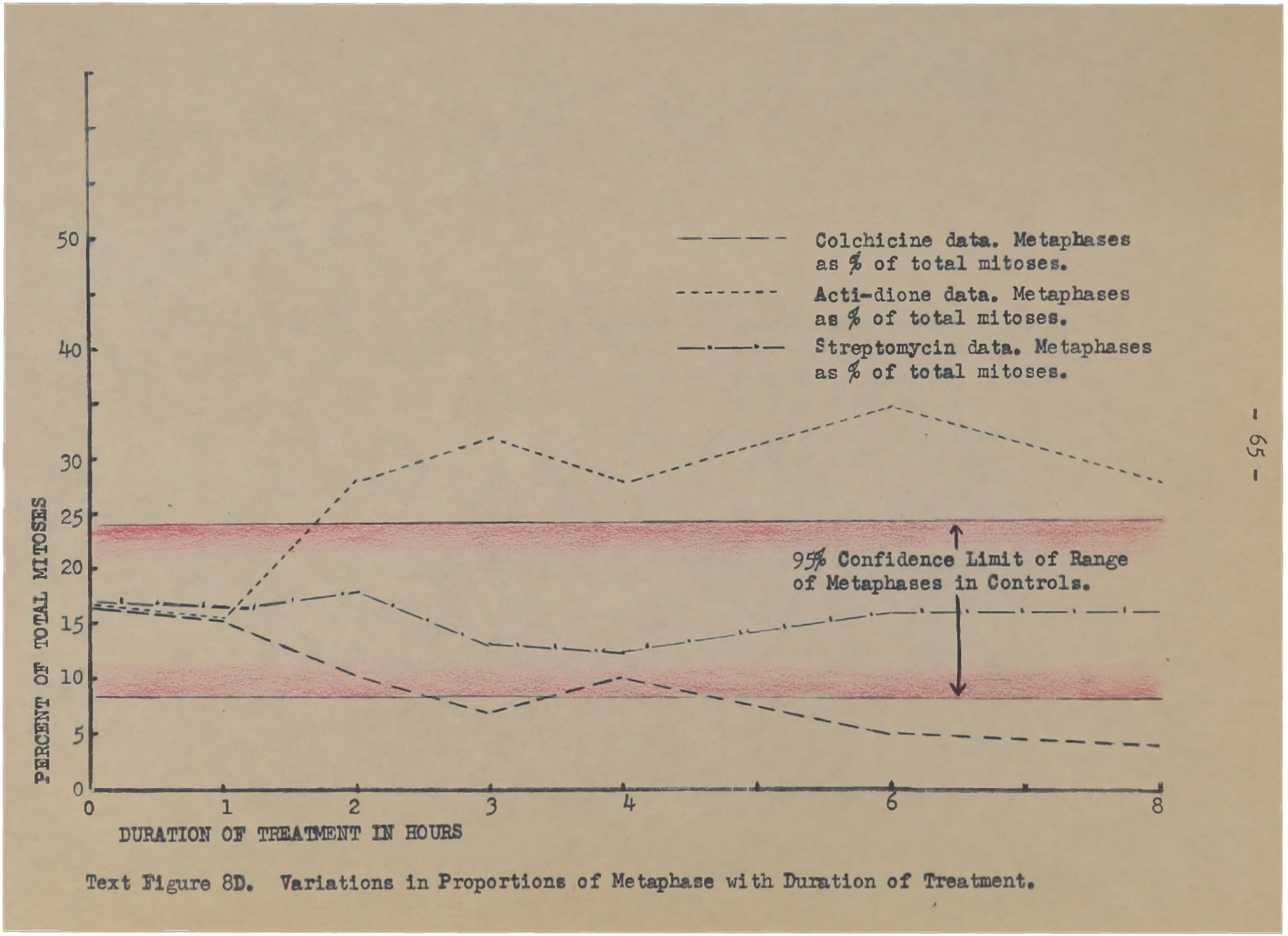
Text Figure 8A. Variations in Proportion of Early Prophase with Duration of Treatment.



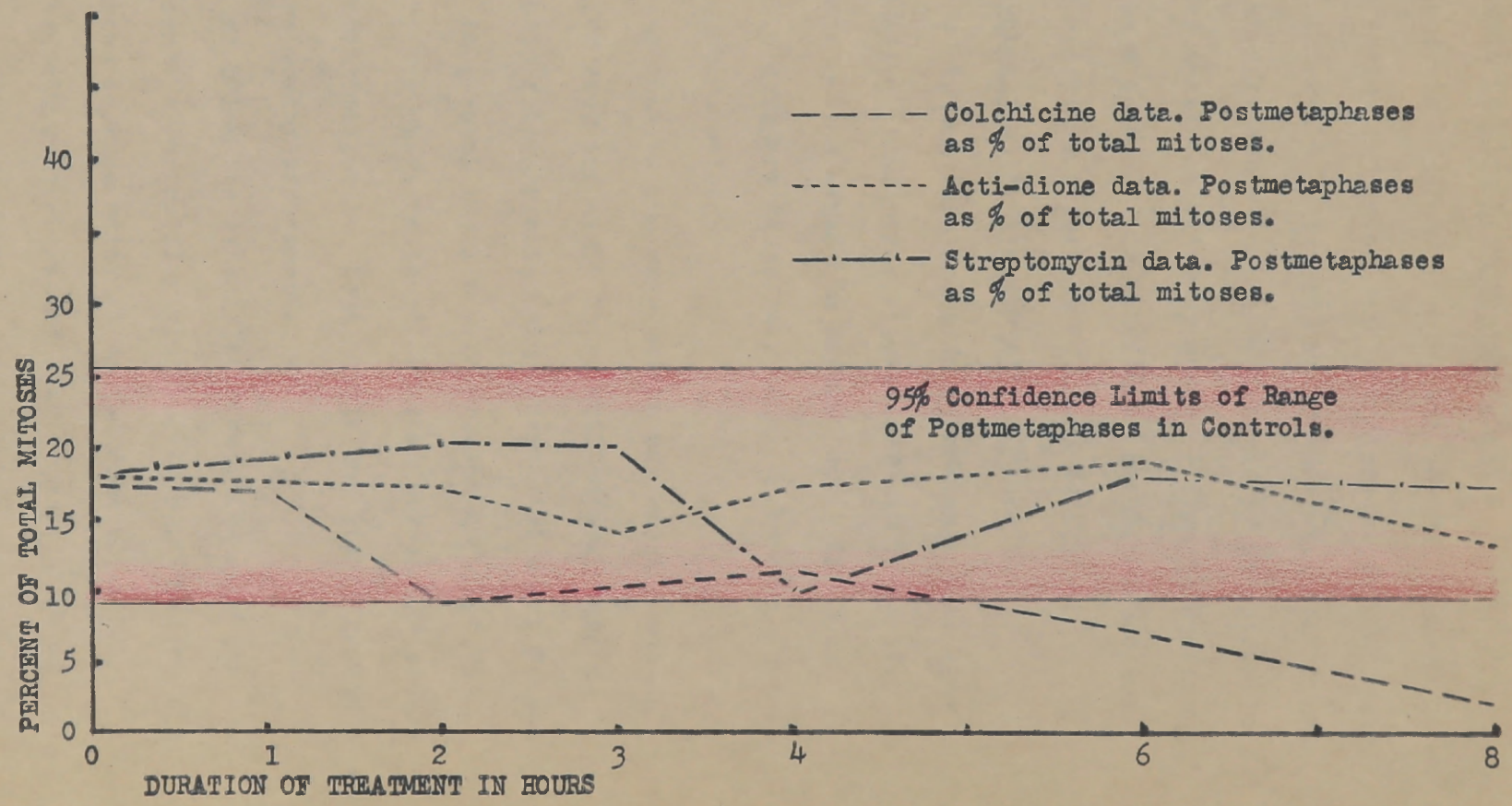
Text Figure 8B. Variations in Proportion of Midprophase with Duration of Treatment.



Text Figure 8C. Variations in Proportion of Prometaphase with Duration of Treatment.



Text Figure 8D. Variations in Proportions of Metaphase with Duration of Treatment.



Text Figure 8E. Variations in Proportions of Postmetaphase with Duration of Treatment.

F. Effects of Treatment with Dihydrostreptomycin

Table III lists the results of runs with several concentrations of dihydrostreptomycin sulfate with respect to cytological and lethal dosage thresholds. It will be noted that these thresholds do not vary significantly from those found for streptomycin sulfate when either distilled water or quarter-strength Hoagland solution is used as a solvent. Qualitatively no difference was noted between the cytological effects of these two drugs.

G. Effects of Treatment with Chloromycetin (Plate VII)

Cytological and lethal thresholds for treatments with synthetic and fermentation chloromycetin are listed in Table III. As far as these data go the effective dosage levels for both preparations of this drug are identical. Qualitatively no cytological differences were evident.

The bulk of the qualitative examination was carried out on material treated for four and eight hours with several concentrations of synthetic chloromycetin using quarter-strength Hoagland as solvent. In general results were erratic and root tips treated identically for the same length of time frequently seemed to give very different patterns. Some fairly consistent trends did emerge that are worthy of comment.

At dosages just below the lethal level about half of the meristems examined showed few or no figures in division. The few divisions that were noted in such material ranged from normal in appearance to moderately overcontracted with occasionally akinetic and unipolar postprophases as well as occasional pyknosis at all

stages. The divisions in the root tips with normal frequency of divisions appeared little different than those in control material except for occasional overcontracted and akinetic figures which generally appeared in conjunction with patches of more or less pyknotic tissue. Cells in such areas resembled pyknotic figures in streptomycin very closely; however, the impression was strong that this necrosis was somewhat less severe than that caused by streptomycin.

Recovered material likewise was difficult to evaluate because forty-eight hours after removal of the drug one would find occasional patches of pyknotic material with the wide variety of grotesque division figures that this entailed. Strangely enough, even at the highest dosages tested it was not unusual to find an occasional very small patch of normally dividing tissue in root tips that were scored as "not recovered".

DISCUSSION

The Pisum sativum Test

In general it may be said that the techniques employed in this investigation produced consistently reproducible data with respect to the cytological effects of certain drugs on meristematic tissue of Pisum. In view of the problems that are encountered in obtaining healthy, uniform, rapidly dividing Allium root tips throughout twelve months of the year, this technique warrants further attention in the search for a standard cytological test. With this method recovery from far stronger treatments can be obtained, and lethal thresholds can be determined with greater precision than with the usual Allium technique. These facts plus the ability to produce an abundance of highly uniform, rapidly dividing root tips at will, seems to more than offset the disadvantages of the relatively small size of the cells and chromosomes in Pisum, and the extra care necessary to insure a good preparation of this material. Vicia faba has the advantage of much larger cells and chromosomes; however, the very bulky, relatively slow germinating and growing seedlings are rather susceptible to fungal and bacterial infections, and cannot be as readily and quickly produced in large numbers as with Pisum.

Colchicine Effects

Colchicine when applied to root meristems of Pisum (at approximately twice the minimum dosage necessary to produce polyploidy after eight hours of treatment) primarily affects metaphases and

postmetaphases. By eight hours of treatment substantially all postprophase figures are akinetic. Metaphases increase in proportion to the other stages. Absolute numbers of divisions were increased. Since this increase could not entirely be accounted for by the increase in proportion of postprophase figures, an actual stimulation of mitosis is suggested as possible.

As has been often reported, colchicine appears to produce its effects by a specific impairment of spindle function. In pea root meristems, the chromosomes appear to proceed through prophase normally as far as prometaphase. The arrangement of chromosomes in a typical tight prometaphase clump persists through the balance of the process, chromatids falling apart in situ and then, without altering position, pass through a typical telophasic relaxation of coiling and dispersion of chromatic material to form a polyploid resting nucleus. This sequence of events may well be illustrated by a series of cells as shown in Plate II, Figs. 1, 2, 3, 4, 8, 12 and 16.

That the impairment of spindle function is not necessarily an all or none process is demonstrated by the fact that threshold dosages result in what appear to be weak or erratic spindles with consequent higher incidence of micronuclei. It will be noticed that dosage at the rate of 15 and 20 ppm for eight hours results in significant numbers of disorganized postmetaphase figures (Text fig. 4A) and it has already been pointed out that these concentrations resulted in the highest numbers of micronuclei. Such a sequence might well be represented by a series of

cells as are shown in Plate II, Figs. 1, 2, 3, 4, 5, 9 or 10, 13 or 14, and Plate III, Fig. 1 or 2.

Since the prophase sequence at all dosages tested is apparently little changed from that of controls (Text fig. 4A) it seems probable that the component of the spindle directing movement to an equatorial plate and that component directing anaphase movement are either both part of the same fundamental process or else are separate processes which are both equally affected by colchicine. The former seems the most likely conclusion to be drawn since there is no evidence that any of the drugs tested in this investigation caused any differential effects on these two theoretical spindle forces. The fact that, at threshold doses, the tight prometaphase organization frequently gave way to scattered akinetic metaphases rather than clumped akinetic metaphases seems to imply at least a degree of plate organizational forces at work. When such figures were abundant, a high percentage of disorganized anaphases could be also observed, thus implying at least a degree of anaphase movement spindle forces at work. Similarly, when substantially all metaphases were of the clumped akinetic type, substantially all of the postmetaphases were scored as unipolar implying that both spindle components were inactivated. It is difficult to find evidence in the literature that is valid on this point. Unfortunately most of the work with colchicine has been done at far greater dosages than was used here, and it is entirely possible that some of the reported effects of colchicine and other true

"C-mitotic" drugs may represent in part the effects of toxic, sub-lethal dosage.

For the latter reason, it is also difficult to compare the data obtained in this study with that of other workers in respect to changes in frequency of divisions. For example, Guttman (1952) reported a decrease in absolute numbers of prophases in Allium material treated for twenty-four hours with 500 and 200 ppm. colchicine which was grouped with material treated twenty-four hours with saturated acenaphthene solution. This may or may not represent a toxic effect of her treatments; however, such a possibility cannot be dismissed. The fact that her conclusions indicate that only eighty-five per cent of metaphases proceed to interphase without passing through normal postmetaphase stages suggests some sort of fixation effect. This possibility is heightened when one considers that in our study far lower dosages and far shorter duration of treatment caused substantially all metaphases to pass directly to the resting condition without anaphase-telophase sequence. Also she reports a doubling of the time required for a cell to complete the whole division cycle. That means a far lower rate of initiation of mitosis and suggests a depressant effect of her treatments. In this investigation nothing of the sort has been noted; in fact it seems likely that the total length of the division cycle in Pisum may be somewhat shortened by 50 ppm colchicine, thus increasing the rate of initiation of mitosis.

Acti-dione Effects

It was noted that, in almost every respect, the effects of Acti-dione at cytologically effective but non-lethal dosage levels are diametrically opposed to colchicine effects. Acti-dione primarily affects prophase causing a tremendous pile-up of overcontracted prometaphase figures in which contraction has proceeded to metaphase length or shorter and where the nuclear "membrane" remains intact. Reversions are frequent, but akinetic figures are not present in significant numbers. The most marked difference is the tremendous drop in frequency of divisions, early prophases becoming practically non-existent by eight hours of treatment.

Acti-dione is a typical "cell poison" in the D'Amato sense. Preprophase inhibition of mitosis at 6 ppm is substantially one hundred per cent after approximately one hour of treatment. Prophases are never able to reach metaphase, and despite extreme contraction chromosomes remain in midprophase arrangement within an intact nuclear membrane. Reversion of prophases to an "artificial interphase" (Levan, 1952) is substantial in amount and may occur at any stage of prophase. Postprophase stages, unlike those in colchicine treated material, show little that is striking in the way of aberrations. Some overcontraction is seen in these stages, but no significant amount of spindle disturbance. Postprophases decrease in total proportion of divisions due to the failure (of at least the great majority) of prophases to pass into metaphase. The sequence of events in prophase may well be

illustrated by such a series as shown by Plate IV, Figs. 1, 3, 8, and 10 with reversions at all stages of prophase shown by Plate IV, Figs. 2, 5, 11 and 12.

Streptomycin Effects

The effects of streptomycin at non-lethal levels appear to bear little resemblance to colchicine effects. On the other hand, streptomycin, like Acti-dione, answers D'Amato's description of a "cell poison", showing effects qualitatively identical with those produced by Acti-dione but considerably weaker from a quantitative point of view. This is seen most strikingly when a comparison is made of Text figs. 3A, B and C. It is apparent that there is little margin between streptomycin dosages that cause marked cytological effects and dosages that cause necrosis as evidenced by appearance of pyknosis. There is only one apparent exception to this conclusion, and that is that preprophase inhibition of mitosis by streptomycin appears significantly at dosages lower than those required to produce cytological effects. Cytological effects with streptomycin might well appear if, for example, treatment with 100 and 150 ppm streptomycin were allowed to continue longer than the eight hours used in this investigation. However, it seems more reasonable to postulate that preprophase inhibition of division, at least as caused by streptomycin, differs fundamentally from the reactions preventing prophases reaching metaphase and causing reversion. The possibility that this may also be the case with Acti-dione is suggested when one examines the data with respect to a dosage of 0.2 ppm and 0.5 ppm as summarized in Text fig. 3B.

While the quantitative information on which these conclusions are based was obtained through the use of streptomycin sulfate, the effects of dihydrostreptomycin sulfate showed no fundamental deviations therefrom and as far as comparisons were made, it seems reasonable to assume that the two drugs are identical in effect.

Chloromycetin Effects

While no quantitative data are at hand with respect to the detailed effects of chloromycetin, it seems apparent that, except for rather erratically expressed inhibition of divisions, clear cut cytological effects of the type produced by the other drugs tested are masked by necrotic changes at all concentrations of the drug able to effect such change. It can be stated that, compared to **Acti-dione** and streptomycin, chloromycetin on a concentration basis is considerably less toxic. However, the threshold of cytological effectiveness is no lower than the threshold of lethality.

The necrotic effects of chloromycetin appear to involve the poisoning of local sectors of meristems rather than a systemic poisoning of the entire meristem, as seems to occur with Acti-dione and streptomycin. Until further investigation is made and many more data are obtained, speculation concerning this apparent difference between chloromycetin and the other drugs seems fruitless.

As far as study was carried out, as might be expected, synthetic and fermentation preparations of chloromycetin appeared identical in their action.

Salt Effects

One of the most striking phenomena noted in this study was

the tremendous degree of antagonism shown by certain mineral salts to streptomycin action. Effective thresholds of the drug were raised by a factor of approximately twenty in going from distilled water to quarter-strength Hoagland solution, and by approximately eighty between distilled water and half-strength Hoagland. Similar salt effects have been reported as antagonizing antibacterial activity of streptomycin in vitro (Pratt and Dufrenoy, 1949). This immediately suggests that a close relationship exists between the antibiotic properties of this drug and the effects we have noted on plant meristems.

As to the significance that this phenomenon has with respect to the mode of action of streptomycin, one can only speculate that either the salt effect is one of decreasing the permeability of the plasma membrane for streptomycin or that the effect involves an alteration of the colloidal properties of protoplasm rendering it directly or indirectly more resistant to the action of the drug.

A salt effect was noted with Acti-dione and chloromycetin, but was not nearly as marked as with streptomycin (a factor of between two and four for Acti-dione and about two with chloromycetin between distilled water and quarter-strength Hoagland solution). Without much more information there is little to be gained in speculating whether or not this has the same basis as salt antagonism of streptomycin. An important observation, however, was that variations in salt concentration did not alter the effective thresholds of colchicine. This fact permits one to

postulate a fundamental difference in mode of action of colchicine compared to the other drugs tested.

C-mitosis

The term "C-mitosis" originally was intended to apply to the altered mitotic pattern caused by colchicine (Levan, 1938). Today, as already pointed out, it is used by most workers to describe any alteration of mitosis where spindle control of postmetaphase chromosomes is impaired. One suspects that highly contracted prometaphases as produced by extended treatment with Acti-dione (Plate IV, Figs. 9 and 10) may frequently be reported as "C-mitotic" by the casual observer. The careful workers in this field have already pointed out (D'Amato, 1949; Hawthorne and Wilson, 1952: Levan, 1952 and others) that, despite the relatively similar appearance of metaphases in a number of different treatments, the fundamental causes and mechanisms may be quite different.

An advantage of the use of Pisum root tips and the technique herein described is that recovery of treated root tips is obtained at a much higher level of effect than with Allium material. Lethal concentration thresholds can be rather precisely determined and, contrary to previous reports (Wilson, 1950; Wilson and Bowen 1951; Wilson and Hawthorne, 1952), these were found to be higher than the thresholds of visible cytological effects in the case of Acti-dione and streptomycin. This precision of determination of non-recovery levels of dosage has led to two observations that may be of fundamental interest. First of all, recovery of tissue was not obtained at any concentration level producing significant

quantities of pyknosis. Secondly, recovery, except with colchicine, was not obtained at any concentration level producing significant quantities of postprophase figures showing spindle impairment.

It is therefore suggested that spindle impairment caused by typical "cell-poisons" is the result of lethal processes, perhaps of "poor fixation", and might well be an early stage in pyknotic degeneration. It may be further pointed out that spindle derangement caused by non-recoverable dosages of Acti-dione (Plate V, Figs. 2, 3, and 4), streptomycin (Plate VI, Figs. 8 and 15) and chloromycetin (Plate VII, Figs. 4 and 5) presents a very different gross appearance compared to that caused by effective polyploidizing dosages of colchicine (Plate II, Figs. 5 through 16). Examination of Pisum root tips treated with polyploidizing concentrations of cyclochlorohexane should prove of interest in this respect.

It is proposed that the use of the term "C-mitosis" and the consequent term "C-metaphase" be reserved for spindle disruption caused by the few true polyploidizing materials. Thus before spindle impairment could be labelled "C-mitosis", it would have to be shown that it was a) a highly specific reaction, b) accompanied by little or no prophase disturbance and, c) productive of substantial amounts of polyploidy. A general term (without any polyploidizing implications) should be used to describe spindle disturbance regardless of cause. "Akinetic mitosis" is suggested as a suitable term to describe all sorts of spindle disruption and "akinetic metaphases" or "akinetic postmetaphases" might be used to describe figures showing spindle disturbance regardless of

cause. This ought not in any way to interfere or be confused with the present term "akinetic chromosome" in limited use synonymously with "acentric chromosome" to describe a chromosome without a kinetochore.

SUMMARY

1. The technique of immersing root meristems of Pisum sativum seedlings in known concentrations of an antimitotic dissolved in a weak balanced mineral solution, produced consistently reproducable cytological aberrations which were readily analyzable on both a quantitative as well as a qualitative basis. This technique possessed certain advantages over the standard Allium cytological test particularly a) in being readily available throughout the year, b) in permitting recovery from stronger treatments and, c) in permitting determination of lethal thresholds with greater precision.

2. Colchicine was observed primarily to affect postprophase stages, eliminating spindle control of chromosome orientation and movement, thus causing formation of polyploid resting nuclei. Prophases generally appeared normal. At concentrations tested, the duration of metaphase was increased resulting in a moderate increase in the proportion of metaphases to other stages. Evidence is presented suggesting a shortening of the time required for a complete division cycle and a consequent increase in frequency of division figures.

3. Differing from colchicine in almost every respect, Acti-dione was shown primarily to affect prophases, preventing at least the greatest part of them from entering metaphase and thus resulting in much overcontraction of chromosomes. Reversions to an artificial interphase were frequent at all prophase stages. Postprophase stages were relatively normal, but decreased in

proportions due to failure of prophases to enter metaphase. Almost complete inhibition of new divisions was observed.

4. Streptomycin effects were similar to those produced by Actidione, but at recoverable dosages were not nearly as strongly expressed. It was shown that little margin existed between the the minimum dosage capable of producing cytological effects and the lethal threshold of this drug.

5. Except for somewhat erratic preprophase inhibition of division, chloromycetin had no cytological effects that could be clearly separated from necrotic processes. Despite this, on a concentration basis this drug was the least toxic of the three antibiotics tested.

6. With the exception of colchicine, the cytological activity of the drugs tested was antagonized by dissolved mineral salts. This was very marked in the case of streptomycin, and with the other two drugs was only moderate. The possible implications of this "salt effect" are discussed. Most important of these is the suggestion that colchicine has a mode of action fundamentally different than the other drugs.

7. In the case of the three antibiotics evidence of necrosis and of spindle impairment was not seen in significant proportions except at dosages high enough to prevent recovery. This and other facts are used to support the hypothesis that the spindle derangements caused by the three antibiotics have a very different basis than the spindle disturbances caused by colchicine. There is some basis for the possibility that spindle impairments

caused by treatment with Acti-dione, streptomycin and chloromycetin are actually early stages in pkynotic degeneration.

8. The term "akinetic mitosis" is proposed as a substitute for the term "C-mitosis" to describe any mitotic process where spindle control of postprophase chromosomes is impaired. "C-mitosis" would be reserved to describe the colchicine type of "akinetic" mitoses where such spindle impairment is a) a highly specific reaction, b) accompanied by little or no prophase disturbance and c) productive of substantial amounts of polyploidy.

PLATE I

Normal Mitoses from Untreated Material

Figs. 1-3 Early prophases

Figs. 4-6 Midprophases

Figs. 7-9 Prometaphases

Figs. 10-11 Metaphases

Figs. 12-13 Anaphases

Figs. 14-16 Telophases

Each division of the scale represents ten microns.

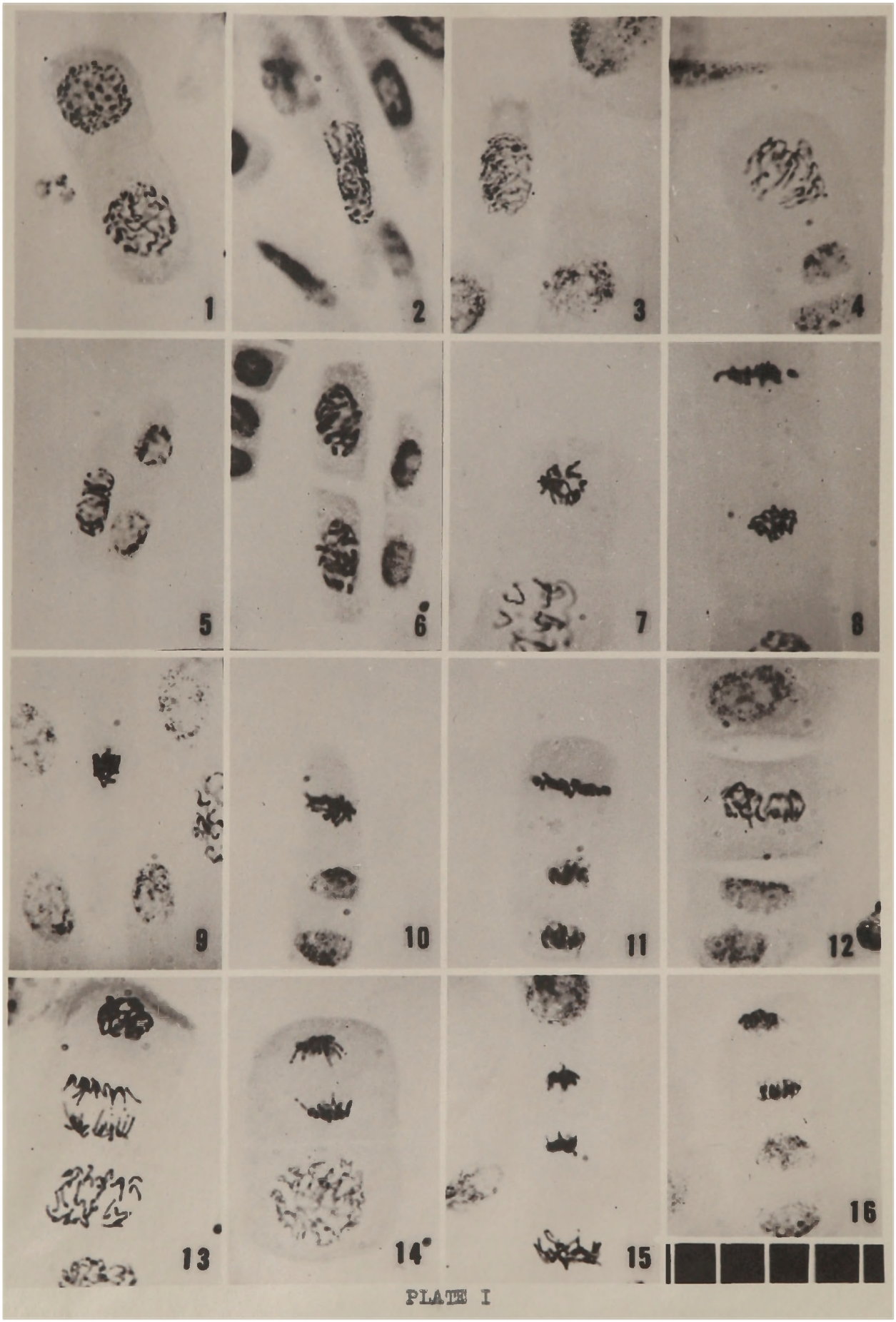


PLATE I

PLATE II

Effects of Colchicine

Mitoses from material treated for eight hours with 50 ppm dissolved in quarter-strength Hoagland solution unless otherwise specified

Fig. 1 Normal early prophase

Fig. 2 Normal midprophase

Figs. 3-4 Normal prometaphases

Fig. 5 Scattered akinetic metaphase

Figs. 6-8 Clumped akinetic metaphases

Fig. 9 Scattered akinetic metaphase and disorganized akinetic anaphase from material treated with 30 ppm

Fig. 10 Disorganized akinetic anaphase

Fig. 11 Unipolar akinetic anaphase from material treated with 30 ppm

Fig. 12 Unipolar akinetic anaphases

Fig. 13 Disorganized akinetic telophases from material treated with 30 ppm

Fig. 14 Disorganized akinetic telophase

Figs. 15-16 Unipolar akinetic telophases

Each division of the scale represents ten microns.

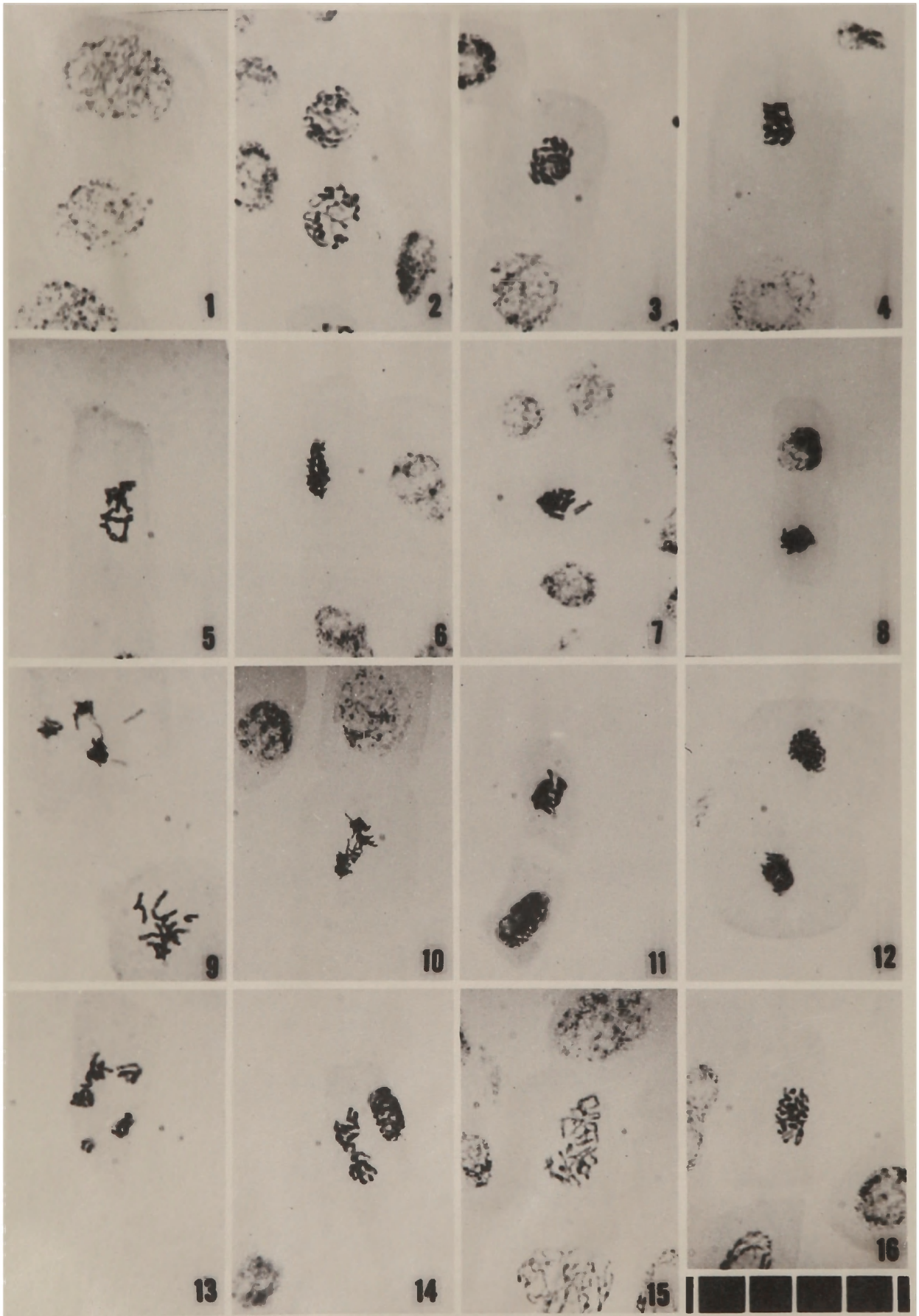


PLATE II

PLATE III

Effects of Colchicine

Mitoses from material treated with colchicine

in quarter-strength Hoagland solution

Fig. 1 Micronuclei from material treated with 30 ppm for eight hours

Fig. 2 Micronuclei from material treated with 20 ppm for eight hours

Figs. 3-6 Polyploid metaphases from material treated with 30 ppm for eight hours and recovered for forty-eight hours

Each division of the scale represents ten microns.

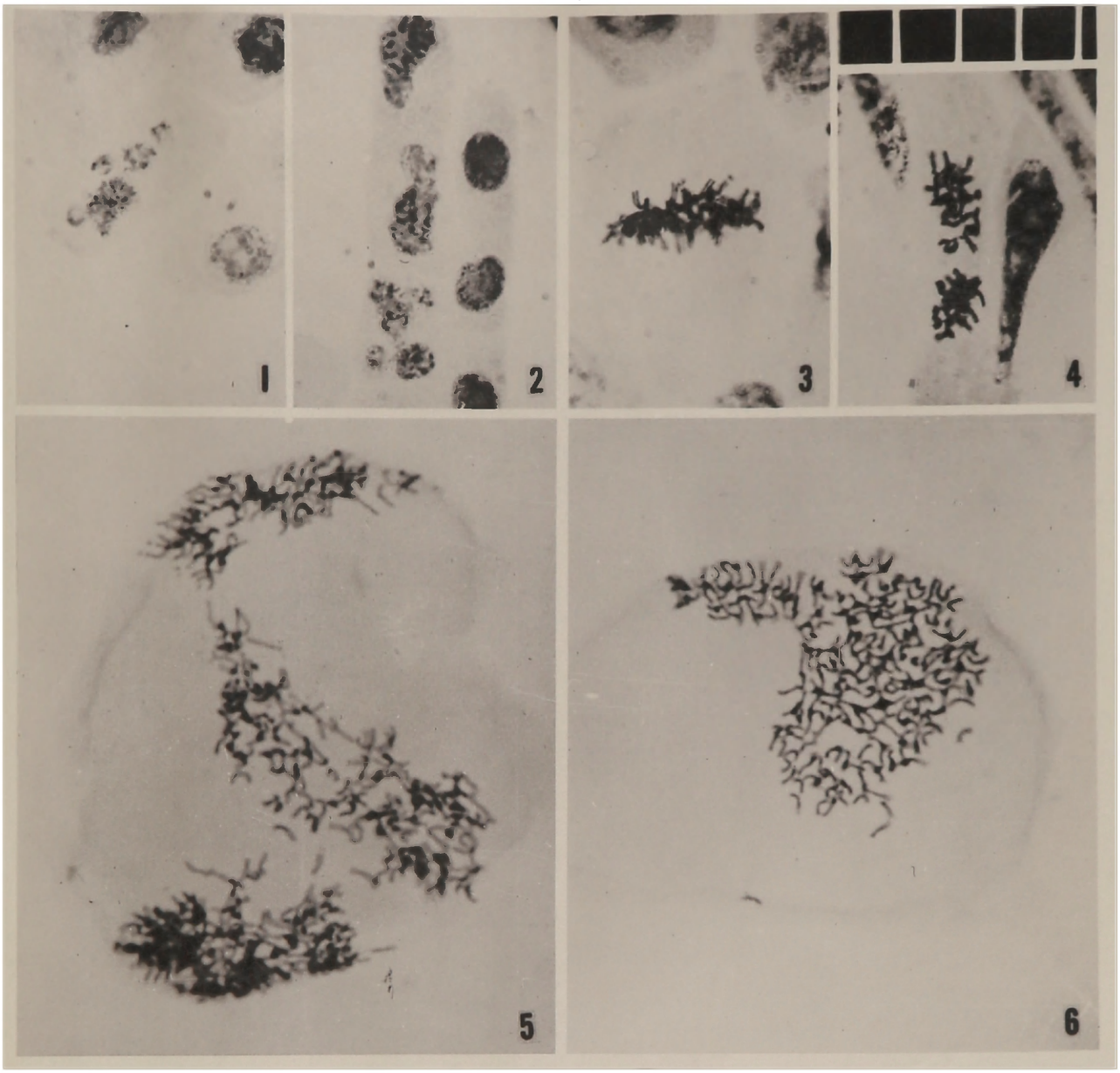


PLATE III

PLATE IV

Effects of Acti-dione

Mitoses from material treated for four hours with 4 ppm Acti-dione in quarter-strength Hoagland solution unless otherwise specified

Fig. 1	Normal early prophase
Fig. 2	Reverting early prophase
Fig. 3	Normal midprophase
Figs. 4-6	Reverting midprophases from material treated with 6 ppm for eight hours
Fig. 7	Ball prometaphase
Fig. 8	Ball prometaphase from material treated with 6 ppm for four hours
Fig. 9	Overcontracted prometaphase from material treated with 6 ppm for four hours
Fig. 10	Overcontracted prometaphase from material treated with 6 ppm for eight hours
Figs. 11-12	Reverting prometaphases from material treated with 6 ppm for eight hours
Fig. 13	Overcontracted metaphase from material treated with 6 ppm for four hours
Fig. 14	Overcontracted metaphase
Fig. 15	Overcontracted anaphase
Fig. 16	Overcontracted telophase from material treated with 6 ppm for four hours

Each division of the scale represents ten microns.

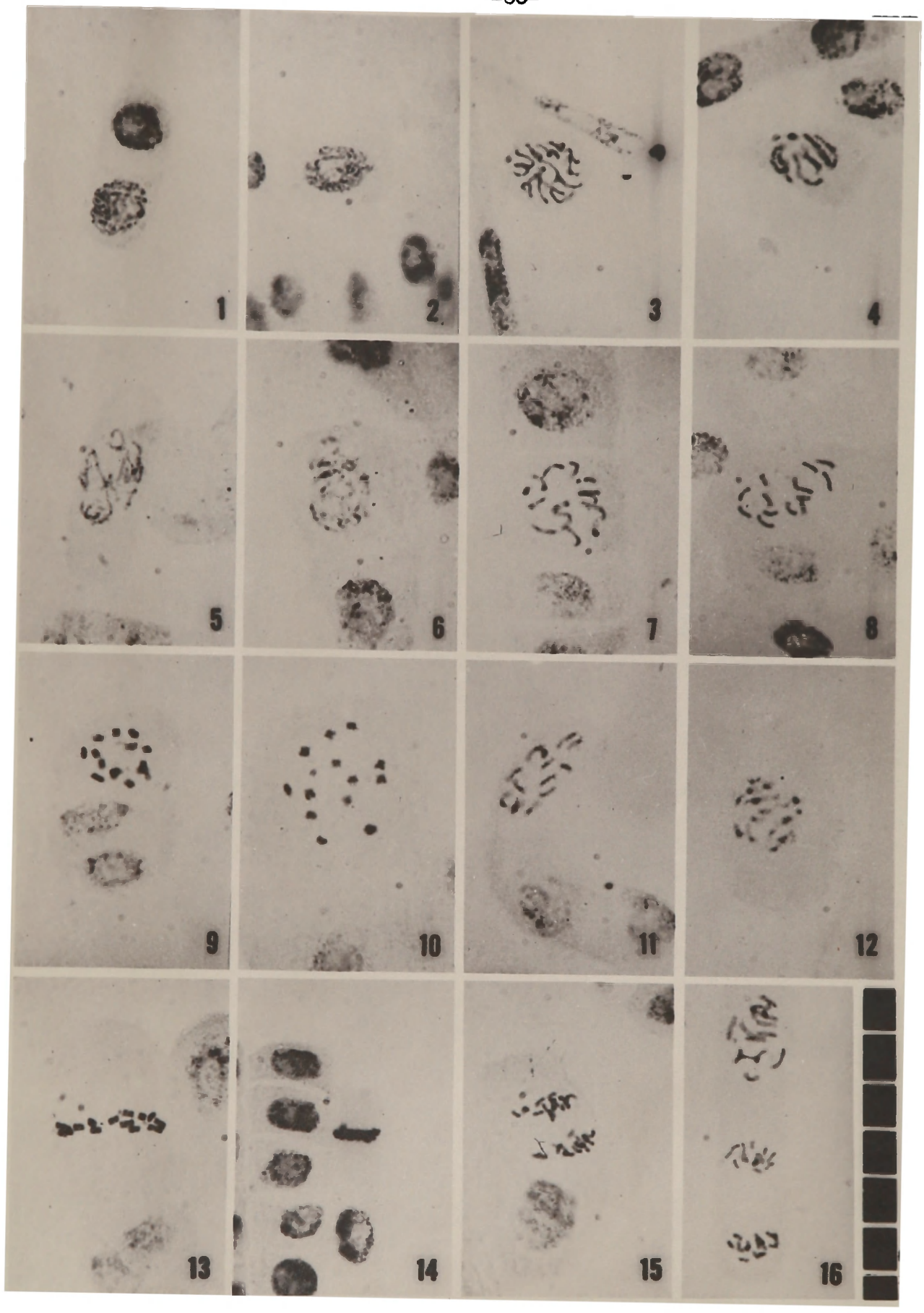


PLATE IV

PLATE V

Effects of Acti-dione

Mitoses from Acti-dione treated material

Fig. 1 Metaphase with normal organization but diplo-chromosomes from material treated for four hours with 16 ppm dissolved in quarter-strength Hoagland solution

Fig. 2 Akinetic (scattered) metaphase from material treated for eight hours with 12 ppm dissolved in distilled water

Figs. 3 & 4 Akinetic (disorganized) anaphases from material treated for eight hours with 12 ppm dissolved in distilled water

Each division of the scale represents ten microns.

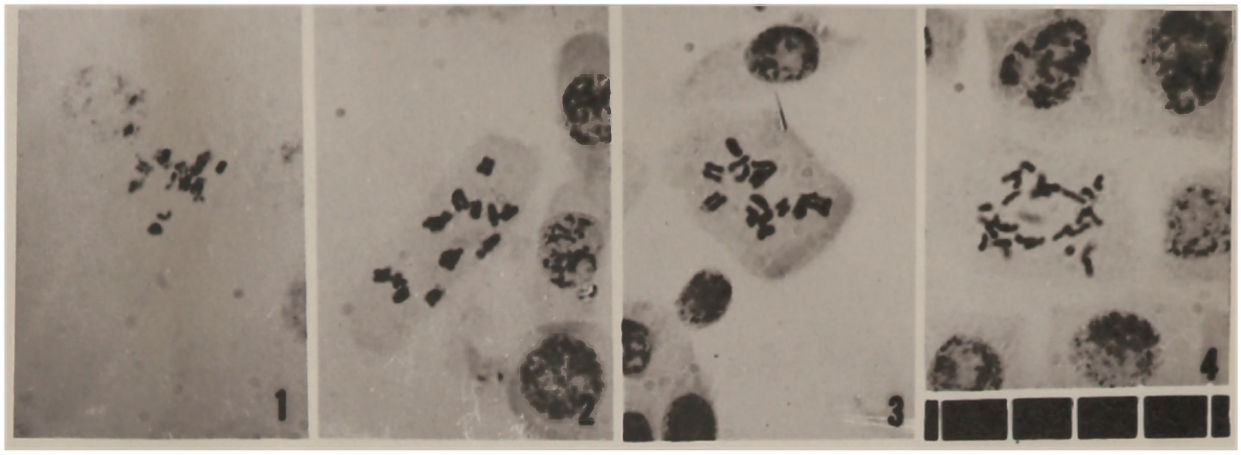


PLATE V

PLATE VI

Effects of Streptomycin

Mitoses from material treated for six hours with 175 ppm Streptomycin sulfate in quarter-strength Hoagland solution unless otherwise specified

Fig. 1 Normal early prophase

Fig. 2 Normal midprophase

Fig. 3 Late normal midprophase and ball prometaphase

Fig. 4 Reverting early prophase

Fig. 5 Reverting midprophase after eight hours treatment with 10 ppm dissolved in distilled water

Fig. 6 Normal metaphase and early prophase

Fig. 7 Normal telophase

Fig. 8 Scattered akinetic metaphase

Fig. 9 Unipolar akinetic telophase and overcontracted anaphase

Fig. 10 Overcontracted telophase

Fig. 11 Pyknotic midprophase

Fig. 12 Pyknotic prophase and resting nucleus after eight hours treatment with 10 ppm in distilled water

Fig. 13-14 Pyknotic metaphases after eight hours treatment with 10 ppm in distilled water

Fig. 15 Pyknotic akinetic anaphase after eight hours treatment with 10 ppm in distilled water

Fig. 16 Pyknotic very early anaphase

Each division of the scale represents ten microns.

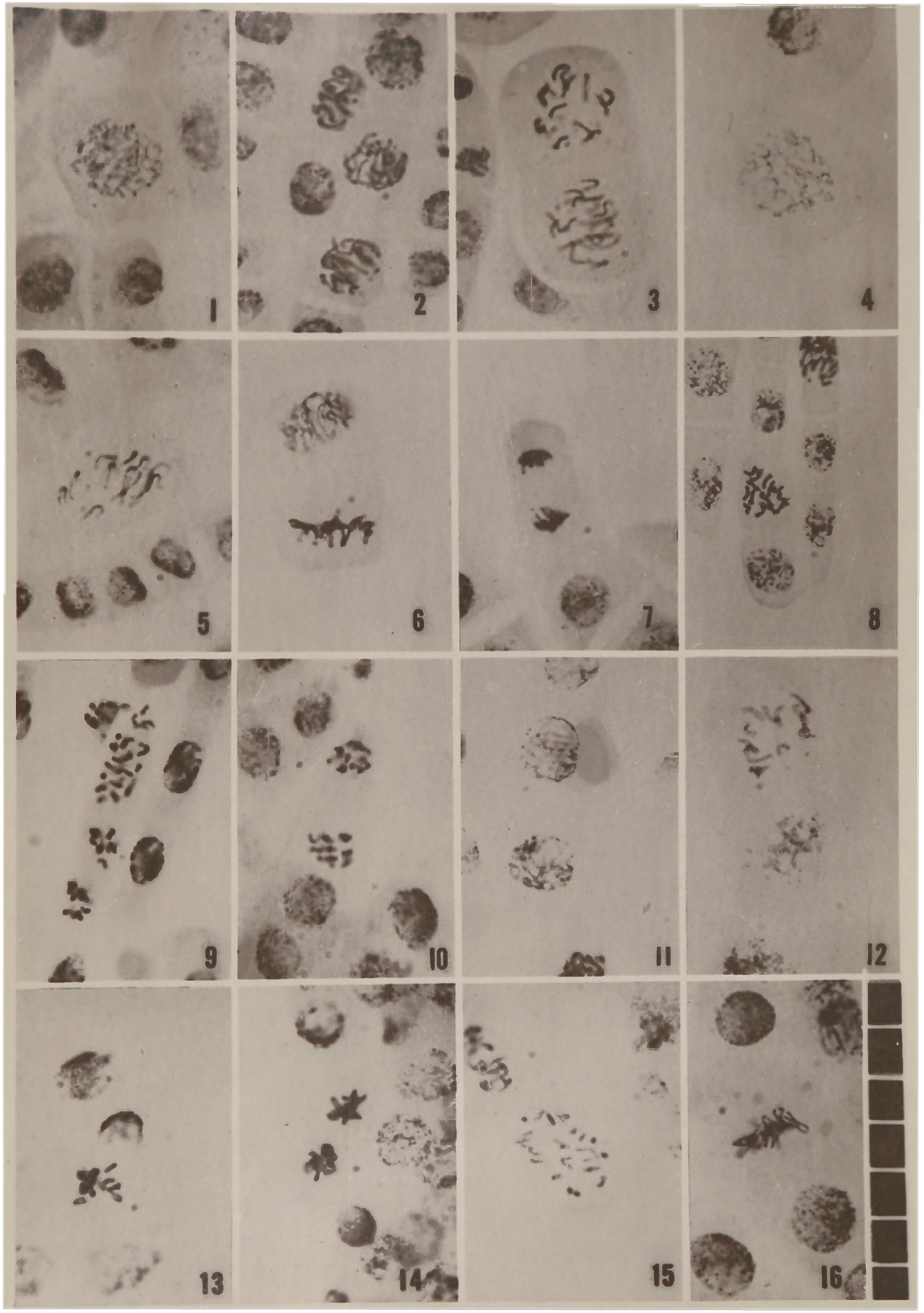


PLATE VI

PLATE VII

Effects of Chloromycetin

Mitoses from material treated for four hours with 1000 ppm synthetic chloromycetin dissolved in quarter-strength Hoagland solution

Fig. 1 Reverting early prophase

Fig. 2 Ball prometaphase

Fig. 3 Reverting midprophase

Fig. 4 Scattered akinetic metaphases

Fig. 5 Disorganized akinetic anaphase

Fig. 6 Pyknotic metaphases

Fig. 7 Pyknotic prophase

Each division of the scale represents ten microns.

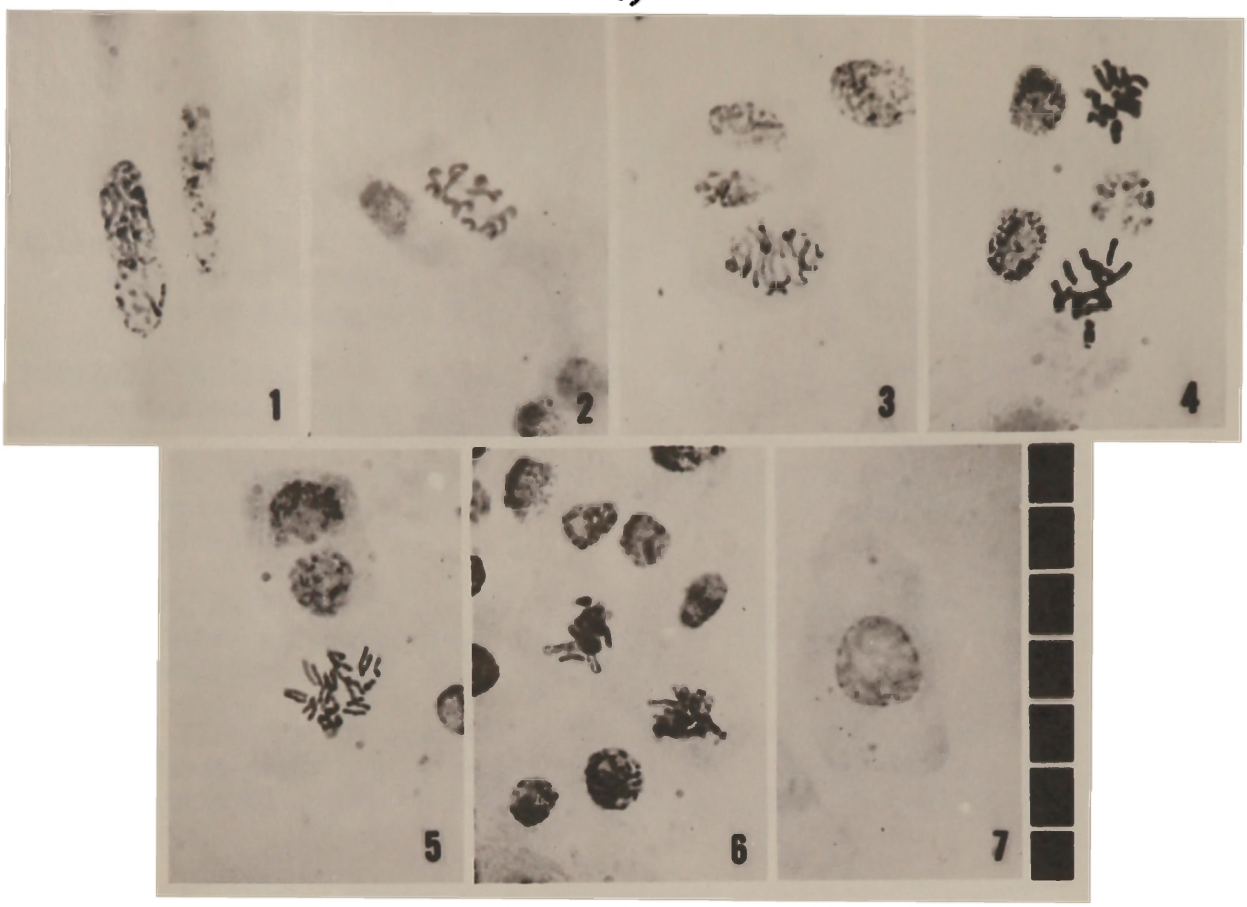


PLATE VII

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TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
ZERO HOUR CONTROLS	30	30	1	30		6				16				17						4.9
TWO HOURS IN ¼STR. HOAGLAND SOLUTION	3	28	1	29		8				15				19						4.2
FOUR HOURS IN ¼STR. HOAGLAND SOLUTION	3	32	1	24		8				19				16						4.8
SIX HOURS IN ¼STR. HOAGLAND SOLUTION	3	29		27		6				16		1		21						3.8
EIGHT HOURS IN ¼STR. HOAGLAND SOLUTION	6	31	1	29		6				16				17						4.8
EIGHT HOURS IN DISTILLED WATER	6	37	1	20		9				19		1		13						2.1

APPENDIX TABLE I. Control Run Summarized Data. Values are percentages of total divisions except as noted.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
EIGHT HOUR CONTROL	6	31	1	29		6				16				17						4.8
5 PPM COLCHICINE	3	31	1	27		6				18		2		15						4.2
10 PPM COLCHICINE	3	29	3	27		8				13		3		17						3.7
15 PPM COLCHICINE	3	24	2	32		7				8		12		12		2	1		++	4.5
20 PPM COLCHICINE	3	25	1	27		7				5		19		6		7	4		++	4.9
30 PPM COLCHICINE	3	24	3	24	1	7					1	29				11			+	5.7
50 PPM COLCHICINE	6	23	1	27	2	5	1					28				13			+	7.0

APPENDIX TABLE II. Colchicine 8 hour Summarized Data. Values are percentages of total divisions except as noted. Quarter-strength Hoagland as solvent.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	HAIL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
FOUR HOUR CONTROL	3	32	1	24		8				19				16						4.8
0.2 PPM ACTI-DIONE	3	18	2	36		4		4		18		1		16		1				2.6
0.5 PPM ACTI-DIONE	3	12	2	35	4	3	1	11		11	2	1		18						2.6
1.0 PPM ACTI-DIONE	3	7	1	26	7	1	1	27	6	7	5			11	1					1.8
2.0 PPM ACTI-DIONE		NOT SCORED																		
4.0 PPM ACTI-DIONE	3	1	3	22	16	1	4	29	5	2	4	1		10	2					2.1
8.0 PPM ACTI-DIONE	3		2	6	18		4	32	3	7	11	1	2	9	4		1			2.5
16.0 PPM ACTI-DIONE	3		3	15	12	3	2	27	3	12	7	11		1	2		2			3.3

APPENDIX TABLE III. Acti-dione 4 hour Summarized Data. Values are percentages of total divisions except as noted. Quarter-strength Hoagland soln. as solvent.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
EIGHT HOUR CONTROL	6	31	1	29		6				16				17						4.8
50 PPM STREPTOMYCIN	3	31	2	36		3				11				17						4.8
100 PPM STREPTOMYCIN	3	26	1	36		4				16				17						2.8
150 PPM STREPTOMYCIN	3	18	2	33	1	7	1			19		1		18						2.0
175 PPM STREPTOMYCIN	3	11	3	35	4	6	1	8		11	2	1	2	15	1					1.4
200PPM STREPTOMYCIN	3	2	5	23	16	2	5	19	1	8	3	2	5	6	2	1				1.8

APPENDIX TABLE IV. Streptomycin 8 hour Summarized Data. Values are percentages of total divisions except as noted. Quarter-strength Hoagland soln. as solvent.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
ZERO HOUR CONTROL	30	30	1	30		6				16				17						4.9
ONE HOUR	3	28	1	31	1	8				4		11		10		1	5			4.0
TWO HOURS	3	25	2	17		11		1		1		27				12	4			4.9
THREE HOURS	3	21	1	26	1	4		1				32				14			+	7.4
FOUR HOURS	3	22	2	23	1	7						27	1			17			+	7.9
SIX HOURS	3	20	1	21		4						35				19			+	7.5
EIGHT HOURS	6	23	1	27	2	5		1				28				13			+	7.0

APPENDIX TABLE V. 50 PPM Colchicine Summarized Data. Values are percentages of total divisions except as noted. Quarter-strength Hoagland soln. as solvent.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORGANIZED	PYKNOTIC		
ZERO HOUR CONTROL	30	30	1	30		6				16				17						4.9
ONE HOUR	3	11	3	47		2		6		11	4			16						3.1
TWO HOURS	3	4	7	43	4	2		20		8	1	1		8	1	1				2.6
THREE HOURS	3	1	6	18	11	1	1	42	2	6		1		6	4	1				2.4
FOUR HOURS	3	1	6	12	10	1	4	39	5	3	7			8	4					1.6
SIX HOURS	3		6	2	21		5	40	14	1	4			4	3					1.0
EIGHT HOURS	3		2		21		16	25	30		4				2					0.5

APPENDIX TABLE VI. 6 PPM Acti-dione Summarized Data. Values are percentages of total divisions except as noted. Quarter-strength Hoagland solution as solvent.

TREATMENT	NUMBER OF SLIDES	EARLY PRO-PHASE		MID PRO-PHASE		PROMETAPHASE				METAPHASE				POSTMETAPHASE					MICRONUCLEI	% CELLS DIVIDING
		NORMAL	REVERTING	NORMAL	REVERTING	NORMAL	REVERTING	BALL	OVERCONTR.	NORMAL	OVERCONTR.	AKINETIC	PYKNOTIC	NORMAL	OVERCONTR.	UNIPOLAR	DISORG.	PYKNOTIC		
ZERO HOUR CONTROL	30	30	1	30		6				16				17						4.9
ONE HOUR	3	28	1	31		5				16				18		1				4.8
TWO HOURS	3	23		33		6				18				19		1				4.8
THREE HOURS	3	23	4	30		7	1	2		13				19		1				4.5
FOUR HOURS	3	18	7	39	2	6		6		10	1	1		9				1		5.7
SIX HOURS	3	8	6	39	2	3	1	7		15		1		18						3.1
EIGHT HOURS	3	11	3	35	4	6	1	8		11	2	1	2	16	1					1.4

APPENDIX TABLE VII. 175 PPM Streptomycin Sulphate Summarized Data. Values are % of total divisions except as noted. Quarter-strength Hoagland soln. used as solvent.

Charles Clark Bowen

candidate for the degree of

Doctor of Philosophy

Final examination: May 20, 1953, 2:00 p.m., Botany Seminar Room

Dissertation: A Comparative Study of the Effects of Several Antimitotics

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