CYTOLOGICAL EFFECTS OF CERTAIN ORGANIC CHEMICALS

Thesis for the Degree of M. S.

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Marilyn Janet Huston

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THESIS

This is to certify that the

thesis entitled

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presented by

Marilyn J. Huston

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G.B.Wilson
Major professor

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CYTOLOGICAL EFFECTS OF CERTAIN ORGANIC CHEMICALS

by

Marilyn Janet Huston

A THESIS

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Thesis Abstract

CYTOLOGICAL EFFECTS OF CERTAIN ORGANIC CHEMICALS

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Marilyn Janet Ruston

It was the purpose of this study to investigate the cytological effects of two insecticides, C-1014 and Systox, and three antibiotics, Rimocidin, Thiolutin and P.A.-96.

The meristems of Allium cepa were treated with solutions of the five organic substances in a continuous treatment up to 12 hours and ranging from concentrations of 1,000 to 500,000 parts per million for C-1014 and Systox and from 200 to 1,000 parts per million for Rimocidin, Thiolutin and P.A.-96. The root tips were collected at hourly intervals and the smear technique used for slide preparation.

In the study of the slide material the occurrence of five things was looked for: (1) spindle abnormalities and c-mitosis, (2) change in the relative numbers of prophase, metaphase and post-metaphase figures, (3) over-contraction of the chromosomes, (4) an increase in the percentage of reductional groupings, and (5) necrosis.

P.A.-96 was found to have no evident cytological effects while the only effects of Rimocidin and Systox were found to be toxic. In material treated with Thiolutin

there was observed an over-contraction of the chromosomes at metaphase which was accompanied by an increase in the relative number of metaphases as compared to prophases and post-metaphases. A correlation was suggested between these two effects. C-1014 also showed evidence of an over-contraction of the chromosomes. At metaphase there was spindle disruption which resulted in a disorganization of the metaphase figures typical of c-mitosis. No cytological effects were recovered from either Thiolutin or C-1014 and these substances were therefore concluded to be toxic at any level of concentration capable of producing mitotic abnormalities.

Approved:

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INTRODUCTION

One goal of an investigation of the cytological effects of different organic chemicals is the formulation of new concepts involved in chromosome reproduction and the mechanics of the mitotic process. Many investigators have made such studies and consequently there is a large amount of literature dealing with the effects of organic substances upon mitosis. Effects similar to those produced by such treatments are to be found under naturally occurring conditions and should be considered, as pointed out by Huskins (1948), as the result of a natural but infrequent process which the influence of chemical treatment makes more evident. The study of the effects, whether naturally or artificially induced, of such mitotic inhibitors is important in the matter of somatic segregation and reduction, the mechanisms of cell division and its cytochemical processes, mutation, elucidation of the nature of cancer or tumor cells and the discovery of useful growth inhibitors.

The discovery by Blakeslee and Avery in 1937 of the polyploidizing action of the alkaloid colchicine has lead to the study of numerous substances with similar properties, with the investigation of the cytological mechanism causing the polyploidy being of prime interest. Since the initial

work with colchicine it has been found that the progress of cell division and the mitotic mechanisms may be suppressed or modified by a variety of physical and chemical means, but colchicine has proven to be one of the most effective of the chemical substances in this respect. This is partly due to the fact that the action of colchicine is specific with regard to the inactivation of the spindle apparatus and partly due to the fact that the inactivation process of the spindle is reversible and not toxic, at least if the exposure to the colchicine is not overly prolonged and the concentrations employed are not too strong (Levan 1943). After removal of the plant from the influence of colchicine, the spindle will recover and the cell, with its increased chromosome complement, will continue to function normally. Cells recovering from colchicine treatment present a wide variety of abnormalities, which, coupled with the fact that its cytological threshold is separate from its toxicity threshold, makes it a good basis for comparison with treatments involving other organic substances.

It has long been known that colchicine has a disturbing effect on the normal course of mitosis. Dixon (1905), working on leucocytes, first noticed its action and interpreted the increase in the frequency of metaphases as being due to the stimulating effect of the drug. Dustin (1934) and Lits (1934) regarded colchicine as a very active agent for increasing the number of mitoses in a tissue, thus also

regarding it as a stimulating substance. However, in 1936 Ludford came to the conclusion that the increase in the number of mitotic figures after colchicine treatment was due to an accumulation of arrested mitoses rather than a stimulation process. He attributed this effect to a failure of the spindle to form and function in the normal manner (Levan 1938). Nebel and Ruttle (1938), who studied the effect of colchicine on stamen hairs of Tradescantia, arrived at a similar conclusion.

Levan, who, with his associates, has made an extensive study of the effects of colchicine, has described its action as consisting of three independent effects, namely: the general poison effect; the c-mitotic effect; and the induction of c-tumors (Levan and Ostergren 1943). The use of the terms "c-mitosis" and "c-tumors" as first used by Levan referred to the typical effects of colchicine upon the mechanics of mitosis and implied the polyploid result in the case of the first-named and to the formation of a tumor at the tip of the onion root in the case of the latter. However, since that time many of the substances tested have shown the typical effects without leading to polyploidy and hence the term "c-mitosis" has come to refer to the two outstanding effects of colchicine treatment which are spindle disruption and a delayed splitting of the kinetochores of the over-contracted chromosomes causing x-shaped figures scattered at random throughout the cell.

Wada (1949) reported that there is an increase in time of duration of c-mitosis over normal mitosis "due to the greatly increased duration of the intactness or staying period of the chromosomes. The increase of time necessary for the formation of chromosomes and uncoiling, namely the time required for the prophase and telophase, was not considerable." This agrees with the findings of Levan (1943) who reports the c-mitotic effect at metaphase in those substances tested.

Although most of the substances tested have been ineffective as polyploidizing agents, there has been accumulated a great deal of information about their abnormal effects on somatic mitosis. These include scattered arrangement of chromosomes at metaphase, "ball" or clumped metaphases; diplo- and contracted chromosomes; "sticky", "lampbrush" and lagging chromosomes; "distributed", "exploded" or "reductional" groupings; "star" or "unipolar" and multipolar spindles; "precocious reversion"; and micronuclei and pycnotic nuclei.

Various groups of workers have attempted to explain the mechanics of "c-mitosis" in terms of physico-chemical relations. Levan and Ostergren (1943) demonstrated that there are many substances which generally fall into the group of spindle inhibitors. These substances include mono- and poly-cyclic hydrocarbons, napthalene acetic acid, nitrogen mustard, insecticides, ethylene glycol, many colchicine derivatives,

and inorganic salt solutions. This discovery led them to the principle that the activity of many c-mitotic agents is conditioned not so much by their chemical properties as especially by their physical ones, and this in turn caused them to propound their theory of the narcotic nature of the c-mitosis. They had noticed that the c-mitotic activity of the substances increased at the same time as their water solubility decreased. This fact, coupled with the wide divergence in the chemical nature of the c-mitotic substances, as well as the fact that many of the narcotics tested were found highly effective, led them to the opinion that the "c-mitotic effect might be regarded as a kind of narcosis." In order to explain the above named observations they formulated the hypothesis, corresponding to the Meyer-Overton theory of narcosis, that "the decisive concentrations are not the concentrations of the substances in the water phase but in the lipoids of the cell." This assumption explains the connection between the solubility and c-mitotic activity which these authors had found to be characteristic of the substances studied. Furthermore, they suggested that the c-mitosis was simply to be considered as a narcosis of certain enzymic functions of the cells. This hypothesis agrees with the work of Dustin (1947) and his suggestion that the various mitotic poisons act as enzyme-inhibitors. Levan and Ostergren also considered the c-tumor effect as a narcosis of the growth control of the cells.

By 1944 Ostergren had arrived at a somewhat modified theory. He laid stress upon the view that the c-mitosis was to be considered simply as a narcotized cell division. The spindle breakdown characteristic of the c-mitosis he supposed to be caused by the fibrous components of the spindle being brought to assume a more or less corpuscular shape by the protein-folding action of the c-mitotic agent, and this in turn would lead to the disorganization of the spindle.

The observations that some of these chemicals, in addition to inhibiting the spindle, had other effects started some new lines of investigation and thinking. Berger and Witkus (1943) reported that under the influence of colchicine Allium root tips produced an unorganized spindle substance which took the form of an achromatic sphere and about which the diplo-chromosomes gathered at c-metaphase. They attributed the many strange shapes of the restitution nuclei as being due to the presence of the achromatic sphere. The work of Kodani with sodium ribose nucleate and Galinsky with phosphates suggested that perhaps more than a single process was involved in all the "c-mitotic" agents. Kodani (1948) noted, among the various effects of sodium nucleate solutions, an inhibition of chromosome re-duplication and the formation of akinetic fragments. Galinsky (1949) found that following his phosphate treatments the deviations from normality were observed during

prophase in the delay of nuclear membrane "breakdown". He pointed out that this effect had not been observed after the use of the c-mitotic poisons. These substances had been reported to permit mitosis to proceed normally until metaphase (Levan 1938) when their specific effect on the spindle mechanism was manifested. Accordingly, he concluded that, "the unoriented prometaphase chromosomes must therefore involve forces other than those associated with the oriented spindle mechanism." D'Amato (1949), after testing a series of chemicals, observed that two different effects must be distinguished: preprophase inhibition and an inhibition of spindle formation giving rise to c-mitosis. He was of the opinion that "the spindle-inhibiting effect, manifested in typical c-mitosis poisons, may be of quite different sort than that by which preprophase poisons induce the c-mitotic effect." Bergner (1950) found an arrangement of chromosomes in the peripheral zone of a hollow sphere, as they were arranged in late prophase before the nuclear membrane solated and interpreted this as indicating that no spindle was formed, and that the chromosomes were not moved from their prophase positions. This suggested that "the compound was exerting its full effect before mitosis began or at least no later than prophase."

It was first noted by Huskins (1948) that some substances being tested for c-mitosis not only were not producing polyploidy but were capable of producing chromosome

groupings within the cell that might lead to somatic reduction. Such substances would be put in the category of "unrecoverable" and in a series of papers (Huskins 1947, 1948, Huskins and Cheng 1950, and Huskins and Chouinard 1949) the occurrence and potential significance of these reductional groupings was discussed. This type of effect has been reported after treatments with sodium nucleate (Huskins 1948), colchicine (Allen, Wilson and Powell 1950) and Acti-dione (Wilson 1950 and Hawthorne, unpublished thesis 1951). Since most of the work on plants with c-mitotic substances has been done with the idea of producing polyploids, many workers have probably missed reductional groupings and those who did note such groupings (Levan 1939, Darber and Callan 1943, Witkus and Berger 1944, Nybom and Knutson 1947, D'Amato 1948 and Bergner 1950) did not realize its implications.

With the publication of the work of Huskins and his associates there became apparent two major points of view. These, as expressed by Allen, Wilson and Powell (1950) were: "(1) that the mechanism involved in the c-mitotic effect is probably the same regardless of the chemical used, and in any event, it is a phenomenon of little theoretical or practical importance, (Levan and Lotfy 1949) and (2) that the mechanisms may be quite different and that there are, in any event, important theoretical and practical implications (Huskins 1948). The separation of the c-mitotic effects into two groups was made by Allen, Wilson and Powell (1950).

They listed five points of difference between the effects of the "mitotic poisons," of which colchicine is the type substance, and the "physiological substances" of which the sodium salt of nucleic acid is the type substance. These were (1) arrangement of chromosomes, (2) types of groupings, (3) chromosome distribution within the groupings, (4) frequency of reductional groupings, and (5) prophase to metaphase ratios. In 1951 Powell (unpublished thesis) showed that the major point of difference between the effects of the colchicine and sodium nucleate treatments in Pisum was the time in the mitotic cycle that the effects were evident. The nucleates showed effects at late prophase while colchicine effects appeared at metaphase. Hawthorne (1951, unpublished thesis) in an analysis of the cytological effects of Acti-dione to be used as a quantitative basis for comparison with other active chemicals, also found that the c-mitotic effects represented a generalized reaction probably the result of prophase or interphase effects which prevented "normal" organization rather than destroying structures already present.

It was suggested by Wilson, Hawthorne, and Tsou (1951), reporting on the cytological effects of Lindane and Acti-dione, that there are two, more or less independent, components of the forces involved in the mitotic mechanism: "(1) a dipolar cytoplasmic orientation possibly characteristic of the cell at any stage; and (2) a nuclear component inherent in and

directed by the chromosome or the kinetochore thereof." Then metaphase organization would be primarily the function of the cytoplasmic component while anaphase separation would be the function of the chromosome itself. Such an hypothesis would explain the presence of "split but organized figures as the result of a weakness in cytoplasmic orientation; multipolar anaphases would represent almost complete breakdown of cytoplasmic orientation without serious disruption of the nuclear component; and completely disorganized metaphases and anaphases would indicate the elimination of both components". As an example of the latter, the unpublished work of Hyppio may be cited. In his preliminary studies with short treatments of Allium with colchicine he has found a reaction typical of the breakdown of both cytoplasmic and nuclear components as evidenced by (1) anaphases coming to a stop and (2) metaphase chromosomes not reaching a plate but beginning disorganized separation. After approximately 5 hours of recovery there is evidence that spindle action has been resumed with the chromosomes disorganized, and the initiation of cytokinesis gives rise to micronucleate cells.

It has been the purpose of this series of experiments with 5 new organic substances; C-1014, Systox, Thiolutin, Rimocidin and P.A.-96; to compare their cytological effects with those of colchicine and other active c-mitotic substances to gain information that will lead to the elucidation of the forces involved in the mitotic mechanism.

MATERIALS AND METHODS

The organic substances tested in this series of experiments were obtained through the courtesy of the Dow Chemical Company of Midland, Michigan and Chas. Pfizer & Co., Inc. of Brooklyn, New York. Systox and C-1014 are both insecticides produced by Dow Chemical Company. C-1014 contains four pounds of Octa-methyl-pyrophosphoramide per gallon, and is a water dispersible concentrate. The active ingredient of Systox is a tri-alkyl-thiophosphate. The antibiotics, Thiolutin, Rimocidin and P.A.-96 were supplied by the Pfizer Company. Although they have shown some antibacterial properties, the main activity of Thiolutin and Rimocidin has been indicated as antifungal in nature, and they are being employed experimentally in the treatment of dermatophytes. P.A.-96, while being devoid of activity against both bacterial and fungal species, has shown a pronounced effect against certain viruses.

All experiments were carried out on a single lot of *Allium cepa* sets. According to routine already established in this laboratory, the sets were rooted in vials of aerated distilled water. When the roots were approximately one to two inches long the root tips were examined and if they showed a high rate of mitotic activity the sets were

transferred to vials of the solutions to be tested. In all cases stock solutions were made with distilled water and kept refrigerated until a series was run, at which time the solution was brought to room temperature before the root tips were transferred. All experiments were conducted at room temperature and the onions were kept under normal conditions of day and night. After treatment the roots were washed with, and returned to vials of, distilled water and a check for recovery was made at four, twenty-four and forty-eight hours.

In all cases a preliminary trial was run with solutions ranging in concentration from 200 to 10,000 parts per million with root tips collected at four, eight and twenty-four hours to determine the most promising concentration to be used in an extended series where root tips were collected at hourly intervals until a level of toxicity was reached. Systox was tested up to a concentration of 500,000 parts per million and a series run at that concentration. The Pestox series was run at a concentration of 10,000 parts per million. The Thiolutin, Rimocidin and P.A.-96 series were taken at concentrations of 1,000 parts per million.

Root tips were collected in a three to one absolute alcohol-acetic acid mixture, fixed for 15 minutes at 60° C., hydrolized in one normal hydrochloric acid for 8 to 10 minutes, stained with Feulgen's stain and smear preparations made. After dehydration in a 95% ethyl alcohol to which a little

fast green for counterstain had been added, the slides were made permanent with diaphane.

At each cutting two root tips were taken and placed on separate slides so as to obtain a duplicate series. Dr. G. B. Wilson counted the slides from one series while the writer counted the slides in the duplicate series as a check upon the consistency of the data.

For counting, according to a standardized procedure, each slide was placed on the calibrated mechanical stage, the count was started at a constant, predetermined point on the vertical axis and this reading recorded on the data sheet. Consecutive high powered fields were counted from edge to edge of the cover slip and these totals were recorded on the data sheet for each axis. The vertical axis was increased by one unit each time and the count continued until a total of approximately 100 division figures were obtained from each slide. In the slides where the 100 figures were totaled in the middle of the axis, the count was continued until the readings on that axis had been completed. In those cases where, in the later hours of treatment, the division figures were less numerous, it was frequently necessary to go back over the slide and take readings at the one-half unit intervals.

It was recognized that, aside from the subjective element involved in the counting of the slides, two factors might influence the counts: (1) the evenness of the

distribution of the material and (2) whether or not the most actively dividing areas of the meristem happen to have fallen within the predetermined area of the count. However, since it can be assumed that all areas of the root tip have an equal chance of being evenly distributed within the predetermined area of the count, it is believed that this technique could be used to obtain an indication of trends and should be interpreted accordingly.

OBSERVATIONS

Controls

Many investigations have been undertaken in this laboratory with control material. In accordance with the established method in these experiments two root tips were cut from every onion to be treated at the zero hour of treatment. From the combined series of experiments 11 control slides were chosen at random and counted to determine the prophase: metaphase: post-metaphase ratio. In Table 1 it will be seen that this resulted in an average frequency of 50.7% prophase figures, 22.4% metaphase figures and 26.9% post-metaphase figures. Plate I (figs. A, B, C) illustrate normal figures of prophase, metaphase and anaphase divisions found in control material.

Insecticides

C-1014

Root tips tested with a solution of C-1014 at a concentration of 1000 parts per million showed no cytological or toxicity effects after 24 hours of continuous treatment. When the concentration of the solution was raised to 5,000 parts per million there was evidence of a very mild cytological effect after 24 hours of treatment. Prophase and and metaphase figures were organized. Metaphase and anaphase

figures showed a more than normal contraction of the chromosomes. The series run at a concentration of 10,000 parts per million showed a mild cytological effect at prophase after two hours treatment. The effect progressed as evidenced by the hourly collections. At four hours the majority of prophases were affected. As is typical with colchicine treatment (Plate I, fig. L), metaphase chromosomes were greatly contracted and were scattered throughout the cell in a disorganized manner (Plate I, fig. E). Post-metaphases were unaffected. After six hours of treatment toxic effects became evident with very "tight" prophases (Plate I, fig. D). By 12 hours of treatment there were very few divisions and these all showed high toxicity effects (Plate I, fig. F).

Recovery of the cytological effects of C-1014 were attempted after treatments of three, four, and five hours with a concentration of 10,000 parts per million. There were no recoverable effects; division stopped completely. Hourly variation in the frequencies of the individual stages of mitosis as compared to control slides was found to be erratic (Table 2; Text fig. 1) and no major trend was observed.

Systox

Systox was tested in solution with concentrations of 10,000; 20,000; 50,000; 100,000 and 500,000 parts per million. In all cases after short treatments of one hour there was evidence of toxicity without cytological effect. Mitosis

came to a complete standstill. Cells in interphase showed toxicity effects with dark staining. Metaphase and anaphase figures were still organized but chromosomes were greatly contracted and showed signs of clumping. Metaphases were roverting to resting stages. At the highest concentration, 500,000 parts per million, there were still many division figures in the material but all figures showed signs of toxicity and reversion.

Antibiotics

Thiolutin

Root tips tested with solutions of Thiolutin at concentrations of 100 and 200 parts per million showed no cytological or toxicity effects after 24 hours of continuous treatment. After four hours of treatment with the concentration raised to 500 parts per million, the roots were still normal but at 12 hours showed signs of toxicity without cytological effect. With the concentration of Thiolutin at 1,000 parts per million and after three hours of treatment there was evidence of a mild cytological effect. Mitotic division figures were still organized but the chromosomes were more contracted than normal. By four hours of treatment at this concentration chromosomes were greatly contracted at metaphase, in some cases showing organization (Plate I, fig. G) and in a few cases lying scattered throughout the cell (Plate I, fig. H). At this time anaphase figures were still organized but were seen only in polar

view (Plate I, fig. I). After eight hours of treatment the first signs of toxicity were becoming apparent and by twelve hours there were few divisions, with the material at all stages of mitosis showing toxicity (Plate I, fig. J).

All attempts to recover cytological effects after treatment with concentrations of 500 and 1,000 parts per million after three, four and five hours were unsuccessful. Hourly variation in the frequencies of the individual stages of mitosis as compared to control slides (Table 3, Text fig. 2) showed a decline in the number of prophase and post-metaphase figures with a complementary rise in the number of metaphases.

Rimocidin

In the series testing Rimocidin in solutions with concentrations of 100, 200 and 500 parts per million, after 24 hours of continuous treatment, all material showed divisions to be entirely normal. With a concentration of 1,000 parts per million division continued normally until, after 12 hours treatment, toxicity was evident. There was no decline in numbers of division but all stages of mitosis showed toxicity, with the chromosomes shortened and beginning to revert.

P.A.-96

An extended series was run with solutions of P.A.-96 at concentrations of 100, 500, and 1,000 parts per million. Root tips were collected in each series at two, three, four,

five, six, eight, twelve and thirty-six hours and after five and one-half days of continuous treatment. In all material examined, there was found normal mitotic division (Plate I, fig. K). Hourly variation in the frequencies of the individual stages of mitosis as compared to control material shows a decline in the number of prophases with an increase in the number of metaphases and post-metaphases (Table 4, Text fig. 3). A count of the number of divisions per 1,000 cells (Table 5) showed no increase or decrease from that of control material.

TABLE 1

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES IN CONTROL MATERIAL

Slide	Prophase No.	Prophase %	Metaphase No.	Metaphase %	Post-Meta. No.	Post-Meta. %	Total
1	53	53	18	18	29	29	100
2	45	45	26	26	29	29	100
3	40	40	29	29	31	31	100
4	45	45	34	34	21	21	100
5	52	52	19	19	29	29	100
6	41	41	25	25	34	34	100
7	66	66	17	17	17	17	100
8	46	46	27	27	27	27	100
9	118	59	33	16.5	49	24.5	200
10	66	66	20	20	14	14	100
11	45	45	15	15	40	40	100
Total							1200
Average %		50.7		22.4		26.9	
Standard Error		± 2.815		± 1.835		± 2.25	

TABLE 2

PERCENTAGES OF TOTAL DIVISION FIGURES OF INDIVIDUAL STAGES IN MATERIAL TREATED WITH C-1014 AT A CONCENTRATION OF 10,000 PARTS PER MILLION

Hour	Prophase			Metaphase			Post-Meta			Total
	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	
¼	142	48.8	- 1.9	53	18.2	- 4.2	96	33.0	+ 6.1	291
2	113	48.5	- 2.2	50	21.5	- 0.9	70	30.0	+ 3.1	233
3	94	55.2	+ 4.5	46	27.1	+ 4.7	30	17.7	- 9.2	170
4	73	49.6	- 1.1	27	18.4	- 4.0	47	32.0	+ 5.1	147
5	131	49.6	- 1.1	113	42.8	+20.4	20	7.6	-19.3	264
6	101	57.0	+ 6.3	38	21.5	- 0.9	38	21.5	- 5.4	177
8	68	50.4	- 0.3	44	32.6	+10.2	23	17.0	- 9.9	135
12	10	62.5	+11.8	2	12.5	- 9.9	4	25.0	- 1.9	16

TABLE 3

PERCENTAGES OF TOTAL DIVISION FIGURES OF

INDIVIDUAL STAGES IN MATERIAL TREATED

WITH THIOLUTIN AT A CONCENTRATION OF 1,000 PARTS PER MILLION

Hours	Prophase			Metaphase			Post-Meta			Total
	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	
2	116	58	+ 7.3	59	29.5	+ 7.1	25	14.5	-12.4	200
3	387	38.7	-12.0	384	38.4	+16	228	22.8	- 4.1	999
4	431	42.3	- 8.4	343	33.6	+11.2	246	24.1	- 2.8	1020
5	75	34.1	-16.6	124	56.4	+34.0	21	9.5	-17.4	220
6	38	38	-12.7	42	42	+19.6	20	20	- 6.9	100
7	36	48	- 2.7	27	36	+13.6	18	16	-10.9	75
8	21	21	-29.7	58	58	+35.6	21	21	- 5.9	100
12	12	24	-26.7	37	74	+52.6	1	2	-24.9	50

TABLE 4

PERCENTAGES OF TOTAL DIVISION FIGURES OF

INDIVIDUAL STAGES IN MATERIAL TREATED

WITH P.A.-96 AT A CONCENTRATION OF 1,000 PARTS PER MILLION

Hours	Prophase			Metaphase			Post-Meta			Total
	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	No.	%	% inc. or dec. from control	
2	92	40.0	-10.7	60	26.1	+ 3.7	78	33.9	+ 7.0	230
3	80	31.5	-19.2	76	29.8	+ 7.4	98	38.7	+11.8	254
4	114	42.5	- 8.2	85	30.6	+ 8.2	71	26.9	0	268
5	102	37.8	-12.9	66	24.4	+ 2.0	102	37.0	+10.1	270
6	94	39.5	-11.2	96	40.4	+18.0	48	20.1	- 6.8	238
8	113	44.6	- 6.1	62	24.7	+ 2.3	77	30.2	+ 3.3	252
12	124	44.3	- 6.4	68	24.3	+ 1.9	88	31.4	+ 4.5	280

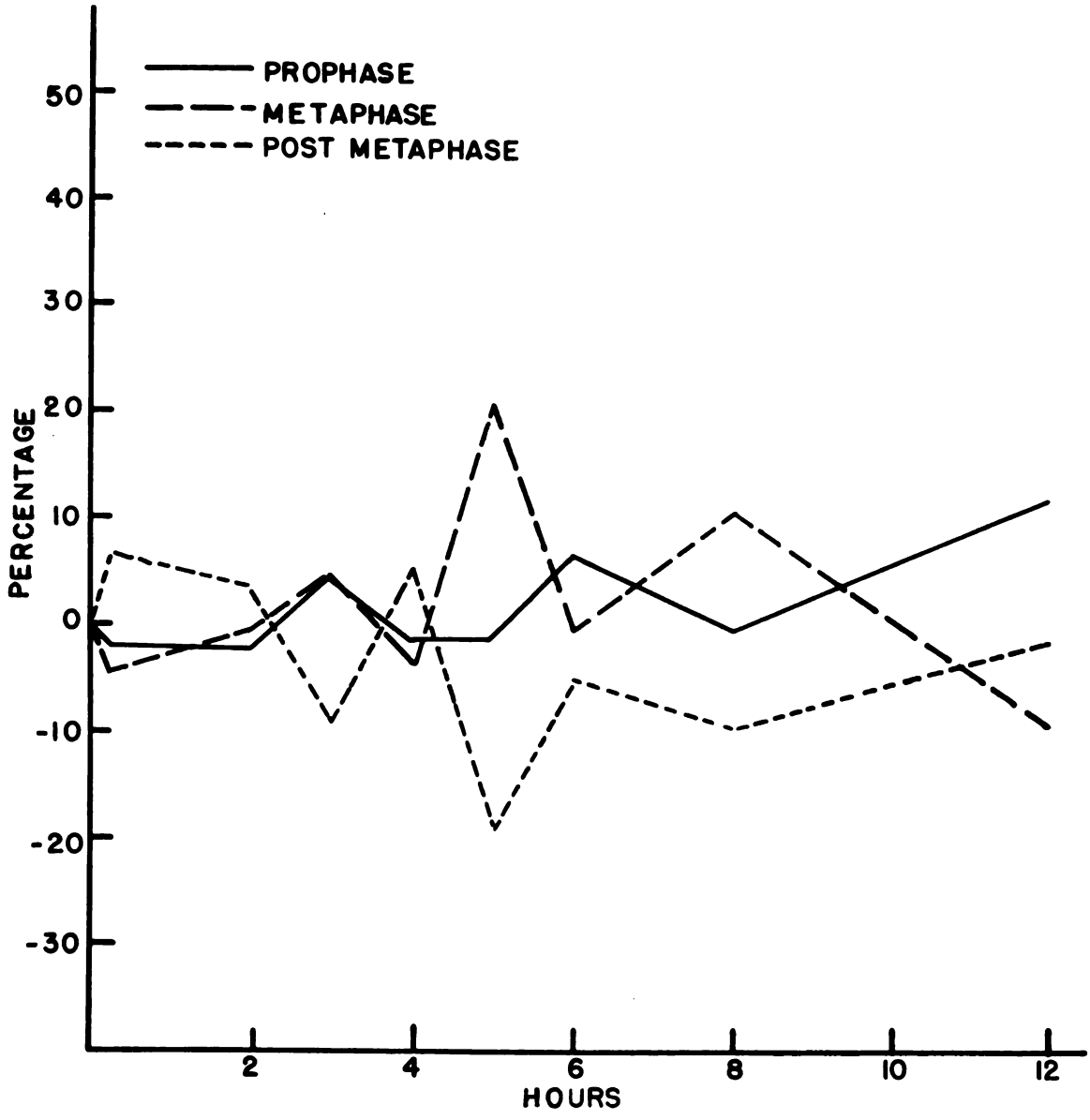
TABLE 5

FREQUENCY OF DIBISIONS IN CONTROL MATERIAL

AND IN MATERIAL TREATED

WITH P.A.-96 AT A CONCENTRATION OF 1,000 PARTS PER MILLION

Axis	Control	P.A.-96 2 hr.	P.A.-96 12 hr.
1	35	43	37
2	41	27	38
3	31	54	41
4	37	36	40
5	41	42	45
6	45	38	36

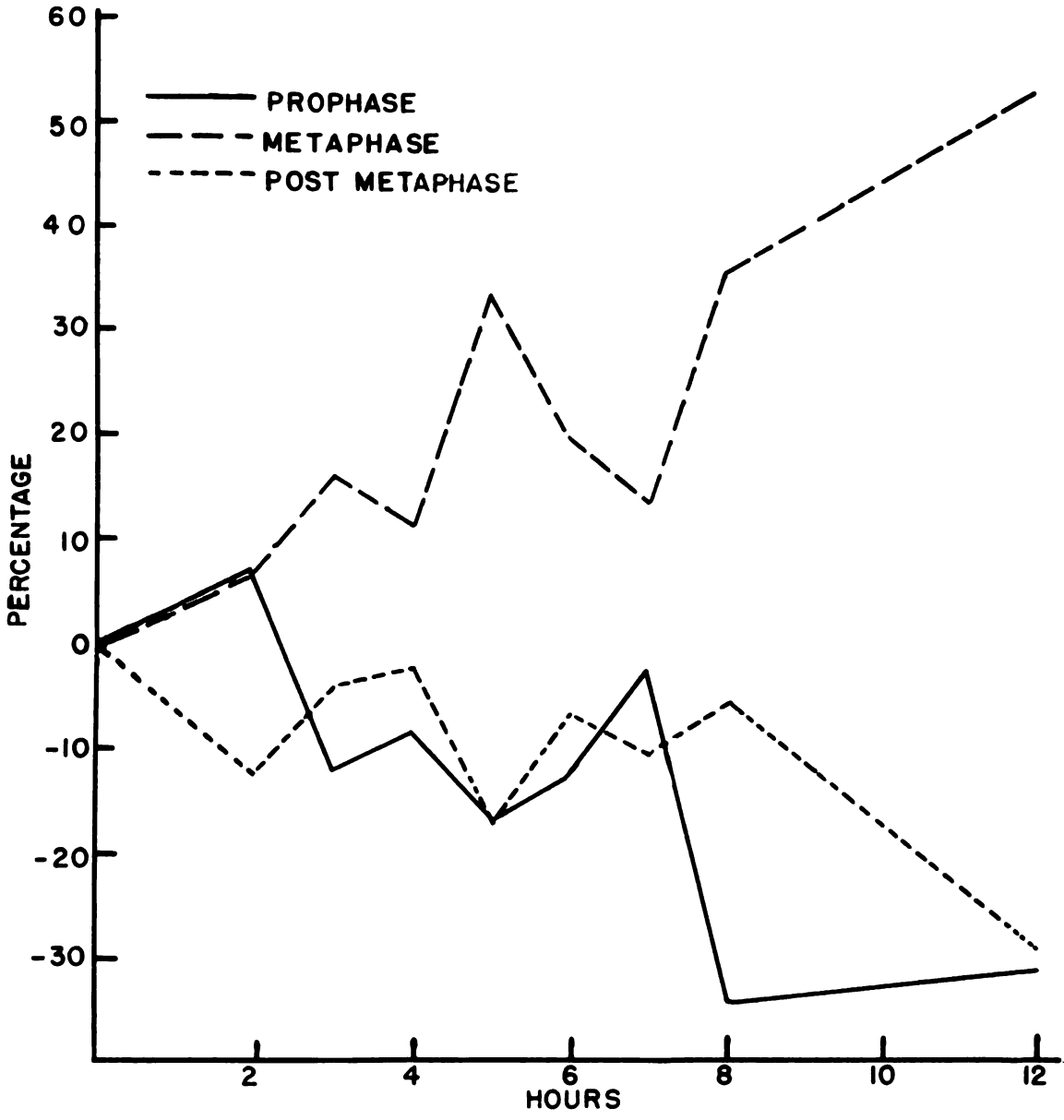


TEXT FIGURE 1

HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES

C-1014

AT A CONCENTRATION OF 10,000 PARTS PER MILLION

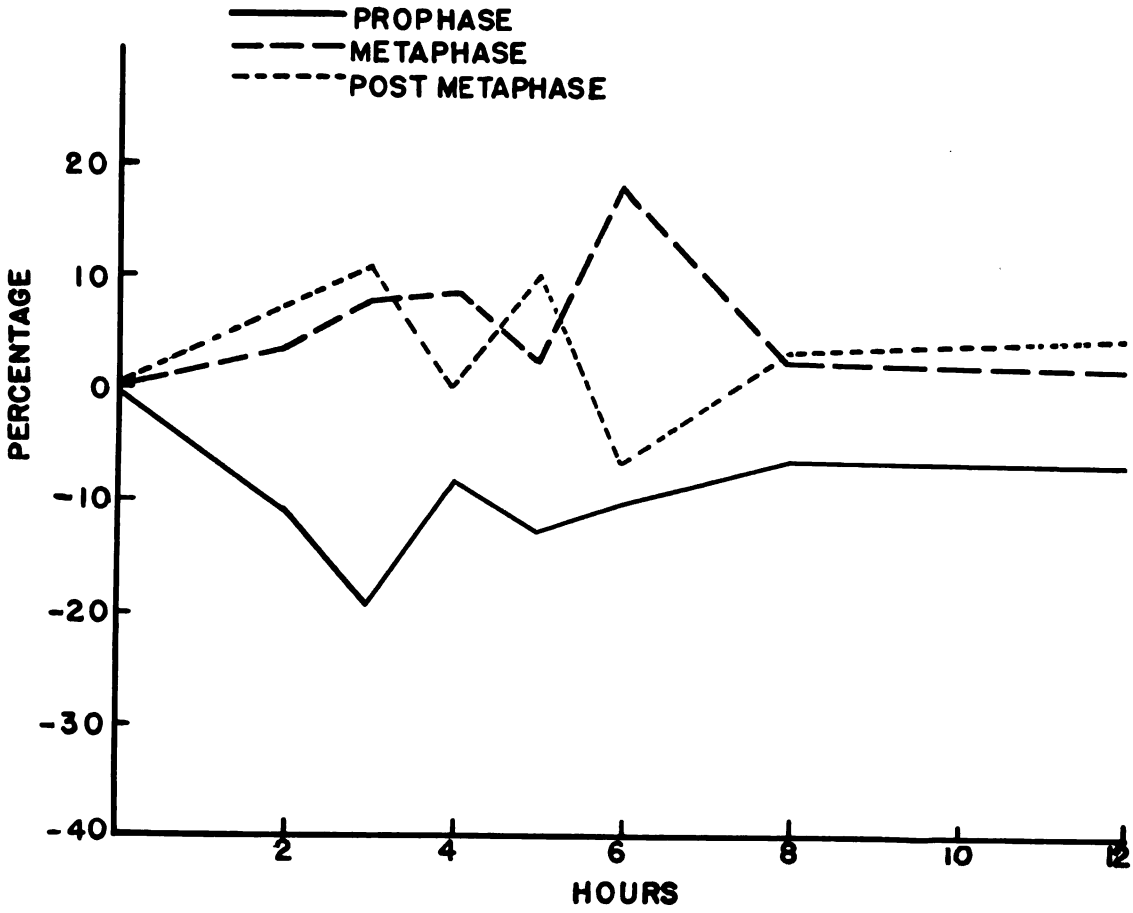


TEXT FIGURE 2

HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES

THIOLUTIN

AT A CONCENTRATION OF 1,000 PARTS PER MILLION



TEXT FIGURE 3

HOURLY VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES

P.A.-96

AT A CONCENTRATION OF 1,000 PARTS PER MILLION

DISCUSSION

As it was the purpose of this investigation to compare the cytological effects of the two insecticides and three antibiotics with those of colchicine and other active c-mitotic substances, in the examination of results the occurrence of five things was looked for:

(1) spindle abnormalities and c-mitosis,

(2) change in the relative numbers of prophase, metaphase and post-metaphase figures,

(3) over-contraction of the chromosomes,

(4) an increase in the percentage of reductional groupings, and,

(5) necrosis.

Spindle Abnormalities and C-Mitosis

Ludford (1936) first suggested that the polyploidizing action of colchicine was caused by its specific effect upon the spindle mechanism of the dividing cell. With the disruption of the spindle the chromosomes, at metaphase, were left lying scattered throughout the cell. At anaphase the chromatids separated, and then, with removal from the influence of colchicine, the spindle became reactivated and the then polyploid cells were able to continue a normal division. The term "c-mitosis", as first used by Levan (1943), referred to the typical effects of colchicine upon

the mitotic mechanism and implied the polyploid result. Since that time, and as used in this instance, "c-mitosis" has come to mean the two outstanding cytological effects of colchicine treatment, i.e., spindle disruption and a delayed splitting of the kinetochores of the over-contracted chromosomes causing x-shaped figures scattered at random throughout the cell. Following the discovery of colchicine as a polyploidizing agent much work was done in the following years with other organic substances by various groups searching for polyploidizing agents. The qualities to be looked for in such substances are: (1) a disrupting effect upon spindle formation as evidenced by disorganized "scattered" chromosomes, and (2) a cytological threshold separate from the toxicity threshold with resumption of normal activity upon removal from the influence of the substance.

P.A.-96 had no effect upon the spindle mechanism. All division figures appeared normal and organized. Rimocidin and Systox showed neither evidence of spindle disruption or other c-mitosis effects. In both cases divisions showing any effects of treatment represented toxicity effects. Thiolutin, at treatment of 1,000 parts per million for three hours, began to show signs of c-mitotic effect with the over-contraction of the chromosomes. After four hours of treatment there were a few divisions at metaphase showing the typical c-mitotic effect with disrupted spindle mechanism and scattered x-shaped chromosomes. However, in the majority

of the metaphase divisions the figures were organized, showing no signs of spindle abnormality. Further treatment produced toxicity effects. It can therefore be concluded that Thiolutin is toxic at any concentration that is capable of inducing mitotic abnormalities. At a concentration of 10,000 parts per million C-1014 began to show signs of c-mitosis after three hours of treatment. After four hours of treatment metaphases showed complete spindle disruption and the chromosomes were over-contracted. This was considered to be of particular importance since, potentially, such figures may lead to a polyploid condition. After six hours treatment toxicity was evident. As all attempts to recover from the c-mitotic effects were unsuccessful it must be concluded that C-1014, like Thiolutin, is toxic at concentrations capable of inducing mitotic changes.

Change in the Relative Numbers of Prophase, Metaphase and Post-Metaphase Figures

Previous experiments have shown that one of the most readily detectable effects of any substance on mitosis is a more or less marked change in the relative frequencies of prophase, metaphase and post-metaphase figures. A detailed analysis of these changes was made from material treated with C-1014, Thiolutin and P.A.-96 (Text figs. 1, 2, 3). As can be seen from the graphs, the only consistent or significant trends in the data was obtained with Thiolutin. C-1014 shows only an erratic increase and decrease for all stages.

It may be noted that with P.A.-96, contrary to the usual trend, there would appear to be an increase in the number of post-metaphase figures in combination with a rise in the number of metaphases and with a decrease in prophases. Thiolutin, however, showed a definite increase in the number of metaphase figures while there was a decrease in the relative number of prophase and post-metaphase figures. Such a change was found in the extensive study of Actidione (Hawthorne, unpublished thesis) at the lowest, cytologically effective concentration used in continuous treatment. With so many variables involved in relation to the frequency changes; e.g. such factors as the absolute frequency of division figures, the rates with which one stage proceeds to the next and the degree of mitotic disruption; it is difficult to draw any definite conclusions from the data, especially in the absence of any other effects from the treatment. However, it may be suggested that Thiolutin had an effect upon prophase which is of a stalling nature and which prevented onset of prophase and, in combination with that, had a stalling effect upon metaphase which prevented that stage from progressing to post-metaphase. This would account for the rise in relative numbers of metaphase division figures in combination with a decrease in the numbers of prophase and post-metaphase figures, and might be correlated with the over-contraction of the chromosomes at metaphase as found with this concentration and time of treatment.

Over-Contraction of Chromosomes

There was no evidence of over-contraction of chromosomes in material treated with P.A.-96, Rimocidin and Systox. With Thiolutin, at a concentration of 1,000 parts per million and after three hours of treatment, metaphases began to show signs of over-contraction and after four hours were "super-contracted". As was suggested above, this may be correlated with the increase in relative number of metaphases. C-1014, at a concentration of 5,000 parts per million, showed signs of over-contraction after a treatment of 24 hours. When the concentration was increased to 10,000 parts per million, the over-contraction of the chromosomes at metaphase appeared after four hours of treatment. As over-contraction of the chromosomes is usually correlated with prolongation of the entire mitotic division, or with prolongation of one stage of division, and since there was no indication of a slowing down of prophase, metaphase or post-metaphase as evidenced in the changes in the relative numbers of these stages, it may be suggested that C-1014 has a general effect of prolongation of the process of mitosis.

Reductional Divisions

The occurrence and significance of reductional groupings has been discussed by the group at Wisconsin (Huskins 1947, 1948, Wilson and Cheng 1949, Huskins and Cheng 1950, and Huskins and Chouinard 1949). Wilson (1950) reported that a variety of substances, unrelated to those previously tested

by the Wisconsin group, were capable of increasing the numbers of such reductional divisions. Since the reductional groupings, as referred to by the above authors, have probably been missed in many cases due to the fact that only the polyploidizing activity of the substances was being tested, a careful check was made on all material treated with C-1014, Systox, Thiolutin, Rimocidin and P.A.-96. In no case was there found an incidence of reductional groupings which exceeded that found in untreated material. From this it may be concluded that these organic substances had no effect on the occurrence of reductional groupings at the degree of concentration and length of treatment employed in these experiments.

Necrosis

Necrosis, in this study, refers to the condition of the nucleus when the prophase chromosomes continue to proceed through their regular morphological changes while the nuclear membrane seems to have been retarded in its activity; and then, having appeared to contract the membrane, has drawn the contents of the nucleus into a tight ball. Such an effect is evidence of toxicity and may arise from a stalling effect of the substance on division or from reversion of the chromosomes to interphase. P.A.-96 showed no signs of toxicity or necrosis. Systox and Rimocidin showed only effects of necrosis at any level capable of inducing changes from the normal. Necrosis was observed

in roots treated with C-1014, with tight prophases, after six hours of treatment with a concentration of 10,000 parts per million, and after treatment of 12 hours all divisions showed necrosis with evidence of tight prophases and metaphases the chromosomes of which were reverting to interphase. Similar effects of necrosis were seen in material treated with Thiolutin after 8 hours of treatment

SUMMARY

1. The cytological effects of two insecticides, C-1014 and Systox, and of three antibiotics, Rimocidin, Thiolutin and P.A.-96, were presented and discussed.
2. Studies were made of continuous exposures up to 12 hours at concentrations ranging from 1,000 to 500,000 parts per million for C-1014 and Systox and from 200 to 1,000 parts per million for Rimocidin, Thiolutin and P.A.-96.
3. The occurrence of five things was looked for: (1) spindle abnormalities and c-mitosis, (2) change in the relative numbers of prophase, metaphase and post-metaphase figures, (3) over-contraction of the chromosomes, (4) an increase in the percentage of reductional groupings, and (5) necrosis.
4. The only cytological effects of Systox and Rimocidin were observed to be toxic.
5. P.A.-96 was found to have no evident cytological effects.
6. The cytological effects of C-1014 and Thiolutin were found to be an over-contraction of the chromosomes at metaphase after some treatments. C-1014 showed signs of unrecoverable c-mitosis.
7. A suggestion of correlation between an increase in the frequency of metaphase figures and over-contraction of chromosomes was discussed with regard to Thiolutin.

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PLATE I

Figure

A. Normal prophase from control material.

B. Normal metaphase from control material.

C. Normal anaphase from control material.

D. "Tight" prophase after six hours treatment with C-1014 at a concentration of 10,000 parts per million.

E. Over-contracted, scattered chromosomes at metaphase after four hours treatment with C-1014 at a concentration of 10,000 parts per million.

F. Toxicity effects from treatment with C-1014 at a concentration of 10,000 parts per million after 12 hours.

G. Organized, over-contracted chromosomes at metaphase after four hours treatment with Thiolutin at a concentration of 1,000 parts per million.

H. Scattered, over-contracted chromosomes at metaphase after four hours treatment with Thiolutin at a concentration of 1,000 parts per million.

I. Polar view of anaphase after four hours treatment with Thiolutin at a concentration of 1,000 parts per million.

J. Toxicity effects after eight hours of treatment with Thiolutin at a concentration of 1,000 parts per million.

K. Metaphase and anaphase from material treated 12 hours with P.A.-96 at a concentration of 1,000 parts per million.

L. Typical c-mitosis from material treated 15 minutes with colchicine at a concentration of 1,000 parts per million and recovered for 5 hours.

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