

CYTOLOGICAL SPECTS OF THE DEPOLIANT ENDOTHAL

Thesis for the Degree of M. S. MICHICAN STATE COLLEGE Arlyn Mario Daniel 1952 This is to certify that the

thesis entitled

The Cytological Effects of the Defoliant Endothal

presented by

Arlyn Marie Daniel

has been accepted towards fulfillment of the requirements for

<u>M.S.</u> degree in <u>Botany</u> (Cytology)

-85n

Major professor

Date March 13, 1953

O-169

CYTOLOGICAL EFFECTS OF THE DEFOLIANT

ENDOTHAL

ЪУ

Arlyn Marie Daniel

.

A THESIS

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

Master of Science

Department of Botany and Plant Pathology School of Science and Arts.

1952



TABLE OF CONTENTS

,

4-9-53 g

Introduction	1
Material and Methods	7
Observations	10
Classification of Aberrant Figures	10
Continuous Treatment Analysis of the Relative Frequencies of	11
Stages of Mitosis	11
Analysis of Laggards	14
Analysis of Chromosomes	10
Contraction of ontono somes	10
Short Period Treatment	18
Three Hour Treatment with Recovery Period	18
Four Hour Treatment with Recovery Period	23
Discussion	32
Formation of Laggards	32
Interrelation of Aberrations	34
Laggards and Mitosis Mechanics	37
Summe ry	39
Bibliography	40
Acknowledgments	42
Description of Plate I	43
Description of Plate II	45

•

INTRODUCTION

The immediate purpose of the research for this thesis was to determine the effects of Endothal upon mitosis. However, beside this immediate aim the type of investigation herein reported has a more general and fundamental purpose, namely that of discovering the force or forces involved in mitosis.

As the psychologist learns of the working of the brain by studying the deviant individual, so too the cytologist may hope to learn something of the mechanics of mitosis by studying the abnormalities. Many theories have been formulated to describe the "normal" movement of chromosomes during mitosis. However, not only do these theories have to account for the ability of the chromosomes to reach the Metaphase plate and the movement of chromatids to the poles at Anaphase but they must allow for the particular break down as seen in the various treatments.

Franz Schrader in his book on "Mitosis" discusses numerous theories set forth from 1878 to 1944. The details of the various hypotheses (pulling, pushing, viscosity changes, electrostatics, etc.) will not be gone into here. There are specific difficulties with each hypothesis in that each breaks down in explaining Metaphase or Anaphase movement or, if one force is used in explaining both movements, how the reversal in direction is accomplished. The case of the fly, Sciara, presents difficulties for all

1

hypotheses which do not have as a basic concept the idea of chromosomal autonomy. In the first spermatocyte division no synapsis occurs, nor is any Metaphase plate formed. At Anaphase the maternal autosomes and sex chromosomes go to the one pole present while the paternal autosomes migrate backwards (with kinetochore pointed toward the pole) to the opposite periphery of the cell where they are budded off.

Carlson (1938) cites Belar, Bleier, Schaede, and Schrader in pointing out the importance of chromosomal autonomy. This was shown by the fact that chromatid separation occurs even when a spindle is not present.

All of the hypotheses rely upon a spindle. The importance of the spindle is pointed out in Carlson's investigation of the mitotic behavior of fragments. It was noticed in the neuroblasts of the grasshopper that acentric fragments moved toward the poles, sister chromatids to opposite poles, and ultimately were incorporated into the resting nuclei. Although the presence of a spindle organization is generally accepted, there is lack of agreement as to its nature. The hydrodynamic and electrostatic hypotheses consider the fibers as lines of force. The viscosity-hydration and streaming hypotheses consider the fibers as regions of differential viscosity. For a long time it was a metter of doubt whether the spindle fibers were an actuality. Since the spindle had not been seen in living tissue, it was suggested that it might well be simply an artifact in fixation. Chromosomes could be extracted from the cells and no evidence of a fiber could be seen. However,

-2-

as Schrader (1944) states "the question of the reality of spindle fibers may be regarded as settled." At the time of his book two cases of a spindle in living tissue had been found. The birefringence of these has been compared to the birefringence of the spindle of fixed material and found to be the same. Therefore, the spindles seen in fixed material would not appear to be artifacts.

We have now two different phenomena which are connected in some way with the process of mitosis. In a report on the cytological effects of Lindane and Actidione by Wilson, Hawthorne and Tsou (1951) it was suggested that two forces were involved--one being a dipolar cytoplasmically initiated mechanism (the spindle) and the other being an autonomous chromosomally initiated mechanism (the kinetochore). They went further to suggest that the Metaphase organization is a function primarily of the cytoplasmic component whereas the Anaphase movement is accomplished by the chromosome itself.

It would seem necessary to point out that Anaphase movement could not be accomplished entirely by the autonomy of the kinetochore. The organized fashion that the chromatids display in moving to the pole would indicate that the spindle must also be an important influence.

It is well here to review some of the work that has preceeded this investigation on antimitotic substances. Substances possessing antimitotic ability are mitotic poisons or interfer in some manner with mitosis. This antimitotic capacity may be toxic or non-toxic depending upon the substance and upon the concentration of that substance.

-3-

The mitotic poison which creates a toxic effect is felt to such a degree that there is no recovery and death of the cell is expected sooner or later. Stickiness and peculiarly shaped and staining nuclei often typify toxic effects. Battaglia (1950) reported on a number of stains---basic fuschin, crystal violet, aniline blue-which have this toxic effect. Houston (1952) reported toxic effects of Systox and Rimocidin. One type of toxic effect is the reversion muclei noted by C. C. Bowen (Unpub.). These were seen to form from any stage of mitosis in his work with Acti-Reversion is the name given the phenomenon of the dione. loosening of the chromosomal spiral and the massing of the chromosome material. A second type of toxic effect is seen in the stickiness and ultimate clumping of the chromosomes. The chromosomes may clump to such an extent that they form a darkly staining solid ball in the cell. This is readily seen in the material treated with high concentrations of Endothal.

The other main type of mitotic poison is the type with a non-toxic effect. Here recovery is possible and the cell will complete the mitotic cycle and may even go on to further divisions. Chloral hydrate (Meyer, 1938) and colchicine are polyploidizing agents of this type. There seem to be several types of effects that can be grouped under this heading of non-toxic. The first could be the mitotic suppressor. This effect is shown by maleic hydrazide (Wilson, unpub.) and five per cent Urethan (Battaglia, 1949). No further division is initiated after the time of treatment so as the cells undergoing mitosis complete the cycle, no

-4-

other mitotic figures are found. A second type of non-toxic mitotic poison may be classed as an Anaphase inhibitor. There is a distinct pile-up of Metaphase figures which after recovery tend to go on to Anaphase. Colchicine is an excellent example of a mitotic poison of this type (Levan, 1938). Colchicine is also the typical example of a substance which produces a third type of non-toxic effect. This effect is known as C-mitosis. Here the chromosomes are scattered throughout the cell. The chromatids contract to such an extent that little X-shaped bodies are formed (the kinetochore remaining undivided). At what is called Anaphase the kinetochores split and the chromatids lie scattered throughout the cell. This is the action through which polyploidy is induced by Colchicins and Lindane. A fourth type of chromosomal abberation is fragmentation. Smith and Srb (1951) reported laggards due to fragmentation after treatments with Beta Propiolactone. There is yet a fifth type of non-toxic mitotic poisons. This effect is noted in the "reductional groupings" found after Actidione and Streptothricin. Sodium nucleate and cold treatment seem to effect mitosis also in such a way that segregated prophases, split Metaphases, and multi-polar spindles are formed. Yakar (1952) reported multi-polar spindles in Vicia treated with Chloranil. These resulted in multinucleate cells.

In applying the previous discussion of the mechanisms involved in mitosis we can see that the cytoplasmic-autonomy hypothesis would fit nicely. The reductional groupings would be the result of an interference with the normal operation of the spindle. It would seem to be a splitting of the spindle into several parts

-5-

through the poles thus making the multiple poles seen at Anaphase and Telophase. C-mitosis could be explained simply by the complete inactivation of the spindle. The laggards formed by the fragments could be explained as being due to the loss of their autonomy through loss of the kinetochore.

The cytological effects of Endothal will now be reported and viewed in the light of this hypothesis.

MATERIALS AND METHODS

The substance used in these experiments was supplied through the courtesy of the Niagara Chemical Division, Middleport, New York. Niagara Experimental Product 3003-Endothal has as the active ingredient Disodium 3, 6-Endoxohexahydrophthalate at a concentration of 19.2 per cent. The solution contains two pounds of technical Endothal per gallon. Endothal is used as a defoliating agent and herbicide and is applied as a dilute spray. The active ingredient has the following structural formula:

The tests were carried out on <u>Allium cepa</u> (2n = 16) in the following manner. The onions were grown in jars of aerated distilled water until the roots were about one-half to three-quarters of an inch long. The mitotic activity was then checked and if mitosis was going on at a good rate treatment was started.

The treatment consisted of growing the onions in a specific concentration of Endothal for a particular length of time. The first experiments were carried out at a concentration of 1000 ppm

and treated for periods of two, three, and four hours. However, this concentration appeared to be very toxic and all the onions died. The concentration was gradually reduced until the threshold for mitotic effect could be separated from the general toxic effect. The concentration for this work was 25 ppm. Two-hour, three-hour, four-hour, and continuous (to twenty-four hours) treatments were carried on.

After the two, three, and four-hour treatments the onions were removed from the solution of Endothal, washed with distilled water, and returned to the jars of aerated distilled water in order that recovery from the effects of Endothal could be checked.

Control root tips were taken at the beginning of each run and two root tips were taken periodically. The root tips were fixed in 3:1 alcohol-glacial acetic acid and the preparations were stained by the Feulgen reaction. The squash technique was used in making the slides.

The slides (made permanent with diaphane) were analyzed according to the phase of mitosis and an attempt was made to classify the type of aberration when abnormal figures were found.

In order to maintain some degree of objectivity with regard to cells chosen for count, scoring of the slide was commenced at the vertical mechanical stage reading of 1000. Horizontal rows ten units apart were scored until approximately one hundred figures had been counted.

The results of analyzing were checked by Dr. G. B. Wilson who also analyzed a series of slides starting at a different

calibration. Since comparable results were obtained it was considered that at least main trends were reliable.

OBSERVATIONS

Classification of Aberrant Figures

Laggard Figures

The classification of laggerd was given to a mitotic figure when all of the chromosomes or chromatids were properly oriented with the exception of a few which were situated quite apart from the rest. The number of lagging chromosomes or chromatids ranged from one chromosome or chromatid to two or three chromosomes or five or six chromatids. Leggards were found in Prophase, Metaphase, Anaphase, and Telophase. Examples of the laggards in different stages of mitosis are shown in Plate I, Figs. D - G.

Segregated Figures

The classification of segregation was given to a mitotic figure if the chromosomes at Metaphase were divided into definite groups. Very often two groupings of chromosomes were formed and at times these groups were accompanied by a laggard. A picture of one such configuration is shown in Plate II, Fig. B. For purposes of analysis segregations and laggards have been lumped.

Scattered Figures

In the previous two categories some sort of Metaphase plate was evident but in the case of scattered figures no apparent plate was formed. The chromosomes of Metaphase or chromatids of Anaphase were freely scattered throughout the cell. Often a few chromosomes

-10-

were seen to be grouped but mostly the chromosomes were quite independent of each other. A scattered Metaphase is pictured in Plate II, Fig. C. A scattered Anaphase is shown in Plate II, Fig. F. <u>Clumped Figures</u>

The classification of "clump" was used to designate Metaphase figures in which the chromosomes either had started to revert (spirals loosening) forming an amorphous mass in the cell or had become sticky and were adhering to each other. Photographs of three types of clumped figures are shown in Plate II, Figs. J, K, and L displaying laggard, unequal clumping, and scattered clumping respectively.

Over Contracted Figures

In both continuously treated and short treatment material the chromosomes were found to contract to a greater degree than in the untreated cells. This over contraction appeared in late Prophase and was noted through the following stages.

Continuous Treatment

Analysis of the Relative Frequencies of Stages of Mitosis

As calculated from the control slide which was used as the base line for Text Fig. 1, it is shown that the relative precentage of Prophase figures gradually decreased from the beginning to the end of the twenty-four hour treatment. Metaphase figures rapidly increased from the start of treatment to seven hours, decreased slightly at eight hours, then increased very slightly to the end of the twenty-four period. Post-metaphase figures decreased rather sharply to five hours then leveled off and increased very slightly to twenty-four hours.

-11-





HOURLY VARIATION IN FREQUENCY OF INDIVIDUAL STAGES

AT 25 PPM

<u>reatment</u>	••		opha se		••	Met	apha se		••	Post	Metaphs	150	••	Total	
		No. of cells	8 2	Å Diff.#		No. of cells	8 2	\$ Diff.•		No. of cells	8 °	g Diff.*			1
ontrol	••	347	36.5		••	250	27.0		••	328	35.5		••	925	
hours	••	Libit	28.7	- 7.8	••	E †18	54.8	27.8	••	250	16.3	-19.2	••	1534	
hours	••	253	21.4	-15.1	••	743	62.6	35.6	••	189	16.0	-19.5	••	1185	
hours	••	210	24.3	-12.2	••	594	68.8	41.8	••	60	7.0	-28.5	••	864	
hours	••	96	20.3	-16.2	••	363	76.9	6.64	••	14	3.0	-32.5	••	473	
hours	••	123	22.3	-14.2	••	419	76.0	0.64	••	10	1.8	-33.7	••	552	
hours	••	75	17.1	-19.4	••	350	79.0	52.0	••	15	3.4	-32.1	••	0111	
hours	••	711	23.8	-12.7	••	365	74.4	47.4	••	6	1.8	-33.7	••	164	
2 hours	••	69	14.8	-21.7	••	361	77.5	50.5	••	36	7.7	-27.8	••	1466	
4 hours	••	14	4.1	-32.4	••	291	85.5	58.5	••	35	10.3	-25.2	••	340	

•

-13-

Figures used in Text Figure 1

Thus it is seen that while the relative frequency of Metaphase figures increases, that of Prophase and Post-metaphase figures decreases.

Analysis of Laggards

Text Figs. 2 and 3 represent graphically the percentage of laggards in Metaphase and Post-metaphase respectively. The perpendicular lines show the range whereas the lines of the graph show the mean.

There is a rapid increase of Metaphase laggards from two to five hours, a decided decrease of laggards at six hours, an equally decided increase at seven hours and then a gradual tapering off after the eight to twelve-hour period.

There is a somewhat slower increase of Post-metaphase than Metaphase laggards to three hours. At four hours the peak of Post-metaphase laggards is reached. A definite decrease in number is seen at five hours and eight hours and then a gradual decrease in number occurs from twelve to twenty-four hours of treatment.

In comparing the frequency of Metaphase with Post-metaphase laggards, the graphs are similar. After a slower increase in number of Post-metaphase than Metaphase laggards the former reach a peak at five hours rather than at six hours as is the case with the latter. Each stage shows a distinct decrease after the peak is reached, this appearing at five hours in Post-metaphase and six hours at Metaphase. The same fluctuations are seen from seven hours to twenty-four hours except that they are at a lower level

-14-





TEXT FIGURE 2



TEXT FIGURE 3



Treatment	••`	1	<mark>Me taphase</mark>			••	Pos	t-me taphase		
		No. of Cells with Leggrids	Total No. of Cells	BE	s Diff.*	•	No. of Cells with Laggards	Total No. of Cells	BE	\$ Diff.**
Control	••	Ŋ	250	2.0		••	0	328	0.0	
2 hours	••	67	643	8.0	6.0	••	2	250	2.8	2.8
3 hours	••	131	753	17.4	15.4	••	19	189	10.0	10.0
4 hours	••	168	594	28.4	26.4	••	20	60	33.3	33•3
5 hours	••	128	. 363	35.2	33.2	••	Ļ	14	7.1	7.1
6 hours	••	59	614	14.1	12.1	••	8	10	20.0	20.0
7 hours	••	104	350	29.8	27.8	••	ſ	15	20.0	20.0
8 hours	••	55	365	15.1	13.1	••	0	6	0.0	0.0
12 hours	••	22	361	19.9	17.9	••	4	36	11.1	11.1
24 hours	••	27	291	9.3	7.3	••	2	35	5.2	5.2
								igures used igures used	in Text in Text	Figure 2 Figure 3

.

TABLE 2

HOURLY PERCENTAGE OF LAGGARDS IN METAPHASE AND POST-METAPHASE

-17-

for Post-metaphase laggards.

It is seen that the same fluctuations are present in both Metaphase and Post-metaphase percentage of laggards. After a slower start the graph of Post-metaphase laggards displays its fluctuations earlier.

Analysis of Clumped Figures

Text Fig. 4 shows the percentage of clumped figures in Metaphase. The percentage of clumps displays a typical sigmoid curve, with a slow increase of clumped figures from two to four hours, then a rapid increase in number from four to eight hours with a leveling off from twelve to twenty-four hours.

Contraction of Chromosomes

The chromosomes at Pro-metaphase we noted as being overcontracted. This condition became more apparent through all stages during the period of treatment.

Short Period Treatment

Three Hour Treatment with Recovery Period

Text Fig. 5 gives a graphical representation of the ratio of relative frequencies of Prophase, Metaphase and Post-Metaphase throughout the three-hour treatment and recovery period. It is seen that Prophase figures tend to decrease in relative number through the treatment period and on to eight hours. By the end of twenty-four hours (twenty-one hours of recovery time) the Prophases have returned to the frequency at which they started. Metaphase figures tend to increase through the treatment an on to six hours (three hours of recovery) with a slight decrease noted

-18-









Treatment	No. of Clumped Figs.	Total No. of Cells	\$	\$ Diff.*
Control	• 0	250	0.0	0.0
2 hours	80	843	9.5	9.5
3 hours	93	753	12.7	12.7
4 hours	98	594	15.0	15.0
5 hours	147	363	24.7	24.7
6 hours	245	419	58.6	58.6
7 hours	206	350	58.8	58. 8
8 hours	287	365	78.6	78.6
12 hours	315	361	87.4	87.4
24 hours	276	291	94.9	94 .9

HOURLY PERCENTAGE OF CLUIPED FIGURES AT 25 PPM

* Figures used in Text Figure 4

,



-+

UPON THREE HOUR TREATMENT AND RECOVERY AT 25 PPM

RATIO OF VARIATION IN FREQUENCIES OF INDIVIDUAL STAGES

·

FREQUENCIES OF INDIVIDUAL STAGES UPON THREE HOUR TREATMENT

AND RECOVERY AT 25 PPM

Treatment	••		Propha	8	••	ę=si	Me taph	8 S O	••	Poi	st-met	a pha se	••	To tal.
	•••••	No. of Cells	₽¢.	Ratio of Treatment to Control*		No. of Cells	b 9.	Ratio of Treatment to Control*		No. of Cells	BR.	Ratio of Treatment to Control		
Control	••	68	33.2		••	61	29.8	I	••	76	37.0	ч	••	205
2 hrs.	••	103	26.8	.81	••	212	56.5	1.90	••	119	16.7	.45	••	384
3 hrs.	••	III	22.4	.67	••	269	53.5	1.80	••	122	24.1	.65	••	502
3 hrs., 1- hr. recov.	••	27	16.3	617.	••	235	1 1 9.8	1.67	••	160	33.9	.92	••.	472
3 hrs. , 3- hrs. reco	• •	78	22.0	.66	•• ••	240	67.7	2.27	•• ••	36	10.2	.28	•• ••	354
3 hrs., 5- hrs. recov	••••	52	11.7	.35	••	266	60.0	2.01	••	126	28.4	27.	••	ヤヤヤ
3 hrs., 21- hrs. recou	••••••••••••••••••••••••••••••••••••••	20	35.7	1.08	•• ••	27	1.8.1	1.62	•• ••	6	16.1	64.	•• ••	56
										₽1E	ures u	sed in Text	1 1 2 1 2	are 5

in the two to four hour period. After six hours the relative number of Metaphases tends to decrease toward the control. Post-metaphase figures decrease in number through the first six hours with the exception of an increase in relative frequency between two and four hours. Between six and eight hours there is an increase, almost reaching the Post-metaphase frequency at the start, which falls away only slightly through the eight to twenty-four hour period.

Thus it is seen that after treatment with Endothal at 25 ppm for three hours, it takes three to five hours before recovery sets in.

In analyzing the percentage of laggards for the three-hour treatment it is noted that there is a gradual increase reaching a peak at six hours and then falling off to zero at Metaphase. The same tendency is seen in the Post-metaphase figures. However, the peak here is reached at four hours. Text Fig. 6 represents this information graphically.

It is seen from Table 6 that the occurrence of clumped figures tends to increase through the treatment period and the degree of clumping is highly variable in the recovery material.

ι..

Four Hour Treatment with Recovery Period

Text Fig. 7 represents the ratio of the relative frequencies of the various stages of mitosis through the four-hour treatment and recovery period. The Prophases are seen to decrease in relative frequency to eight hours and then to recover gradually by the end of twenty-four hours. The frequency of Metaphase figures

-23-



TEXT FIGURE 6

TREATED THREE HOURS WITH RECOVERY PERIOD AT 25 PPM

PERCENTAGE OF LAGGARDS IN METAPHASE AND POST-METAPHASE

v	`
TABLE	

FREQUENCY OF LAGGARDS IN METAPHASE AND POST-METAPHASE

TREATED THREE HOURS WITH RECOVERY

25 PPM

Treatment	••	2	etanhase			••	Pos	t-metaphase	_	
	••	No. of Cells with Laggards	Total No. of Cells	BR.	\$ Diff.*		No. of Cells with Laggards	Total No. of Cells	R.	\$ Diff.*
Control	••	2	61	3.3		••	O	76	0 •0	
2 hrs.	••	28	212	12.9	9.6	••	Г	79	1.6	1.6
3 hrs.	••	53	269	19.7	16.8	••	16	122	13.1	13.1
3 hrs., 1- hr. recov	••	55	235	23.4	20.1	••	58	160	36.3	36.3
3 hrs., 3- hrs. recov	•••••	59	240	24.6	21.3	•• ••	~	36	19.3	19.3
3 hrs., 5- hrs. recov	••••	51	266	19.2	16.9	•• •	22	126	17.5	17.5
3 hrs., 21- hrs. recov		o	32	0.0	0.0	•	1	19	5.2	5.2

* Figures used in Text Figure 6

PERCENTAGE OF CLUNPED METAPHASES IN SHORT PERIOD TREATWENT

AND RECOVERY AT 25 PPM

•

F	hree	e Hour Treatmen	10 4	nd Recove:	b		••		Fou	r Hour Treatment	an S	d Recover	ы	
Treatment		No. of Clumped Figures	••	Total No. of Cells	••	8 8.	••	Trea tment	••	No. of Clumped Fig ures	E⊣ o ••	otal No. f Cells	••	₽¢
Control	••	0	••	61	••	0.0	••	Control	••	o	••	43	••	0.0
2 hrs.	••	39	••	212	••	18.0	••	2 hrs.	••	21	••	241	••	8.7
3 hrs.	••	36	••	269	••	13.4	••	3 hrs.	••	38	••	183	••	20.8
3 hrs., 1- hr. recov	••	Γ η	••	235	••	17.5	••	4 hrs.	••	63	••	209	••	30.1
	••	v	••	Olco	••	и С	••	1 bra	••	ſ	••		••	r 0
hrs. reco	••)	••	042	••	•	••	hrs. reco	•	ſ	••	0	••	
3 hrs. 5- hrs. reco	•	43	••	266	••	16.2	••	4 hrs., 4- hrs. reco	•••	16	••	47 T	••	14.0
	••		••		••		••		••		••		••	
3 hrs., 21	T	Ч		32		3.1		4 hrs., 20	1	0		20		0°0
hrs. reco	:•		••		••		••	hrs. reco	 Þ		••		••	

-26-



FREQUENCIES OF INDIVIDUAL STAGES UPON FOUR HOUR TREATMENT

AND RECOVERY AT 25 PPM

Trestment	••	-7	Propha	8 51	••		<u>Me taph</u>	836	••	Pos	st-mete	10hase	••	<u>lo tal</u>
	••	No. of Cells	R	Ratio of Treatment to Control*		No. of Cells	R.	Ratio of Treatment to Control*		No. of Cells	₩.	Ratio of Treatment to Control'	•	
Control	••	91	46.6	г	••	43	22.1	ч	••	()	31.3	T	••	195
2 hrs.	••	141	30.8	.66	••	541	52.5	2.38	••	27	16.8	.54	••	459
3 hrs.	••	58	18.7	017.	••	183	59.4	2.69	••	67	21.8	.70	••	308
4 hrs.	••	472	25.4	.54	••	209	71.9	3.25	••	ω	27.5	.88	••	291
4 hrs., 2- hrs. reco	• •	27	18.8	041.	••	011	76.4	3.46	••	9	4.2	.13	••	143
4 hrs., 4- hrs. reco	•••••	21	13.8	.30		411	75.0	3.40	•• ••	17	11.9	.38	•• ••	152
4 hrs., 20 hrs. reco	 ⊥ Þ	37	47.5	1.02	••	20	25.6	1.16	••	5	27.0	.86	••	78
										• 1716	ures u	used in Text	i. H	gure 7

-28-

FREQUENCIES OF INDIVIDUAL STAGES UPON FOUR HOUR TREATMENT

AND RECOVERY AT 25 PPM

Treatment	••		Propha	86	••		<u>Me taph</u>	836	••	Pos	st-me ta	inha se	••	To tal
	••	No. of Cells	R	Ratio of Treatment to Control*		No. of Cells	PK.	Ratio of Treatment to Control*	••	No. of Cells	b R	Ratio of Treatment to Control	••	
Control	••	91	46.6	- 7	••	43	22.1	ч	••	61	31.3	1	••	195
2 hrs.	••	141	30.8	.66	••	142	52.5	2.38	••	27	16.8	.54	••	459
3 hrs.	••	58	18.7	017.	••	183	59.4	2.69	••	67	21.8	•70	••	308
4 hrs.	••	44	25.4	.54	••	209	71.9	3.25	••	ω	27.5	. 88	••	291
4 hrs., 2- hrs. reco	 ⊳	27	18.8	017.	••	011	76.4	3.46	••	9	4.2	.13	••	143
4 hrs., 4- hrs. reco	•••••	51	13.8	.30	•• •• ••	4 II	75.0	3.40	•• ••	17	11.9	• 38	•• ••	152
4 hrs., 20- hrs. reco	••	37	47.5	1.02	••	20	25.6	1.16	••	51	27.0	.86	••	78
										₩ 1976	ures u	ised in Text	••• •••	care 7

-28-

.

increases sharply to six hours then decreases to practically normal by the end of the recovery time. Post-metaphase shows a rather fluctuating tendency in that the figures decrease in relative frequency by two hours, increase in the two to four period, decrease from the four to six hour period and gradually increase to nearly normal by the end of the recovery period.

The effects of treatment seem to last for two hours beyond the period of treatment and then the relative frequencies of the various stages return to that of the control slide.

There is a gradual increase in percentage of laggards up to four or six hours then a gradual decrease during Metaphase. A much higher peak is seen in Post-metaphase and this comes in the period of from six to eight hours after start of treatment. This is represented graphically in Text Fig. 8.

Here again there is a lag of at least two hours before the cells start to recover from treatment.

In analyzing the clumped figures it is seen in Table 6 that the frequency of clumped figures increases through the time of treatment and after this period varies highly. This effect was also noted in the material treated for three hours.



TEXT FIGURE 8

TREATED FOUR HOURS WITH RECOVERY PERIOD AT 25 PPM



TREATED FOUR HOURS WITH RECOVERY PERIOD AT 25 PPM

PERCENTAGE OF LAGGARDS IN METAPHASE AND POST-METAPHASE





TEXT FIGURE 8

TREATED FOUR HOURS WITH RECOVERY PERIOD AT 25 PPM

•

FREQUENCY OF LAGGARDS IN METAPHASE AND POST-METAPHASE

TREATED FOUR BOURS WITH RECOVERY

25 PPM

•

Treatment	••	ΣI	le tapha se			••	Pos	t-metaphase		
	••	No. of Cells with Laggards	Total No. of Cells	be	¢ Diff.*	••	No. of Cells with Laggards	Total No. of Cells	BE	g Diff.*
Control	••	Ч	64	2.3		••	0	61	0.0	
2 hrs.	••	13	142	5.4	3.1	••	Ч	27	1.3	1.3
3 hrs.	••	18	183	9.8	7.5	••	2	42	4.8	4.8
4 hre.	••	1 6	192	17.7	15.4	••	r-1	ω	12.5	12.5
4 hrs., 2- hrs. recov	•• ►	18	011	16.4	14.1	••	N	9	33.3	33.3
4 hrs., 4- hrs. recov	•• •• ~	15	119	13.1	10.8	•• ••	9	17	35.2	35.2
4 hrs., 20- hrs. recov	ŗ _	o	ω	0.0	0 . 0		o	2	0.0	0.0

* Figures used in Text Figure 3

DISCUSSION

The point of primary interest in these experiments is the production of an unusually large number of figures exhibiting laggards. Occasionally laggards are found in untreated root tips and a few were found in treatments with Actidione and Streptothricin. However in none of these cases have such a number been noted before.

Formation of Laggards

Figures D, E, F, G, and H, Plate I would seem to indicate a developmental sequence of laggards--starting at Prophase, passing through Metaphase, Anaphase, Telophase and reaching Interphase with one or more micronuclei present. This truly may be the case in some instances, since if the lagging chromosome does not reach the plate at Metaphase when the other chromosomes do, it will very likely continue to be late through Anaphase and Telophase (as is frequently the case with univalents in meiosis). At times it would still be lagging when the nuclear membrane is formed after Telophase and would be incorporated in the cell as a micronucleus.

However, from the current data it is seen that this does not appear to be the complete story. In the first place, Prophase laggards do not seem to occur very frequently, certainly not frequently enough to give rise to the high number of Metaphase laggard figures. In the second place, if Anaphase laggards developed

-32-

only from Metaphase laggards, the peak in number of Metaphase laggards should appear before that of Anaphase. Text Fig. 3 shows that this is not the case. At the end of four hours of treatment the percentage of Anaphase laggards has reached its peak whereas it is not until five hours of continuous treatment that Metaphase laggards are found at the highest percentage. The possibility of toxic effects from the continuous treatment might present a difficulty here. It might be pointed out that the reason for the earlier peak in Anaphase laggards could be due to clumping (toxicity effect) that appears at Metaphase, thus cutting down the number of laggards seen at Metaphase. Therefore, the information on the short treatment is of value. In the material treated for three hours and then allowed to recover, the Anaphase peak occurs before the Metaphase peak. In the material treated for four hours, however, it is found that the Anaphase peak does not occur until after the Metaphase peak, in the recovery period. This piece of information keeps the evidence from being complete and therefore, it is probably best to say that the indication is strong that Anaphase laggards reach a peak before Metaphase laggards. In viewing Text Figs. 6 and 8 a third piece of evidence against the idea that the developmental sequence of laggards from Prophase on is the complete story for the formation of laggards is found. There appears to be a much higher number of Anaphase than Metaphase figures with laggards in both the three hour treatment and the four hour treatment. Here the consideration of clumping can be disregarded. For if clumoing tended to reduce the formation of

-33-

laggards at Metaphase it would also tend to lower the precentage of Anaphase laggards to the same degree. It must be that the effects of Endothal are felt at each stage independently of another stage, i.e. a chromosome could be effected and a laggard initiated at any time during the mitotic cycle. This would account for the additive effect and it would be expected that more laggards would be seen at Metaphase than at Prophase and also more at Anaphase than it Metaphase.

Thus it is seen that there could easily be two methods by which laggards are formed--a developmental sequence of events from an affected Prophase and from an immediate effect, regardless of the stage and ouite independent of the developmental sequence.

Interrelation of Aberrations

At the beginning of the section on observations a concise listing and description of the different classifications was set down. However, it is not as simple a process to place a specific mitotic figure into one of these classifications. It did not take very long to realize that the variation from one category to another was continuous rather than discontinuous.

The easiest figure to classify is the Metaphase figure with laggards. There may be one or more laggards but a definite Metaphase plate is formed and some of the chromosomes do reach it. This is shown in Plate II, Fig. A. However there does come a time when so many chromosomes are lagging that a plate does not seem apparent. Then the figure is considered scattered. Plate II, Fig. C shows this scattered condition which is analogous to the

-34-

C-mitotic effect produced by Colchicine. Midway between these two extremes is found a condition which was labelled segregation. This classification was lumped with laggards for the purposes of analysis because it most frequently seemed to be a case of several of the laggards congregating at one point other than the ecuatorial plate. However, segregations also occur when the rest of the chromosomes are in a scattered condition. In addition to this laggards are found. Should Plate II, Fig. B then be called a scattered, segregated, laggard Metaphase? In doing this a large number of categories would have to be set up, and it would seem that there would always be a figure that would be part way between categories.

There was also a difficulty in classifying the affected unoriented Anaphases and oriented Anaphases. After the dividing of the kinetochores of a scattered Metaphase the chromatids fall apart with no apparent orientation toward the poles. How far one can go in considering these unoriented is purely subjective. For instance, Plate II, Figs. E and F show two degrees of the same phenomenon. Yet one is classified as oriented and the other as unoriented. In Fig. E it is noted that the kinetochores of the chromatids are for the most part directed toward the poles. Thus it was recorded as oriented. Fig. F was classified as unoriented because the kinetochores did not seem as well directed---although there is an indication that they eventually would become oriented toward the pole.

Telophases also posed problems. Nicely oriented Telophases with one to many laggards were found. These fit very well into

-35-

the conception of Telophase laggards. There were also found unequal Telophases in which more chromatids congregated at one pole than at the other. This might be explained by the fact that some of the chromatids were lagging to such an extent that through the movement of the organized chromatids to the poles the two sister chromatids were gathered onto the same pole. Plate II, Fig. H illustrates an unequal Telophase with a laggard. It then presents a problem whether to call this figure a laggard Telophase or an uneoual Telophase. Another difficulty arises when considering unipolar Telophases. Often a unipolar Telophase seems to be segregated or it might be that the two poles of the spindle are very close together. At times laggards are found. Again the question arises -- which is the main disruption, the laggard or the unipolar Telophase? In most questions of this type the abnormal figure was classified under the heading other than laggard so as not to deliberately overbalance the laggard category.

Clumping since it is an indication of toxicity is not too important in the analysis. Yet is interesting to note that this classification too presents the same difficulties of placement among the laggard, segregation, scattered categories. But this is to be expected since they are simply expressions of the type of configuration of Metaphase from which they are reverting.

For these reasons it would seem that laggards, segregated figures, and scattered figures are all variations of the same phenomenon to a lesser or greater degree.

-35-

Laggards and Mitosis Mechanics

It was concluded in the last section that laggards, segregated figures, and scattered figures seemed to be different degrees of the same phenomenon. If this is the case, we are then saying that the force or mechanism which breaks down and produces laggards is the same breakdown that produces scattered Metaphases (C-mitosis).

The problem then arises as to whether the laggards are due to a weakening of the spindle (perhaps a breakdown of a single spindle fiber) or the kinetochore being affected. For the most part the spindle is still in operation. This is indicated by the fact that most of the chromosomes reach the Metaphase plate. It is not easily seen why just one or two fibers out of the whole spindle mechanism should be effected and not the rest. Perhaps it is assuming too much, but it would seem that the cytoplasmically initiated spindle would be uniform and any effect that is felt ought to be felt simultaneously throughout. Now in chromosomes we have a lack of uniformity. We would expect the reaction to an artificial condition to be different. Thus a laggard could easily be explained as an inactivation or partial inactivation of the autonomy of the kinetochore. Walters (1952) indicates that this is the case in the laggerds of Bromus.

The scattered Metaphase or C-mitotic figure could be explained as an inactivation of all the kinetochores but it would be best to include also an effect on the spindle whereby it had been delayed in its formation. For if the spindle had been formed, it would be expected that, at least, some of the chromosomes would have been

-37-

pushed onto the plate. The use of the word delayed in referring to the formation of the spindle stems from the fact that in some of the scattered Anaphases some orientation is seen. The chromatids are pointed toward and headed by the kinetochore in the direction of one of the poles. Therefore, it would seem that the spindle had been set up but at some time later than at Prometaphase or in a less well organized fashion.

Segregated Metaphase figures could then be explained as an inactivation of a large number of kinetochores and the chromosomes just by chance congregating at common places in cases where some chromosomes do arrange themselves on the Equatorial plate. In cases as seen in Plate II, Fig. B the explanation would more than likely be an inactivation of the spindle formation and chance congregation..

The completely scattered Anaphases and unipolar Telophases would then be due to the complete non-functioning of the spindle and to the affected power of the kinetochore that has retained its ability to divide and perhaps even retained its power of motion but now with the spindle gone it has no where to go.

Laggards at Anaphase, Telophase, and the micronuclei could be interpreted as affected kinetochores which have been only partially inactivated. They seem to have retained their abilities but are operating at a lowered efficiency.

-38-

SUMMARY

- 1. The cytological effects of Endothal were investigated.
- 2. Endothal was found to possess antimitotic activity. At high concentrations it was found to be toxic. At 25 ppm at which the runs for this research were carried out nontoxic effects were displayed. These were shown through the production of laggards and scattered Metaphases.
- 3. The types of mitotic deviations were observed and discussed as to the possible relation between them.
- 4. An attempt was made to integrate the deviations found with the theories of the mechanisms of mitosis now prevailing.

BIBLIOGRAPHY

- Allen, N. S., G. B. Wilson and S. Powell (1950) Comparative Effects of Colchicine and Sodium Nucleate on Somatic Chromosomes of Allium and Tradescantia Journ. of Hered. 41: (No. 6) 159-163.
- 2. Battaglia, Emilio (1950) Osservazioni Sull'Azione Citologica di Alcune sostanze coloranti Caryologia, II:223-228.
- 3. Battaglia, Emilio (1949) Sull'Azione Citologica Dell'Etil-Carbamato (Uretano) e del Cicloesil-Carbamato. Caryologia I:229-247.
- 4. Carlson, J. Gordon, (1938) Mitotic Behavior of Induced Chromosomal Fragments Lacking Spindle Attachments in the Neuroblasts of the Grasshopper. Proc. of Nat. Acad. of Sci., Vol. 24: (No. 11) 500-507.
- 5. Hawthorne, Mary Elizabeth, (1951) The Cytological Effects of the Antibiotic Acti-dione. Ph. D. Thesis for Degree at M.S.C.
- Houston, Marilyn Janet (1952)
 Cytological Effects of Certain Organic Chemicals.
 Unpub. Thesis for Degree of M.S. at Mich. State College
- Huskins, C. Leonard and K. C. Cheng (1950) Segregation and Reduction in Somatic Tissues IV. Reductional Groupings Induced in Allium cepa by low Temperature. Journ. of Hered. 41: (No. 1) 13-18.
- 8. Levan, Albert (1938) The Effect of Colchicine on Root Mitoses in Allium. Hereditas Vol. 24:471-486.
- 9. Meyer, James R. (1938) Modification of Mitosis by Chemicals. Science 108:188.
- Schrader, Franz (1944) Mitosis, Columbia University Press, 110 pp.

- 11. Smith, Harold H. and Adrian M. Srb (1951) Induction of Mutations with Beta-Propiolactone. Science 114:490-492.
- Walters, Marta Sherman (1952) Atypical Chromosome Movement in Meiotic Anaphase of Bromus Pitensis x B. Marginatus. Amer. Journ. of Bot. 39: (No. 9) 619-625.
- Wilson, G. B., (1950)
 Cytological Effects of Some Antibiotics.
 Journ. of Hered. 41: (No. 9) 227-231.
- 14. ______ and C. C. Bowen (1951) Cytological Effects of Some More Antibiotics. Journ. of Hered. 42: (No. 5) 251-255.
- 15. Mary E. Hawthorne, and Te May Tsou (1951) Spontaneous and Induced Variations in Mitosis. Journ. of Hered. 42:(No. 4) 183-189.
- 16. Yakar, Hebahat (1952) Mitotic Disturbances caused by Chloranil. Amer. Journ. of Bot. 39: (No. 8) 540-545.

ACKNOWLEDGMENTS

The writer wishes to express her sincere thanks to Dr. G. B. Wilson without whose help this thesis would not have been completed. Yet thanks is a rather inadequate way of expressing gratitude for his help and interest, his ability to provoke thought, and incentive for doing well.

The writer also wishes to thank Mr. Philip G. Coleman for his photomicrography, but mainly for his interest and help.

The writer is greatly appreciative of those who encouraged and sympathized during the writing of this thesis.

DESCRIPTION OF PLATE I

Figure

- A. Prophese from untreated cell.
- B. Metaphase and Anaphase from untreated cell.
- C. Telophase from untreated cell.
- D. Prophase with laggard from material, treated three hours, after one hour recovery time.
- E. Metaphase with laggard from material, treated two hours, after two hours recovery time.
- F. Anaphase with leggard from material, treated three hours, after one hour recovery time.
- G. Telophase with laggard from material, treated three hours after one hour recovery time.
- H. Micronucleate cell from material, treated four hours, after twenty hours recovery time

All treatments were run at 25 ppm.

Scale--one division represents ten microns.



PLATE I

DESCRIPTION OF PLATE II

Figure

- A. Metaphase with three laggerds from material, treated three hours after one hour recovery time.
- B. Segregated Metaphase from material, treated three hours, after five hours recovery time.
- C. Scattered Metaphase from material, treated three hours after five hours recovery time.
- D. Anaphase with two laggards from meterial, treated two hours after one hour recovery time.
- E. Somewhat oriented but disorganized Anaphase, treated three hours after five hours recovery time.
- F. Unoriented Anaphase from material, treated three hours, after five hours recovery time.
- G. Telophase with four lagging chromosomes from material, treated three hours after one hour recovery time.
- H. Unequal Telophase with laggard from material, treated three hours after five hours recovery time.
- I. Unipolar Telophese from material, treated three hours, after one hour recovery time.
- J. Clumped figure with laggard from material treated for eight hours continuously.
- K. Segregated clumped figure from material treated eight hours continuously.
- L. Scattered clumped figure with laggard from material treated eight hours continuously.

All treatments were run at 25 ppm.

Scale--one division represents ten microns.



PLATE II



MAY 11 1962 - ROOM USE ONLY

