VARIATIONS IN THE C-MITOTIC ACTIVITY OF COLCHICINE SOLUTIONS

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Edward A. Greenberg

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This is to certify that the

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Edward Arthur Greenberg

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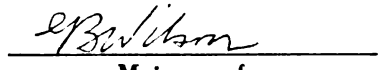

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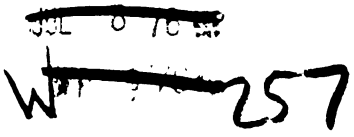

ABSTRACT

VARIATIONS IN THE C-MITOTIC ACTIVITY OF COLCHICINE SOLUTIONS

by Edward A. Greenberg

This study sought to determine whether variations in the c-mitotic activity of colchicine solutions would occur when stored under certain "standard" laboratory conditions, and when exposed to ultraviolet light. It was hoped that if modifications did occur that these could be quantified.

The experimental materials were: seedlings of Pisum sativum var. Alaska; and the alkaloid colchicine in concentrations of 400 ppm (1×10^{-3}M) for exposure to a fluorescent light source and ultraviolet light, and 200 ppm (5×10^{-4}M) for treatment.

It was found that solutions exposed to ultraviolet light lost appreciable c-mitotic activity. Exposure to fluorescent light did not give conclusive results.

The results obtained suggest that it is probable that the beta and gamma photoisomers of colchicine are not very active and may even be toxic.

It was again observed, as in a previous study (Greenberg, 1962), that different samples of the dry powder form of the alkaloid vary in their ability to induce the desired effects in our test system.

VARIATIONS IN THE C-MITOTIC ACTIVITY OF COLCHICINE SOLUTIONS

By

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Plate I. Plants of the genus Colchicum growing in the Horticulture Gardens of Michigan State University.

The Genus Colchicum (Hybrid "The Giant").

Plate I.

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INTRODUCTION

This study has been carried out as a continuation of a previous study (Greenberg, 1962); both have dealt with determining the biological activity remaining in colchicine solutions kept under various "standard" laboratory conditions and after irradiation with ultraviolet light.

The first study (Greenberg, 1962) dealt with colchicine solutions kept in the dark at room temperature (21-23 °C) and under refrigeration (0-5 °C), for predetermined lengths of time.

The present investigation was made to determine whether modifications occurred in solutions of colchicine exposed to fluorescent and ultraviolet light sources. If modifications were observed in c-mitotic activity, it was hoped that these could be quantified by the number and kind of specific cytological configurations produced in a biological test system.

Colchicine solutions have been reported to be unstable by several investigators. Eigsti and Dustin (1955) state that solutions may lose as much as 20 per cent of their activity after 5 weeks. Powell (1951) mentions that the alkaloid maintains its activity rather well when refrigerated. Epstein (unpublished,1955) reports using a solution of colchicine that had been kept under refrigeration for over 7 months, and noted no difficulty in obtaining the desired results in treated Tradescantia staminal hairs. Wood (1957) found through spectrophotometric and chemical

studies, that when colchicine solutions were kept in the dark at room temperature and tested at regular intervals, up to seven months, they did not show appreciable changes. Van't Hof (1961) suggests that colchicine solutions should be prepared five minutes prior to being utilized, because the compound deteriorates rather rapidly in aqueous solutions.

Hadder and Wilson (1958) carried out studies to relate particular concentrations of fresh colchicine solutions to specific effects observed in mitotic cells of Pisum root tips. They proposed a mathematical model which could be used to measure the c-mitotic potency of a compound. This model was utilized in part to analyze the data of this investigation.

Aside from the report of Eigsti and Dustin (1955), and the studies of Wood (1957) and Hadder and Wilson (1958), it appears that no others have been carried out to obtain qualitative and quantitative comparative data on colchicine solutions to be employed in cytological work. Our experience demonstrates that neither the fresh nor the stored colchicine solutions produce a constant effect.

In the previous study (Greenberg, 1962) it was found that:

a. Colchicine solutions stored in the dark at 0-5°C and at 21-23°C for more than 48 hours, showed a progressive loss of activity.

b. The dry powder form of the alkaloid, once

exposed to light and moisture, also showed a loss of activity over a 3 month period.

c. Different batches of the alkaloid appeared to differ in their ability to produce the expected cytological effects.

Our test system was the meristematic portion of Pisum primary roots; specifically those cells which are actively engaged in mitosis.

According to Bowen and Wilson (1954), and Hadder and Wilson (1958), colchicine produces two quite characteristic cytological configurations in Pisum. All other configurations have been found by Hyppio (1954) to be an expression of one of these two configurations. These are the characteristic "clumps" of Powell (1951) and D'Amato (1948a) and "scatter" of Levan's (1938-1939) c-mitosis (colchicine mitosis), reported extensively in the literature. The number of "clumps" and/or "scatters" are a measure of the alkaloid's biological activity at a given time (Hadder and Wilson, 1958).

LITERATURE REVIEW

Colchicine is a compound whose biological, pharmacological and physico-chemical characteristics and activities are complex.

van Tamelen and co-workers (1961) have been able to synthesize the compound, but state that colchicine possesses some unusual features which, combined into a single structure, offer a unique challenge in synthesis.

The substance has been extracted from members of the Liliaceae (Eigsti and Dustin, 1955), especially from the autumn crocus or meadow saffron, Colchicum autumnale L. It has also been extracted as a natural product, together with several other derivatives, from related genera of the sub-family Wurmbaecideae (Cross, 1964 and Kaul, 1964).

The history of the use of colchicine is extensive and reaches far into ancient times, apparently having been used by the Egyptians for the treatment of gout. More recently, after Pernice (1889) discovered its action on dividing cells (Eigsti, Dustin and Gay-Winn, 1949), it has been employed as an antimitotic agent.

Hyppio (1954), Hadder (1957) and Biesele (1958) have reviewed the different aspects of the effect of the alkaloid on mitosis. Stetten (1958), Copeman (1964) and Eschner (1964) have considered the medical aspects of colchicine. A complete review of the importance of colchicine in agriculture, medicine, biology and a discussion of its chemistry, by Loudon, may be found in a book by Eigsti and

Dustin (1955).

Since the discovery of Pernice (1889) many observations have been made on the effects of the drug on mitosis, but the fact still remains that very little is known as to how the compound acts biochemically on mitosis. Eigsti and Dustin (1955) suggest that the compound acts on the spindle by entering into a chemical combination with an intracellular receptor. Mazia (1956) suggests that colchicine affects the "secondary bonding mechanism" of the spindle. Levan and Östergren (1943), and Östergren (1944) mention that colchicine may act as a narcotic which may affect certain metabolic mechanisms involved in the formation of the spindle.

The two principal cytological effects of the compound, which are the most important for this investigation, are the "balled metaphases" of Powell (1951), D'Amato (1948a), Barber and Callan (1943) and Berger and Witkus (1943); and the "scattered" configurations characteristic of Levan's (1938-1939) c-mitosis. Hadder (1957), and Hadder and Wilson (1958) have pointed out that the two configurations, which express different degrees of the same primary effect, are dependent on time and concentration.

Gaulden and Carlson (1949-1951), Levan (1938-1939) and Hindmarsh (1952) believe that the aberrations are a result of the specific affinity of colchicine for the precursors of, and/or the fully formed achromatic figure. The same investigators and others (Levan and Östergren, 1943, Östergren, 1944, Eigsti and Dustin, 1955, Mazia, 1956, and

Morrison and Wilson,1958) are of the opinion that the action of colchicine is to change the spindle from a fibrillar to a corpuscular structure, and this seems to be related to the compound's physico-chemical activities.

Not only has it been difficult to define the biological and pharmacological activity of the alkaloid, but the chemical synthesis of colchicine has proven to be just as difficult.

Early investigators of the chemical nature of colchicine were Houde who first isolated the alkaloid in 1887 (Cohen and Cook, 1940), Windaus (1911-1924), who proposed a phenanthrene ring system (Cook, 1944), Cook (1944), who established that ring B is seven membered, and Dewar (1945), who proposed that ring C was also seven membered (see Figure 1). Thus, with this initial characterization, colchicine, was placed among the tropolone compounds.

Early biosynthetic work carried out to determine the pathways followed in the elaboration of the compound by the plants was done by Walaszek et al. (1952). These investigators used $C^{14}O_2$ and obtained labeled colchicine from the exposed plants.

Leete and Nemeth (1960) administered DL-phenylalanine-3-C^{14} to Colchicum bizantinum and obtained colchicine labeled in ring B. A year later (1961), these investigators reported using sodium acetate-1-C^{14} and obtained the label on the N-acetyl group, and with L-methionine-methyl-C^{14} they obtained colchicine labeled on the O- and N-methyl groups.

Leete (1963) using DL- phenylalanine-2-C^{14} observed activity in C_6 of ring B.

Battersby et al. (1964), state that methods using tracer experiments establish that ring A and carbons 5, 6 and 7 of ring B are derived phenylalanine and cinnamic acid.

Leete (1965) proposes a biosynthetic pathway for colchicine and also mentions that Battersby (1964) was able to show that tyrosine-3^1-C^{14} is incorporated into C_{12} of ring C. He also found that feeding DL-tyrosine-4-C^{14} to sprouting Colchicum bizantinum corms labeled colchicine at C_9 of ring C.

Pertinant to this investigation has been the knowledge of the apparent instability of colchicine solutions, especially when exposed to sunlight and ultraviolet light. There appears to be a correlation between the particular structure of the isomers and their capacity to act as effective antimitotics, the configuration of ring C being of most importance.

Grewe (1946) reported that when a colchicine solution was irradiated with ultraviolet light, the characteristic absorption peaks of 250 and 350 mu gradually disappeared and a new one appeared at 270 mu. He found that the new peak represented the formation of a photoisomer of colchicine named "Lumicolchicine". In 1951 the same investigator and W. Wulf exposed a 0.2 per cent colchicine solution to sunlight, obtaining after 5-7 weeks a crystalline precipitate. This precipitate could be separated into three fractions on

the basis of differential solubilities in alcohol. The fractions turned out to be the alpha, beta and gamma-lumicolchicine isomers, representing 95 per cent of the original colchicine solution.

Forbes (1955), through degradative and spectroscopic studies, has gathered evidence suggesting that beta and gamma-lumicolchicine are stereoisomers having tetracyclic structures (Figure 1). This isomerization occurs through the rearrangement of ring C into a four carbon C ring and a five carbon D ring. He also stated that he was able to obtain all three isomers by exposing a solution to sunlight, having first excluded air from the solution with nitrogen. The beta form was obtained in the largest amounts.

Gardner et al. (1957), in their investigation, noted the conversion of beta-lumicolchicine to the gamma form, thus lending support to the hypothesis of a stereoisomeric relation between them. They also reported isolating the alpha form but were later unable to repeat this (Chapman, 1963).

Schenk, Kuhn and Neümuller (1961) transformed alpha-lumicolchicine into the the beta and gamma photoisomers.

Chapman et al. (1963) have established the structures of alpha and beta-lumicolchicine and present good evidence for the structure of the gamma isomer. Their studies involved use of nuclear magnetic resonance techniques and deuteration. Most interesting is the establishment of alpha-lumicolchicine as a dimer of the beta isomer of colchicine. They also found that the alpha form is not easily obtained

by irradiation with a mercury arc lamp as previously reported. A cyanine dye filter is required to obtain at least a 20-30 per cent yield.

Investigations have also been carried out with a stereoisomer of colchicine, isocolchicine, by Steinegger and Levan (1947). The only difference structurally is the exchange of positions of the =O and $-OCH_3$ groups of ring C. Such an exchange of positions has been noted by these investigators to reduce the biological activity from 70-100 times in comparison to colchicine. The suggestion is put forth that the activity of colchicine and related compounds is regulated by their thermodynamic activity. Colchicine, it is claimed, looses its specific activity upon being transformed into isocolchicine, which then acts only by its physical activity.

Dauben and Cox (1963) photoisomerized isocolchicine to the lumiisocolchicines in order to obtain evidence for the particular structures of beta and gamma-lumicolchicines.

Chapman *et al.* (1963) have also carried out studies on the photoisomerization of isocolchicine and obtained 40-50 per cent yields of lumi-products having ultraviolet light absorption peaks similar to those of beta and gamma-lumicolchicine.

The synergistic and antagonistic effects of other compounds on the antimitotic activity of colchicine have been reported by Eigsti and Dustin (1955), Frezzi and Balin (1956), Morrison and Wilson (1958) and Biesele (1958).

Figure 1. Structures of Colchicine and its Isomers.

I. Colchicine (Chapman *et al.*, 1963).

II. Isocolchicine (Steinegger and Levan, 1947).

III. Lumiisocolchicine (Chapman *et al.*, 1963 and Dauben and Cox, 1963).

IV. Gamma-lumicolchicine (Chapman *et al.*, 1963).

V. Beta-lumicolchicine (Chapman *et al.*, 1963).

VI. Alpha-lumicolchicine (Chapman *et al.*, 1963).

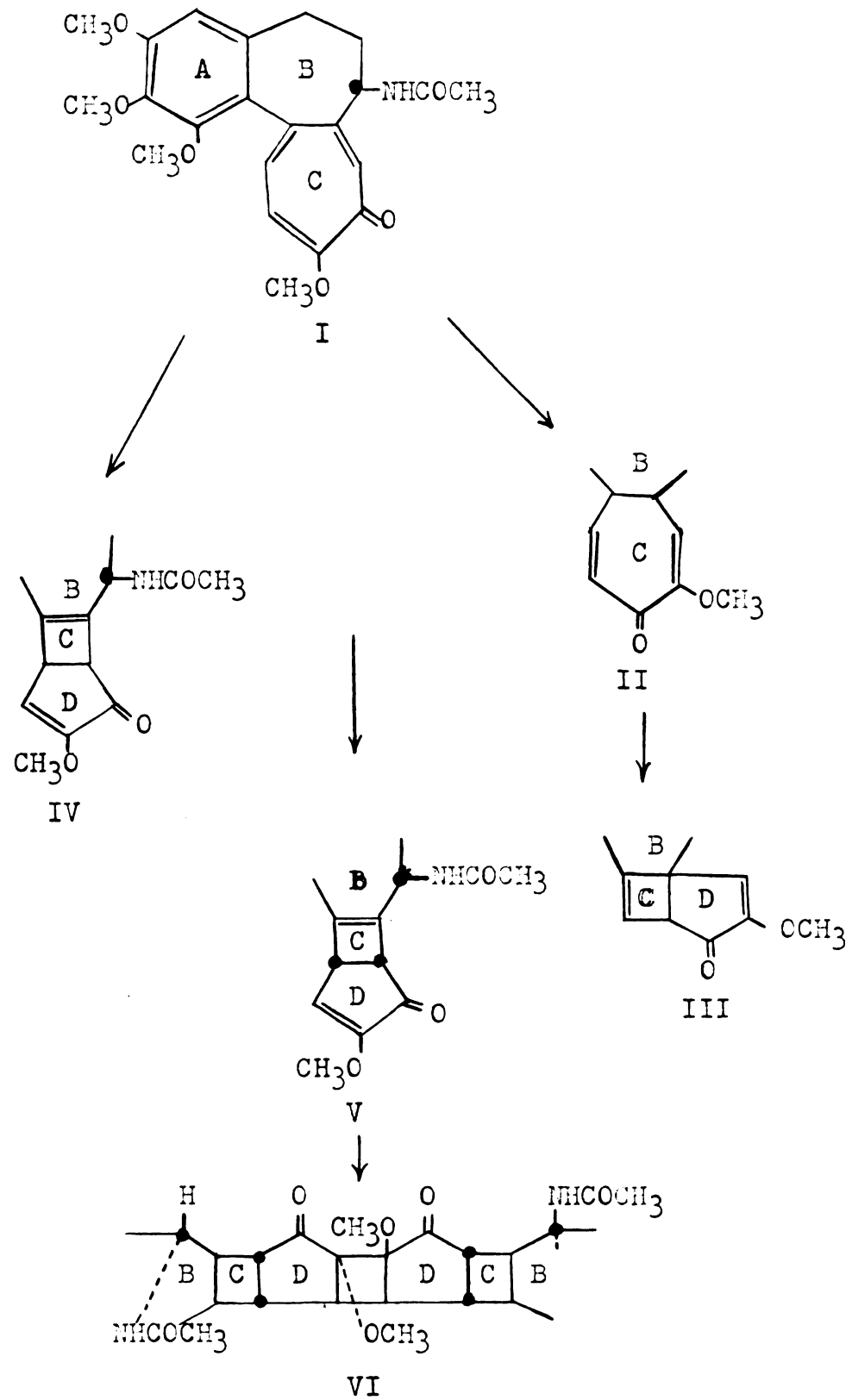
CH3O
CH3O
CH3O
A
B
C
NHCOCH3
O
CH3O
I
B
C
OCH3
O
II
B
C
D
O
CH3O
IV
NHCOCH3
B
C
D
O
CH3O
V
B
C
D
OCH3
O
III
H
O
O
NHCOCH3
CH3O
B
C
D
D
C
B
NHCOCH3
OCH3
VI

Figure 1.

Although of general interest in providing comparative data, they still are not readily interpretable.

Studies on the mass spectra of colchicine compounds have been carried out by Wilson et al. (1963), and optical rotation measurements of the same by Irbek et al. (1964).

Cross (1964) and Kaul (1964) have recently isolated colchicine, lumicolchicines, and other colchicine-like compounds from some genera of the sub-family Wurmbaeoideae. They have thus shown that some compounds made synthetically exist as natural products. Forbes (1955) reports that Šantavý isolated beta and gamma-lumicolchicine as a natural product in 1951-1952.

Physical and chemical studies have contributed much information as to the particular structures and physico-chemical activities of colchicine and its isomers. Comparative quantitative studies should be performed to relate specific isomeric configurations to the ability of inducing certain effects on biological systems, as has been done by Steinegger and Levan (1947). Hadder and Wilson (1958) have proposed a model system which could possibly be correlated with our present knowledge of the compounds and which could relate specific structures to biological activity or function.

MATERIALS AND METHODS

GENERAL EXPERIMENTAL PROCEDURES

The meristematic root tip of the common garden pea, Pisum sativum var. Alaska, was used as the biological test system in this investigation. The peas were guaranteed to be free from chemical treatment by the supplier, the Ferry Morse Seed Company.

The chemical, colchicine, was obtained from the Light's Chemical Company Ltd., Bucks, Colnbrook, England.

The investigation was divided into two parts. The first was concerned with exposing a 400 ppm (1×10^{-3}M) colchicine solution to a fluorescent light source at room temperature; the second part consisted of exposing a solution of the same concentration to ultraviolet light. The light experimental runs were grouped into 12 hour, 3 week and 15 week exposure periods. The ultraviolet light experiments were carried out for only 24 hour exposure periods.

When solutions were exposed for as long as 3 to 15 weeks, it was necessary to autoclave all glassware and Seitz-filter the solutions. Previous experience (Greenberg, 1962) demonstrated that the solutions were capable of supporting bacterial and fungal organisms over a range of temperatures.

The solutions and glassware utilized in the ultraviolet experiments did not have to be treated in any special manner.

In order to insure that the dry powder stock of col-

chicine would not be modified in any way, it was stored in an evacuated desiccator and kept at 0°C.

Fluorescent Light Experiments:

Each 400 ppm colchicine solution for these experimental runs was divided into three aliquots. One was used as the fresh control treatment, a second one exposed to the light source, and the third, when run, was placed in the dark and kept at 22.5°C for as long as the solution exposed to light.

Exposure of the solutions was carried out in a lined orchidarium to insure that the light from another source would not fall on them. The temperature was kept at 22.5°C. The solutions exposed to light were placed two feet beneath two 20 watt cool-white Kenrad Fluorescent Lamps set parallel and one foot apart.

Three exposure periods (12 hours, 3 weeks and 15 weeks) were chosen in accord with previous experience (Greenberg, 1962).

The third aliquot, whenever run, was put in a flask covered with aluminum foil and placed under a small cardboard box, also lined with foil. This solution was also kept, under the conditions mentioned, in the orchidarium.

At the end of the exposure period each solution was mixed with the appropriate amount of nutrient to give a 200 ppm (5×10^{-4} M) solution with which to treat the peas.

Ultraviolet Light Experiments:

Again each 400 ppm colchicine solution was divided into three aliquots. These were a fresh treatment control, a solution to be exposed to ultraviolet light, and one to serve at times as the dark room temperature control.

Four experiments were performed: one at 34°C, and the other three at 22.5°C.

Three hundred milliliters of solution to irradiated were placed in 100 x 50 mm pyrex crystalizing dishes and exposed to an ultraviolet source for 24 hours. The source was placed 1 cm above the free surface of the solution.

Two different energy sources were utilized. The solutions used in the first two runs (one at 34°C and the other at 22.5°C) were exposed to a short wave, mercury quartz-type table lamp (Model V 41) manufactured by U V Products Inc. of San Gabriel, California; who specify that the lamp delivers a peaked beam of 2537 Å at two feet. The other two runs were irradiated with a 17"-15 watt G.E. Champion Germicidal Lamp which also delivers a beam at 2537 Å.

To guarantee that the solutions were evenly irradiated a magnetic teflon-covered stirring bar was activated by a magnetic stirrer. The variable resistance of the stirrer was by-passed when it was found that it would heat the solutions above 22.5°C over a 24 hour period. Thus, the knob on the stirrer was set at its highest reading (zero resistance) and the system was connected to a Powerstat. This system provided excellent stirring velocity control and eliminated the heating problem.

The whole system was placed in a standard chemical hood with the glass gate lined with aluminum foil. This was done to prevent either sunlight or artificial light from falling on the solutions. A constant stream of air going through vents in the gate and out through an exhaust fan in the hood kept the solutions at 22.5 °C.

Agitation, temperature and air flow caused some evaporation from the surface of the solutions. Since a measured quantity was irradiated each time, the volumes were easily adjusted at the end of the exposure period with distilled deionized water.

Preparation of the Biological Test Organism:

The seeds were soaked in distilled deionized water at 25 °C for six hours in a germinator prior to germination. These were then rolled in moist paper toweling and placed upright in beakers with an inch of distilled water. Each paper roll was covered with a layer of waxed paper to prevent excessive evaporation. The peas were then placed in a germinator at 25 °C for from 36 to 40 hours allowing the seeds to grow primary roots 2.5-3.0 cm in length.

At the end of the germination period the seedlings having the required root length were suspended on 1/4 inch waxed wire meshes and the roots allowed to remain in a 1/4 strength Hoagland nutrient solution for two hours.

After a two hour equilibration period one dish of peas was allowed to remain undisturbed to serve as the un-

treated control. The remaining seedlings were transferred to the 200 ppm test colchicine solutions, and maintained in them for 30 minutes; after which they were removed, washed with distilled water and returned to a nutrient solution. All solutions were filter-aerated for the duration of the experimental run. This was done to aerate and agitate the solutions so that all the seedlings received the same amount of oxygen and nutrients.

The concentrations chosen, especially the ones used in treating the seedlings, are based on past experience and the suggestion by Van't Hof that the most successful concentration for treating Pisum is 150 ppm (3.75×10^{-4}M). Because difficulty was encountered in obtaining the desired effect at this concentration a 200 ppm solution was chosen. Good results were obtained and a fairly rapid recovery period was observed, without evidence of toxicity with the fresh control solutions.

A 400 ppm (1×10^{-3}M) solution was chosen as a stock and experimental solution, because it was close to the treatment range and easily adjustable for experimental purposes.

Five primary roots were taken from the seedlings in each culture dish every two hours after the initiation of treatment for ten hours. The excised primary roots were placed in Pinaar's fixative, consisting of a 6:3:2 mixture of absolute methanol, chloroform and propionic acid, and evacuated for ten minutes. The appropriately coded vials were then capped and placed in a refrigerator for 24 hours.

The primary root meristems were prepared for analysis by the Feulgen "squash" technique after the roots had been hydrolyzed in 1N HCL at 60°C for 18 min. The prepared slides were then dehydrated in 9:1 tertiary-butyl alcohol and absolute ethanol for at least 12 hours, then made permanent with diaphane. These slides were then examined and scored for effect.

The characteristic "clump" and "scatter" configurations (Plate II, Figures A-B) induced in dividing cells of Pisum root meristems (Powell, 1951) were used as the diagnostic index in this investigation. Each "altered" solution of colchicine was compared to a solution kept in the dark at room temperature, and to a fresh "unaltered" solution, by recording the number of "clumps", "scatters" and normal mitotic figures occurring in the meristematic tissues. Untreated control seedlings were also examined and scored so as to have a control index of mitosis.

The "clumps" and "scatters" were either recorded per one thousand cells scored at random or per two-hundred post-prophases ("clumps", "scatters" and normal post-prophases). Four out of every five slides prepared per sampling period were scored for the particular configurations mentioned, and the averages recorded.

Indices of effect (Hadder and Wilson, 1958) were obtained at two hour intervals up to six hours after the initiation of a 30 minute treatment with colchicine. Curves were plotted for these points and the area under these

curves served as a measure of effect. These areas, in arbitrary units, were then used to compare fresh treatment solutions with "altered" colchicine solutions.

OBSERVATIONS

Colchicine produces several well known and specific aberrations in mitosis. Specifically its action seems to be on the precursors of, and/or the achromatic figure. This, then, places the action of the compound at the prometaphase, metaphase and anaphase stages.

A characteristic effect observed in the cells of treated pea seedlings is the "scattered metaphase" of Levan's c-mitosis. It has been suggested that this is a partial effect on the spindle (Levan,1938-1939, Barber and Callan, 1943, D'Amato, 1948a, Eigsti and Dustin, 1955, Hadder, 1957). The second effect noted and believed to be due to a complete inactivation of the spindle is the "clumped configuration" of Powell (1951), Bowen and Wilson (1954), Hadder and Wilson (1958), Van't Hof (1961) and Greenberg (1962) for Pisum, which eventually leads to polyploidy (Van't Hof, 1960-1961).

Hadder and Wilson (1958), based their investigation upon the degree of effect observed after treatment of pea seedlings with fresh colchicine solutions of different concentrations. Their study suggested that "scatters" appear first in time and "clumps" later, providing the dose is high enough to produce them. They emphasized that "scatters" can not give rise to "clumps", since with continuous low dose treatments only "scatters" are observed. They conclude that,"'scatters' and 'clumps' express different degrees of the same effect, and that the degree of effect is dependent upon dosage, which may be expressed as duration of exposure

to a given concentration."

The activity of the colchicine solutions was measured by means of a "Colchicine Index of Effect" devised by these investigators. The configurations appearing after treatment had to be distinguished from each other. Since "scatters" appear before "clumps", and are the only configuration appearing with low doses, and since it was assumed they represent a less severe cytological effect than "clumps", they were multiplied by a factor of one and "clumps" by a factor of two.

Configuration	Symbol	Weight
Clumps	Z	2
Scatters	Y	1
Normal Post-proph.	X	0

The total number of post-prophases (n) scored per slide was approximately 200 in all cases.

The Index of Effect is expressed by the following formula:

$$\text{I. E.} = \frac{1(Y) + 2(Z) + 0(X)}{n}$$

It was shown that this index changes smoothly with time, and the rate of change is dependent upon dose, giving a measure of activity or potency at any given time.

Van't Hof (1960-1961) determined that concentration and duration of treatment are important in producing the best reaction and recovery from the effects of the alkaloid.

The reaction time is that period during which chromosome configurations ("clumps" and/or "scatters") leading to tetraploidy are observed in significant numbers, and recovery period the interval from peak reaction (which occurs at two hours after the initiation of treatment) to the point where ten or less per thousand figures showing the effect are observed. He concludes, "...ideally the duration of the treatment should be as short as is consistent with a rapid rise in and recovery from the effect of the alkaloid."

In this investigation, as well as in the previous one (Greenberg, 1962), a modification of Hadder and Wilson's (1958) study and the incorporation of Van't Hof's (1960) ideas were followed in obtaining measurements of the effect of the colchicine solutions.

Indices of effect were plotted at intervals of two hours from 0-6 hours after the initiation of treatment. From the curves for these indices areas were obtained in arbitrary units. These areas give a measure of the total reactivity of the colchicine solutions and were used to compare fresh solutions with each other and to their respective "altered" solutions. Instead of treating the seedlings continuously for the duration of the experiment as had been done by Hadder and Wilson, the seedlings were exposed to a half hour pulse treatment as was suggested by Van't Hof.

Data and information have been included in the tables in the appendix of this manuscript from the previous study

(Greenberg, 1962) dealing with observed variations in the effectiveness of colchicine kept under a variety of "standard" laboratory conditions. They have been included in order to present a general picture of observed modifications of colchicine effectiveness under these conditions.

The data for the indices of effect and areas under the curves for these indices are found in Tables III and IV of the appendix.

Representative curves are shown in Figures 2-7 for the data taken from Tables III and IV of the appendix and included in Table 1. They were chosen because they represent what has been observed for some time by several investigators in our laboratory, but have not been rigidly quantified and compared.

Figures 2, 3 and 4 represent data obtained for a fresh treatment solution, a solution stored 24 hours in the dark at 22.5°C, and a solution irradiated 24 hours at 34°C, with ultraviolet light.

When the solution which was stored for 24 hours in the dark at 22.5°C is compared with its fresh counterpart a loss of 15 per cent in activity is observed. The solution irradiated for 24 hours shows a loss of as much as 74.5 per cent in activity.

Figures 5, 6 and 7 represent data for a fresh solution, a solution stored 3 weeks in the dark at 0-5°C, and a solution stored in the dark at 22.5°C for the same length of time.

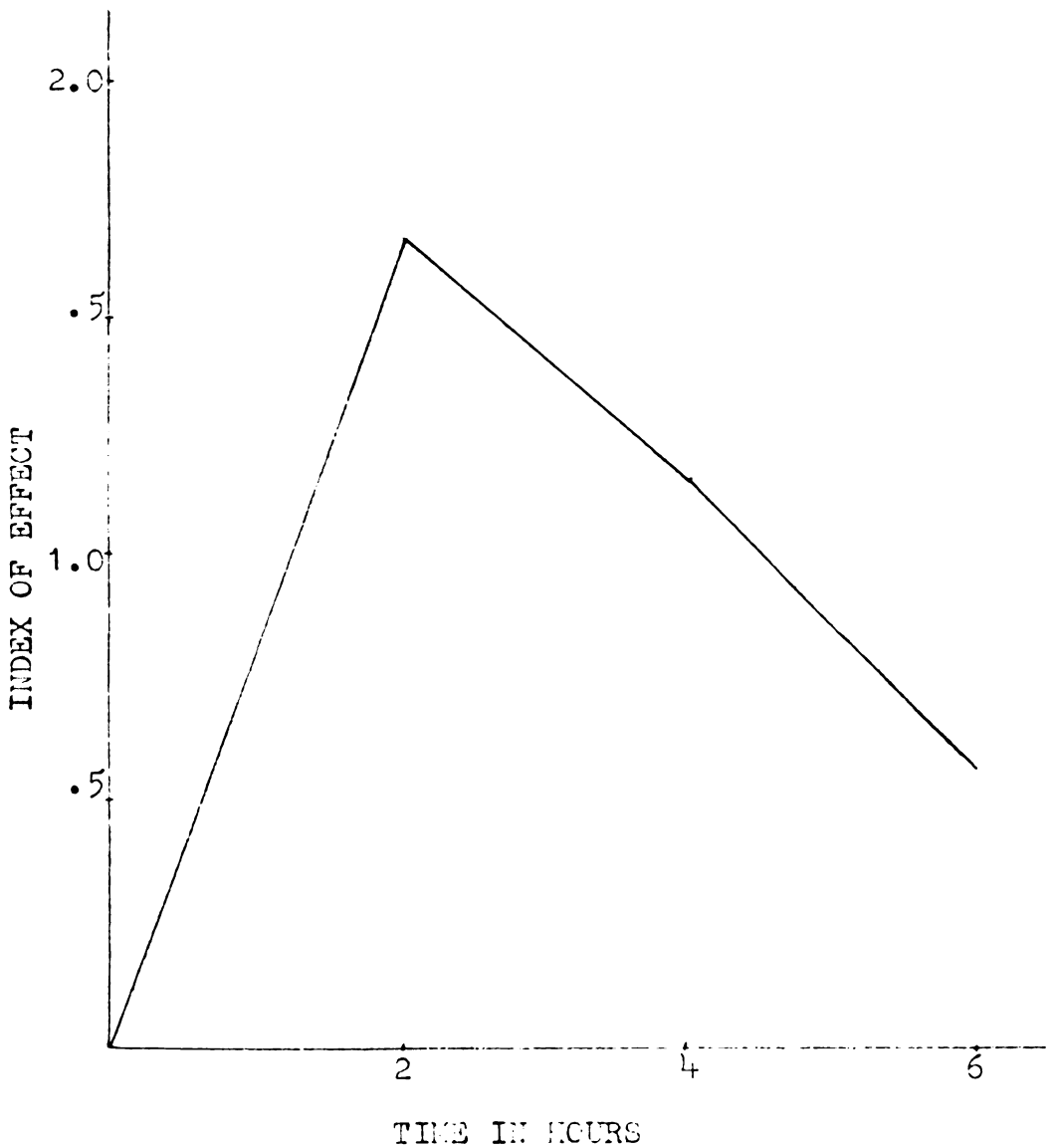

Figure 2.

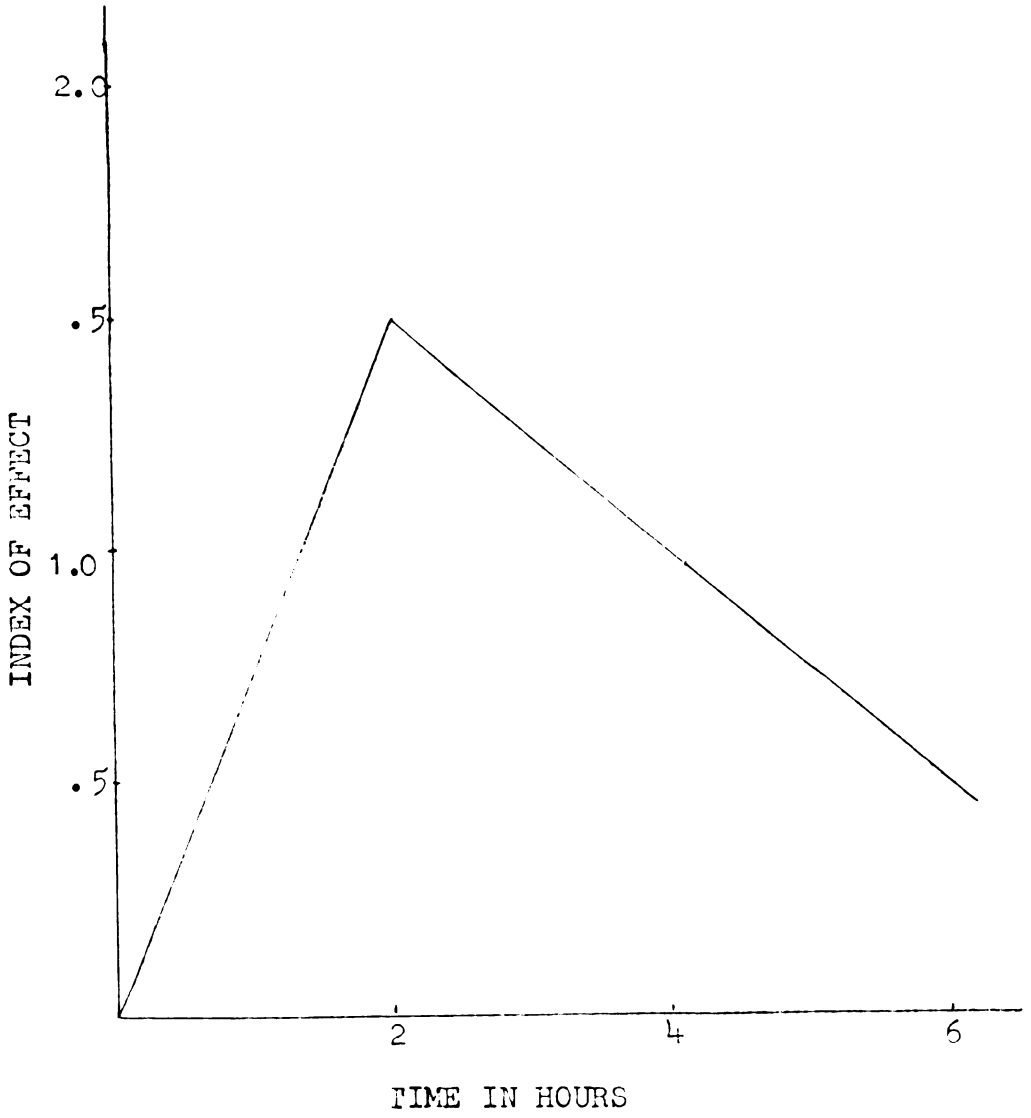

Figure 3.

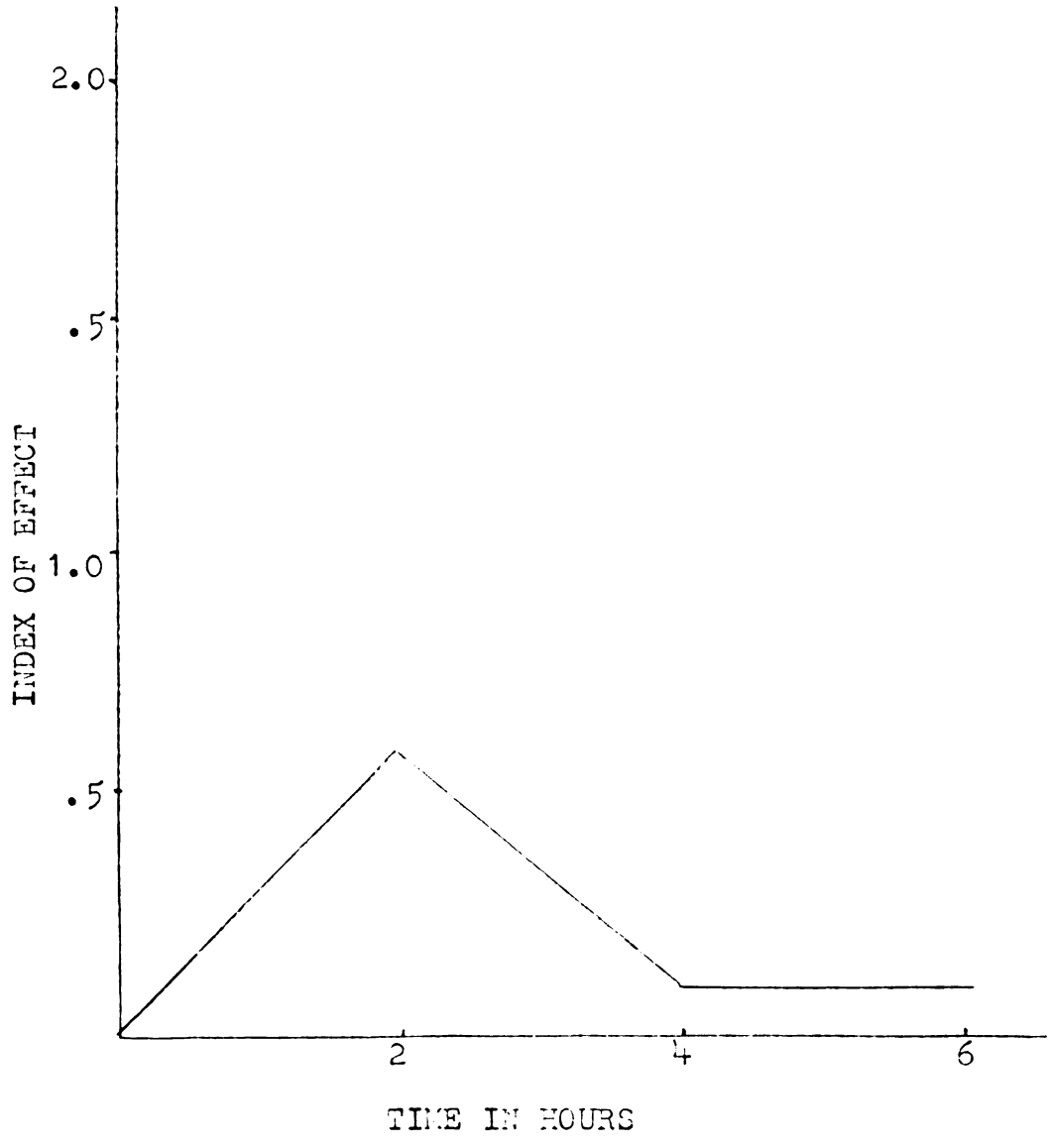

Figure 4.

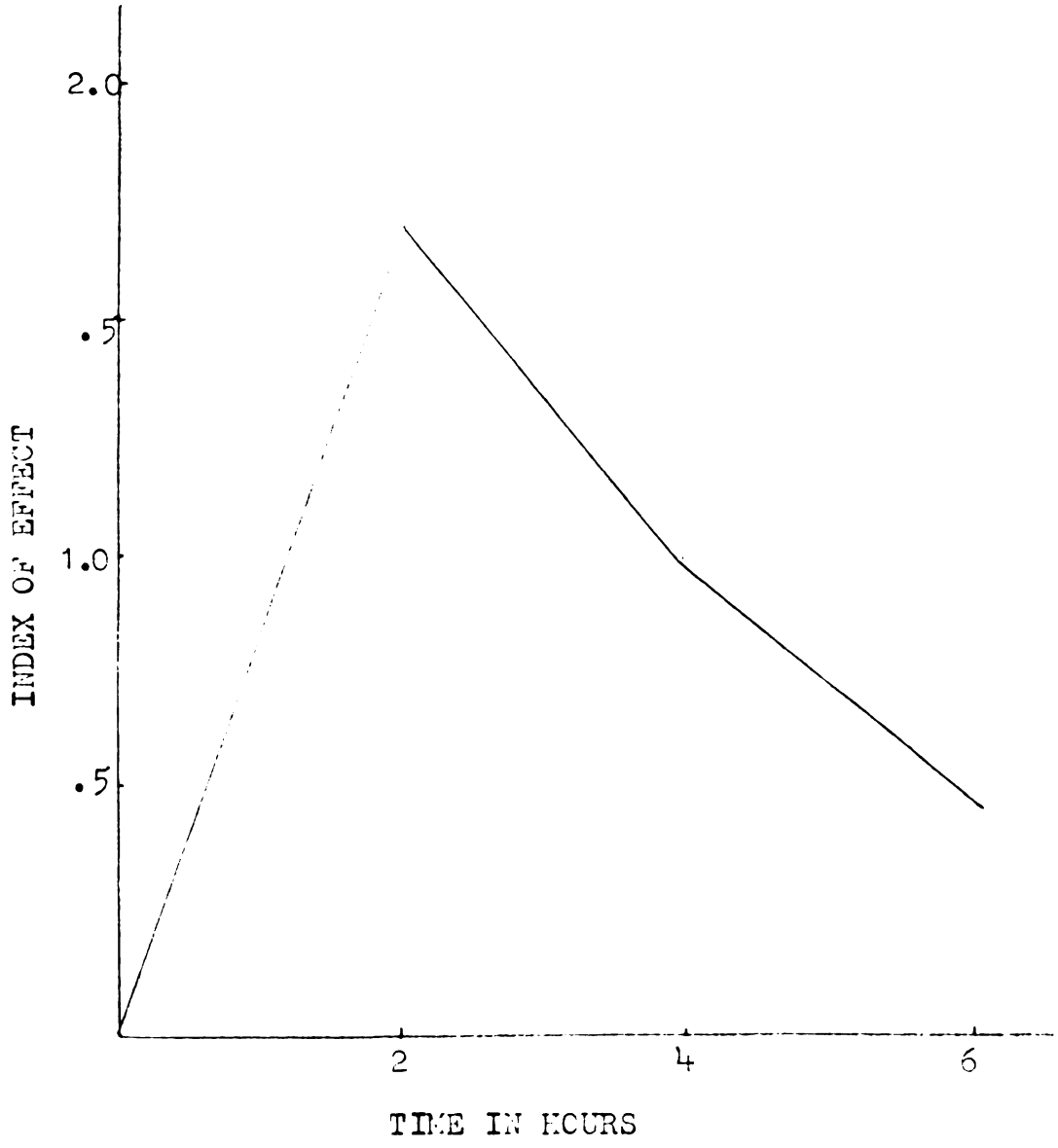

Figure 5.

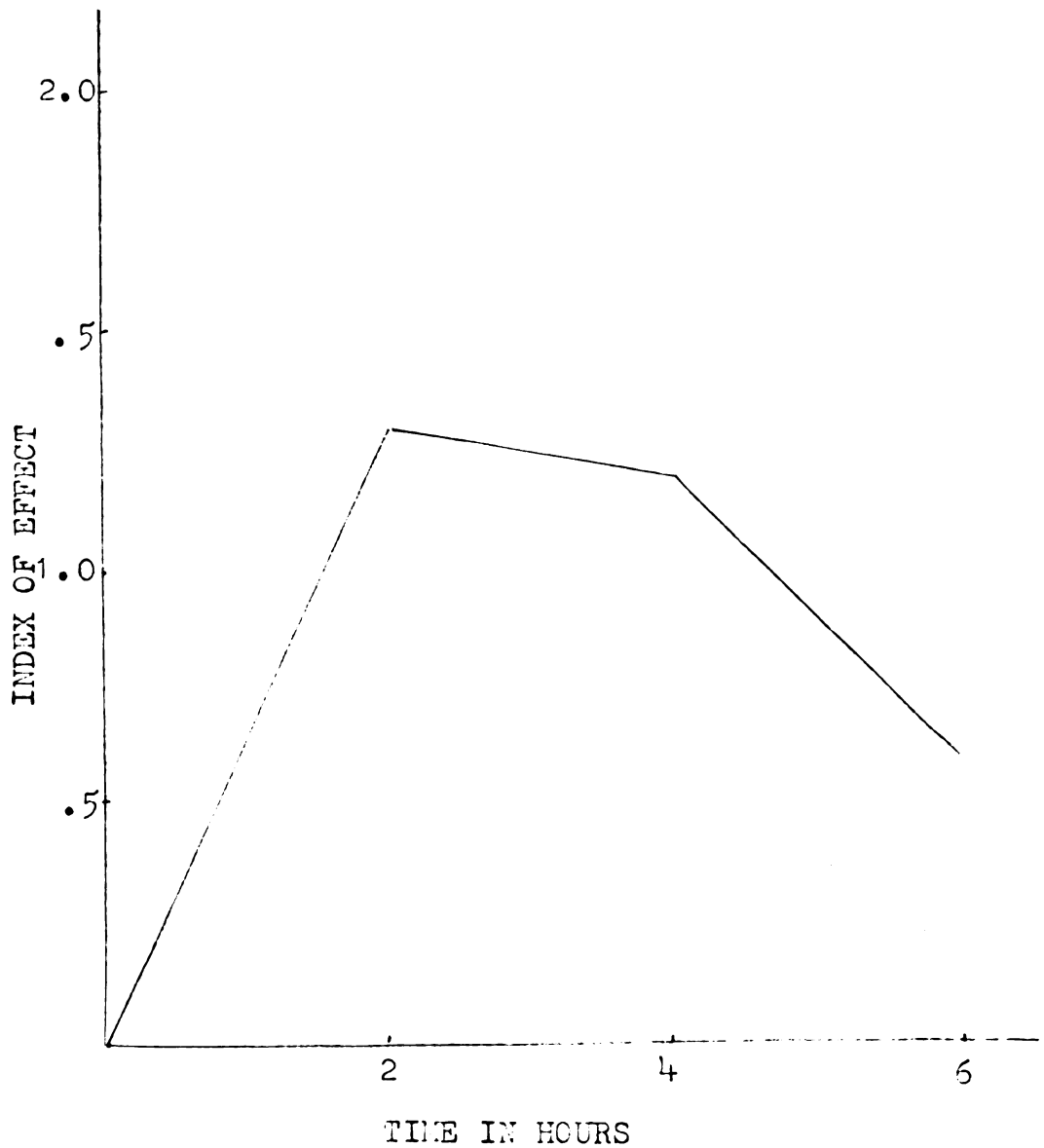

Figure 6.

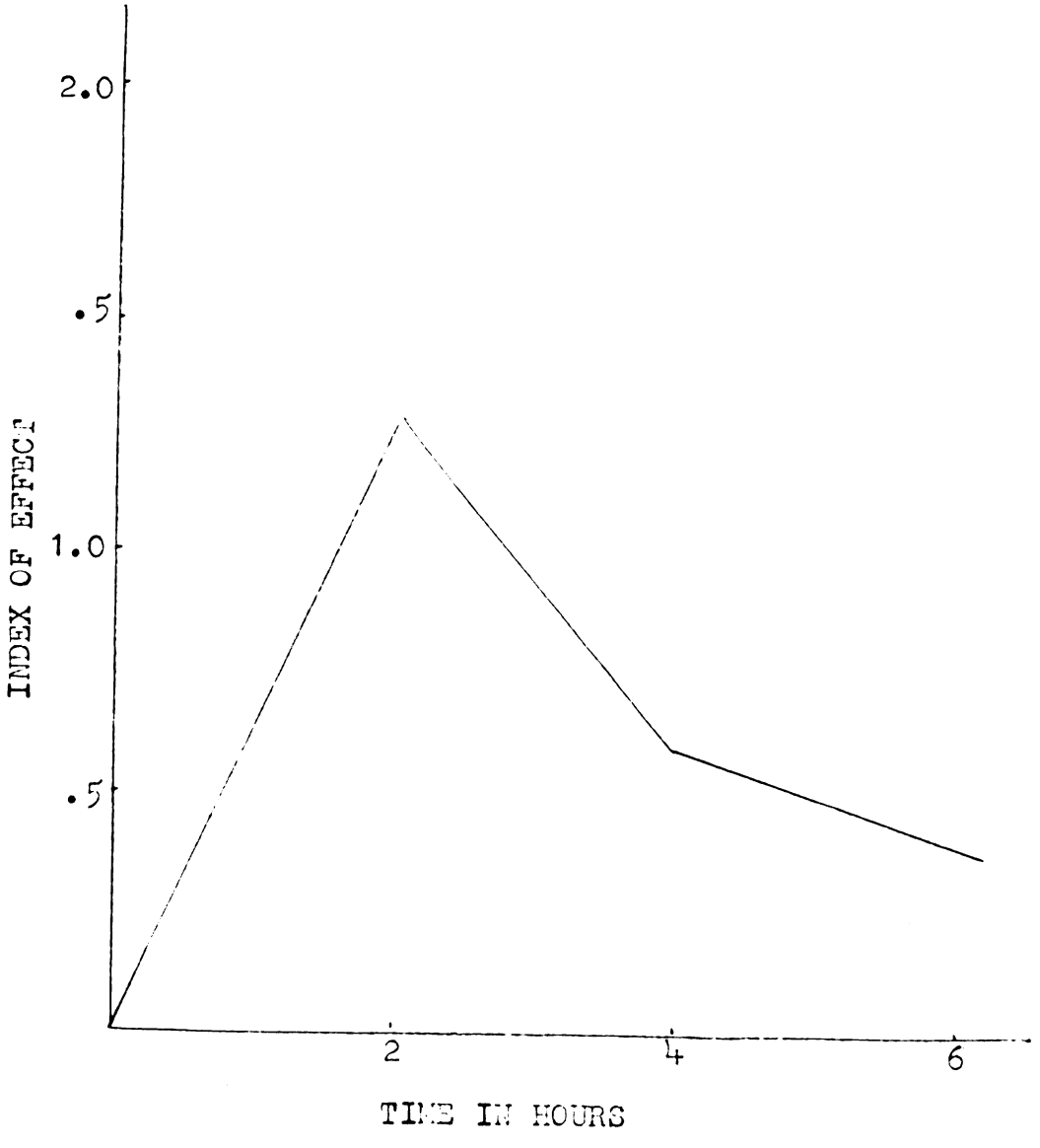

Figure 7.

TABLE I

		Index of Effect	% Loss	Area in Arbitrary Units
Hrs. 2	Fresh.	1.7		
4		1.2		12.0
6		.6		
Hrs. 2	Stored 24 hrs. in the dark at 22.5 C.	1.5		
4		1.0	15.0	10.2
6		.5		
Hrs. 2	Irradiated 24 hrs. with uv at 34 C.	.6		
4		.1	74.5	2.7
6		.1		
Hrs. 2	Fresh.	1.7		
4		1.0		11.0
6		.5		
Hrs. 2	Stored 3 weeks in the dark at 0-5 C.	1.3		
4		1.2	5.0	10.4
6		.6		
Hrs. 2	Stored 3 weeks in the dark at 22.5 C.	1.3		
4		.6	29.0	7.8
6		.4		

Again it may be observed that the refrigerated solu- seems to have lost about 5 per cent activity, while the solution kept at 22.5°C has lost about 29 per cent of its activity.

The percentage losses were obtained by using the following formula:

$$\text{Per cent loss} = \frac{S_1 - S_0}{S_0} \times 100$$

S_0 = area under the fresh solution curve.

S_1 = area under the "altered" solution curve.

In general, there is a slight decrease in activity with an increasing length of time of exposure to higher temperatures and artificial light. When solutions are exposed to ultraviolet light source there is a considerable loss of activity.

Solutions exposed to artificial light for three weeks and fifteen weeks showed no apparent loss of activity, the solution exposed for twelve hours showed 9 per cent loss of activity. Since the observed results give conflicting data they are not explainable at this time.

No fresh control was run for solution (3) exposed to light for 3 weeks and for solution (2) exposed for 15 weeks. Since they were prepared from the same sample of dry powder and at the same time as their respective solutions (2) and (1), it is believed that they are comparable.

This investigation has shown that different batches

of the dry powder do not have the same biological activity. This may be observed when the activities of the fresh solutions are compared. There may be as much as 57 per cent difference in activity.

Table II of the appendix contains data on the Mitotic Indices, which represent the number of dividing cells per thousand scored at random, for the experimental runs. This particular measurement serves as an indicator which would show-up any discrepancies in the test organism. Any variability in the test organism would modify the experimental results. It has been found and confirmed many times in our laboratory that the average mitotic index of the "Alaska" variety of Pisum sativum is close to 71.

DISCUSSION

The purpose of this investigation, initiated to determine in a quantitative manner changes in the biological activity of colchicine when kept under certain "standard" laboratory conditions and when exposed to ultraviolet light, was attained in part.

Answers were sought to such questions as: "Does the compound remain stable either in the dry powder form or in solution?" "What laboratory storage conditions are best suited for an unstable compound such as colchicine?" "How long may the compound be stored before any detectable changes are evident or of major significance?"

The action of colchicine on mitosis has been difficult to assay and even today biochemical evidence for its "singular" action is conspicuously lacking. Complicating the issue are the effects of a vast number of chemicals, some related to colchicine and others not, which seem to behave as mitotic poisons in a manner similar to colchicine. This has caused much controversy over when and how the chemicals act on mitosis. Biesele (1958) points out that mitotic poisons have been classified not so much on the basis of their chemical structures, but rather on their effects on mitosis or by the type of aberrations they produce in different phases of mitosis. This author points out that the difficulty encountered in the classification of mitotic poisons consists in the fact that a given agent may induce more than one type of aberration, and that these may vary in intensity of ex-

pression and the time at which they appear. They have been found to be dependent on the time of exposure and concentration utilized.

Hyppio and Wilson (1955), interpret mitosis as a process composed of a number of cycles of which spindle formation, function and dissolution comprise a part. It is to these particular phases of the mitotic process that Bowen and Wilson (1954) assign the action of colchicine. The process of spindle formation and function should not be considered a single cycle, but rather the integration of several metabolic cycles which eventuate in spindle formation and function. Evidence for this comes from Biesele (1958), who states that there are three possible points of attack on the spindle formation:

a. Inhibition of the formation of the apparatus from sulfur containing protein, RNA and polysaccharide.
b. Inhibition of disulfide-bridge formation (Mazia, 1955) by agents which disturb the oxidation-reduction condition of the cells, and by sulfhydryl reactants.
c. Inhibition of the "secondary-bonding mechanism" described by Mazia (1956) as essential in spindle formation.

Interference with any one of these stages would lead to inactivation of the spindle, and thus of chromosome movement in mitosis.

The uniqueness of colchicine may be seen when it is compared with the many compounds which are known to affect the spindle. Colchicine is not toxic over the range of concentrations and conditions in which other compounds with alleged c-mitotic activity are very toxic or ineffective (Eigsti and Dustin,1955). Eigsti and Dustin (1955) also make it quite clear that when comparing the effects of colchicine on the spindle, no general statement may be made which can be applied to all living organisms. An example is the finding by Levan and Östergren (1943) that colchicine is active at lower doses on Pisum than it is on Allium.

Although considerable studies have been made with many compounds which seem to confuse the issue, much has been learned from them. Eigsti and Dustin (1955) and Biesele (1958) point out that studies have been carried out to determine what groups are necessary for the activity of the colchicine molecule. They also report that modifications of the molecule have been made in order to determine which ones, if any, lead to an increase in the effectiveness of the compound in inducing polyploidy or in inhibiting neoplastic growth without being toxic to the organism.

Eigsti and Dustin (1955) summarize the findings of many investigators in four points characterizing the activity of colchicine.

a. It is of importance that the esterified side chains of rings B and C are at a proper distance from each other.

b. At least one methoxy group appears to be indispensable in ring A.

c. Esterification of the amino-group of ring B may increase activity, but this is not necessary for activity.

d. Ring C must be seven membered and the hydroxy group should be either esterified or replaced by an amino-group which is itself esterified.

They present as an example the different activities of colchicine and its isomer, isocolchicine, observed by Steinegger and Levan (1947). The reasons for the differences, they state, may lie in the formation of hydrogen bonds between the side chains of ring C and B; in particular the methoxy groups. Thus, it seems these side chains are chemically active, and the same investigators proposed that a chemical combination between the alkaloid and intracellular receptors leads to spindle inactivation.

Mazia (1956) gives a probable explanation of the reactions leading to spindle inactivation. He subjected sea urchin eggs to colchicine and then isolated the "mitotic apparatus". He found that the contracted chromosomes were embedded in a gel-like substance which showed no semblance of organization. He proposed that the formation of the spindle from precursors involves two processes; the formation of a gel and the orientation of the gel into a highly organized fibrillar structure. Colchicine, according to him,

seems to attack the latter process, and in doing so specifically seems to attack the "secondary bonding mechanism". The possible targets seem to be the cell centers and kinetochores.

It has been known for some time that colchicine isomerizes to its alpha, beta and gamma photoisomers when exposed to ultraviolet light and sunlight (Grewe, 1946, Grewe and Wulf, 1951 and Forbes, 1955). It has also been found that if colchicine solutions are left standing and exposed to air they become toxic. It has been proposed, but not fully established, that the observed toxicity is due to the formation of a compound called "oxydicolchicine" resulting from the chemical combination of two colchicine molecules and an atom of oxygen (Eigsti and Dustin, 1955).

Chapman et al. (1963) have established the structures of alpha and beta-lumicolchicine and have presented good evidence for the structure of the gamma isomer. They have also found that the alpha isomer is not formed when solutions are exposed to a mercury arc lamp. Alpha-lumicolchicine, a head to head dimer of beta-lumicolchicine, is formed only when a cyanine dye filter is interposed between the source and the solution.

Forbes (1955) found that there was a preponderance of the beta isomer formed when solutions were exposed to sunlight. There was also present in the solution a mixture of the alpha and gamma-lumicolchicne.

The solutions in this investigation were exposed di-

rectly to an ultraviolet light source without the cyanine dye filter. It is quite probable that very little alpha-lumicolchicine was formed in the irradiated solutions. The beta and gamma isomers should have formed, and since the solutions showed losses of biological activity it is very likely that the two isomers are not as effective as colchicine.

There was evidence of toxicity as illustrated by the presence of pycnotic nuclei in considerable numbers in the first 2-4 hours after the initiation of treatment of the seedlings.

Three possibilities may be considered here to explain the toxicity observed. Since the solutions were exposed to air, ultraviolet light irradiation may accelerate the formation of the compound "oxydicolchicine", mentioned by Eigsti and Dustin (1955); another possibility is that the photoisomers themselves are toxic; and lastly it may well be that a toxic compound, which may or may not be related to colchicine, was formed in the solutions through irradiation.

Solutions maintained either under refrigeration or at room temperature in the dark or exposed to artificial light showed progressive loss of activity with increase in storage time. No evidence of toxicity was observed up to three weeks; but those solutions kept for a longer period of time under artificial light did show some toxicity. Again air was not excluded from these and the possibiltiy that "oxydicolchicine" or some other toxic agent may have been pre-

sent cannot be ruled out.

Thus, from these results and those from physico-chemical observations of Forbes (1955), Gardner et al. (1960), and especially Chapman and co-workers (1963), the point may be made that the transformation of colchicine from a tricyclic structure to its tetracyclic isomers has caused a reduction in c-mitotic activity. This supports Eigsti and Dustin's (1955) ideas that the proper distance must be maintained between the side groups of ring B and C, and that the integrity of ring C must also be maintained. These relationships are changed when ring C is transformed into a 4 carbon C ring and a 5 carbon D ring, and the molecule of colchicine takes on new steric configurations. These changes might possibly affect the reactivity of the side groups and their ability to act as hydrogen acceptors, which would be in accord with Eigsti and Dustin's (1955) and Mazia's (1956) hypothesis on the action of colchicine on the spindle.

Although not an integral part of this investigation, observations were made on whether the "altered" solutions were capable of inducing the c-tumor (colchicine-tumor). The results were not quantified, but it was noted that the irradiated solutions were capable of inducing the c-tumor in seedling roots. Eigsti and Dustin (1955), describe the c-tumor formation induced by colchicine and several other compounds. They state that it has been well established that c-tumor formation and c-mitotic activity are two different effects of the compound. In this case c-mitotic acti-

vity was low and the c-tumor activity was comparable to that induced by fresh solutions.

The other solutions subjected to various "standard" laboratory conditions also gave evidence of c-tumor activity even when their c-mitotic activity was reduced compared to fresh solutions.

From evidence gathered thus far it may be suggested that further work should be carried out to determine the biological activity of the different photoisomers of colchicine. With support from the physical sciences it has become evident that the relationship of the side groups to a molecule and to themselves plus the structural configuration of the whole molecule determines biological activity; such is probably the case with colchicine.

Supporting evidence for this suggestion comes from work done in our laboratory in the past by Tsou (1954) and Hyppio, Tsou and Wilson (1955) on the c-mitotic action of Technical Lindane.

Eigsti and Dustin (1955) reported that both the gamma and delta isomers of Technical Lindane (hexachlorocyclohexane) have been found to be active antimitotics by Carpentier and Fromegeot (1950).

Tsou (unpublished) tested all five isomers of Technical Lindane and found that only the gamma isomer was active. This then points to the necessity of knowing which isomer of colchicine is active and which one(s) are responsible for loss of activity and toxicity.

It would be helpful if, through physico-chemical studies, a method of treating colchicine solutions could be devised by means of which one could be assured that the isomer one had in solution was the most active and would give more or less the same results everytime it was used. This would be of great importance when using colchicine in tagging populations of synchronous cells as proposed by Van't Hof, Wilson and Colon (1960); inasmuch as variability of the colchicine solutions would be eliminated.

Gout, a metabolic disease, has been and still is treated with colchicine (Eigsti and Dustin, 1955, Stetten, 1958 and Copeman, 1964). It would be of interest to know if colchicine per se or one of its isomers is better in the treatment of the metabolic disease.

In conclusion then, it has been found from the previous study (Greenberg, 1962) and from this investigation that colchicine solutions begin to show loss of activity after storage for 24 hours in the dark at room temperature (22.5°C) and after 48 hours in the dark under refrigeration (0-5°C). Solutions exposed to a fluorescent light source gave inconclusive results. The solution exposed for 12 hours shows a loss of about 9 per cent activity, while the solutions exposed for from 3 to 15 weeks are slightly more active than the fresh treatment solution. The controls themselves were not as active as desirable. Solutions exposed to ultraviolet light demonstrated considerable loss of activity after 24 hours of exposure.

It is also suggested that the dry powder form be tested before use since it was found that it showed significant variation from batch to batch.

Increasing concentration and exposure time to colchicine solutions will only increase the probability of encountering toxicity. This is very important to consider when working with cells and tissues of warm blooded animals, since it is known that colchicine is toxic to them at concentrations which are innocuous to plants (Eigsti and Dustin, 1955).

SUMMARY AND CONCLUSIONS

Solutions of colchicine were subjected to incident irradiation from two radiant energy sources. This was done with the hope of determining what conditions ordinarily found in "standard" research laboratories would be most suited for the storage of an unstable compound such as colchicine. The data obtained could then be compared quantitatively.

The results of the two investigations, Greenberg (1962) and this one, indicate that:

a. Colchicine solutions may be stored up to 48 hours at 0-5°C in the dark without showing appreciable loss of biological activity.

b. Solutions subjected to irradiation by ultraviolet light showed considerable loss of activity.

c. The dry powder form of the alkaloid obtained from the suppliers is not constant in the degree of effect induced, and that individual samples of the powder, when stored for long periods of time in the dark at 0-5°C, also lose biological activity.

d. With supporting evidence from the literature it is possible that the beta and gamma-lumicolchicines are not biologically active and may even be toxic.

e. It is suggested that further studies be

carried out using the Hadder and Wilson (1958) test system to test the activity of purified photoisomers and other colchicine derivatives to set up a classification system, with which to correlate the results observed with known physico-chemical data.

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APPENDIX

PLATE II

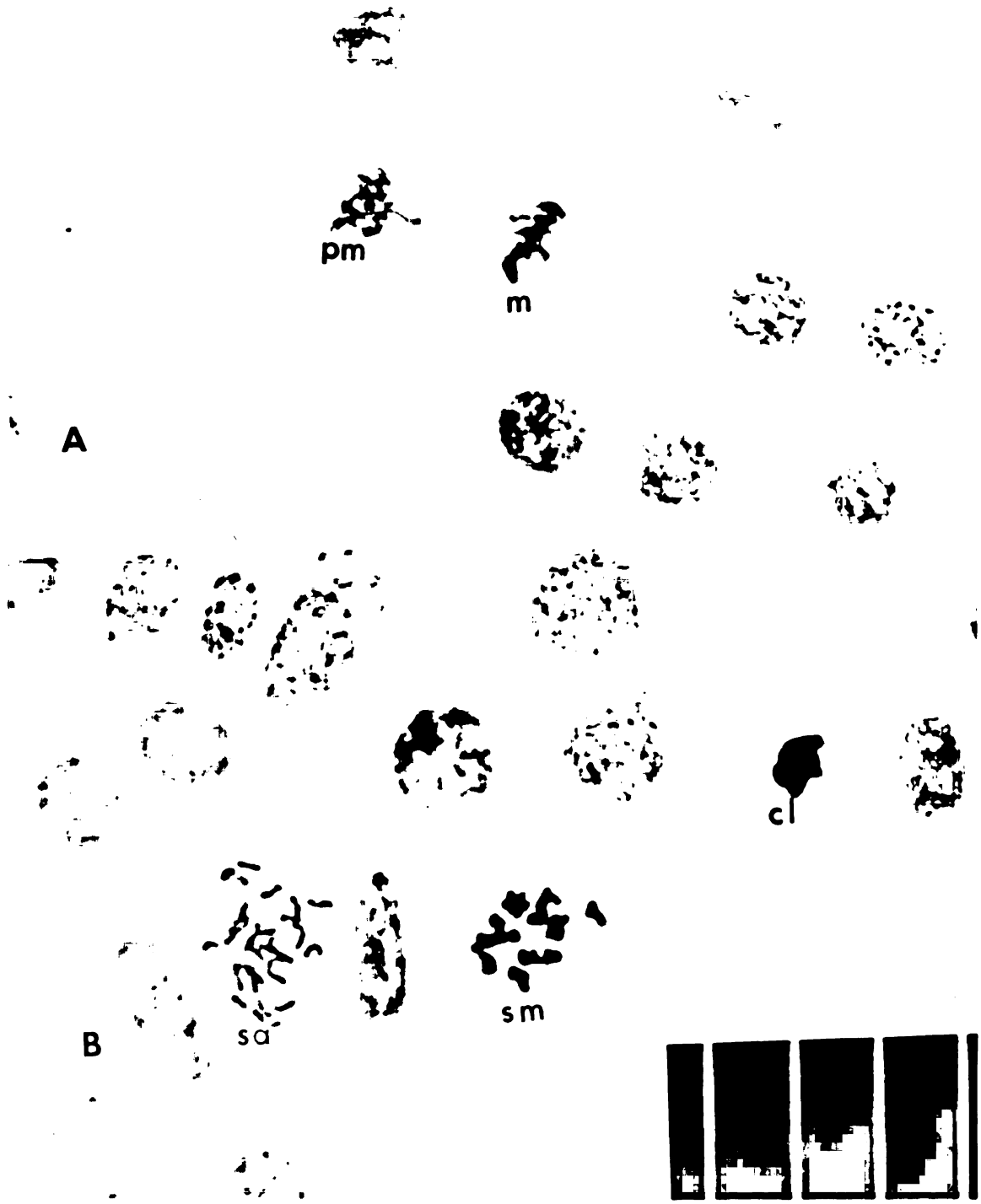
Figures A - B. Mitosis in *Pisum sativum*. Normal cytological configurations, and figures showing effect of colchicine.

Figure A. Normal prometaphase (pm) and metaphase (m).

Figure B. Clump (cl), scattered metaphase (sm) and scattered anaphase (sa).

Each division of the scale represents 10 microns.

PLATE II



EXPERIMENTAL NOTE

Spectrophotometric measurements were made on solutions which were stored under various "standard" laboratory conditions in this investigation and the previous one (Greenberg, 1962). It was hoped that observed modifications in the biological activity of colchicine solutions could be correlated to observable changes in the ultraviolet light absorption peaks of the solutions.

Aqueous solutins at 16 ppm were measured with a B&L 505 spectrophotometer and no variations could be detected between fresh solutions and those stored under different conditions for varying lengths of time, even when biological activity had been modified as measured by the Pisum test.

No measurements were performed on the solutions exposed to ultraviolet light.

Further studies should be carried out in a more systematic manner to correlate physical and chemical changes with observed modifications of biological activity.

Special thanks are extended to the Biophysics Department of Michigan State University for permitting the use of their spectrophotometer.

Table I. Data* on the Normal Post-prophases, Clumps, Scatters and Per Cent Effects of the Fresh and "Altered" Colchicine Solutions.

Fresh**:

Hrs.+	n.	post-proph.	clumps	scat.	% eff.
2		8.00	38.00	1.25	83.07
4		22.20	24.80	7.00	58.83
6		25.50	5.75	18.25	48.43
8		34.60	1.20	5.20	15.61
10		32.25	.25	2.50	7.86

Stored 12 hours in the dark at 5°C.

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		4.85	36.00	4.50	89.30
4		18.75	20.75	3.75	61.14
6		23.25	6.25	23.75	55.69
8		20.75	5.00	9.25	40.71
10		34.75	1.25	2.00	8.55

Fresh:

Hrs.				
2	4.50	33.25	8.50	89.30
4	16.50	22.00	16.25	70.31
6	26.50	4.75	22.50	57.94
8	28.25	2.75	15.50	39.24
10	31.00	2.00	9.50	27.05

Stored 12 hours in the dark at 22.5°C.

Hrs.				
2	4.67	34.00	7.67	89.92
4	18.25	29.50	14.00	84.05
6	21.00	4.50	17.25	50.87
8	38.00	2.50	10.25	25.12
10	25.75	1.75	18.50	44.02

Fresh: (same as above).

Hrs.				
2				
4				
6				
8				
10				

Stored 12 hours in the light at 22.5°C:

Hrs.				
2	2.00	30.25	14.00	95.77
4	25.00	7.00	14.00	45.65
6	23.33	1.67	22.33	50.70
8	39.75	.75	8.00	18.22
10	30.75	.50	5.75	16.39

* Data: Each figure represents the average of 4000 cells scored at random per sampling period.

** Fresh: is the treatment with fresh colchicine solutions corresponding to the treatment with the "altered" or stored solutions.

\+ Hrs.: Means hours after the initiation of treatment at which samples were taken and scored for effect.

Table I. (Cont'd).

Fresh:

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		4.75	34.75	6.25	89.61
4		15.75	19.50	10.25	65.38
6		26.25	3.00	13.25	38.23
8		26.00	1.75	16.75	41.57
10		33.25	.50	3.75	11.33

Fresh:

Hrs.				
2	5.25	30.25	4.00	86.70
4	15.50	36.60	11.40	75.59
6	25.00	5.80	30.60	59.28
8	38.00	.80	14.40	28.57
10	42.25	.25	5.25	10.99

Fresh: (same as above).

Hrs.				
2				
4				
6				
8				
10				

Fresh:

Hrs.				
2	8.00	29.67	14.33	84.61
4	12.00	17.00	21.33	76.15
6	31.50	2.25	11.50	24.08
8	31.50	1.00	2.50	10.00
10	32.75	1.50	5.75	18.12

Stored 24 hours in the dark at 5°C.

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		6.00	36.25	6.75	87.75
4		13.25	10.00	12.25	62.68
6		22.50	5.25	16.75	49.44
8		33.00	2.25	7.50	22.81
10		26.00	0.00	2.25	7.96

Stored 24 hours in the dark at 22.5°C:

Hrs.				
2	5.75	37.12	6.12	88.26
4	20.50	22.00	18.00	59.40
6	32.40	3.40	35.00	54.23
8	45.20	1.40	5.00	12.40
10	49.00	.86	5.14	10.91

Irrad. 24 hours with uv at 34°C:

Hrs.				
2	21.62	6.12	19.50	54.23
4	42.40	2.43	4.86	14.67
6	43.83	1.50	4.50	12.04
8	50.00	.50	3.33	7.11
10	42.20	.80	1.40	4.95

Irrad. 24 hours with uv at 22.5°C:

Hrs.				
2	13.25	3.25	19.75	63.44
4	21.00	.33	4.33	18.16
6	26.00	.50	1.33	6.57
8	22.20	.40	1.40	7.50
10	30.00	.00	1.67	5.27

Table I. (Cont'd.).

Fresh:

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		7.60	11.00	11.60	74.83
4		19.40	4.00	11.20	43.93
6		25.51	5.50	18.00	47.94
8		39.25	.25	3.25	8.18
10		35.00	1.67	4.33	14.34

Fresh:

Hrs.				
2	6.00	21.60	14.60	85.78
4	27.00	10.33	11.33	44.51
6	34.00	.75	2.75	9.33
8	37.50	.75	2.50	8.00
10	41.75	1.00	2.00	6.70

Fresh:

Hrs.				
2	2.75	23.00	6.75	21.54
4	11.25	15.00	10.50	69.39
6	22.25	4.00	14.75	46.87
8	24.75	1.75	5.75	22.05
10	27.25	3.00	6.00	25.53

Fresh:

Hrs.				
2	3.25	26.50	6.00	90.91
4	15.50	16.00	8.50	61.25
6	54.57	4.00	27.50	36.50
8	29.50	3.00	11.50	32.29
10	33.00	.50	4.50	13.16

Irrad. 24 hours with uv at 22.5°C:(2)

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		15.00	0.00	3.67	19.66
4		29.60	0.00	1.80	5.73
6		37.75	0.00	7.50	16.74
8		-- --	- --	- --	-- --
10		-- --	- --	- --	-- --

Irrad. 24 hours with uv at 22.5°C: (3)

Hrs.				
2	3.25	2.25	15.00	84.14
4	23.00	1.00	6.67	25.00
6	33.00	.50	4.50	13.15
8	36.33	1.00	2.00	7.62
10	25.67	0.00	2.33	8.96

Stored 3 weeks in the dark at 5°C: (1)

Hrs				
2	4.80	14.20	14.40	85.63
4	17.75	7.50	13.50	57.14
6	29.50	1.75	7.50	23.87
8	33.00	1.25	12.25	31.81
10	39.00	0.00	8.75	18.13

Stored 3 weeks in the dark at 5°C: (2)

Hrs.				
2	2.75	13.50	12.50	90.43
4	8.75	18.00	10.00	76.19
6	31.50	3.00	19.00	39.25
8	27.50	2.75	9.75	31.25
10	24.75	1.50	8.25	28.25

Table I. (Cont'd).

Fresh: Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		3.25	26.50	6.00	90.91
4		15.50	16.00	8.50	61.25
6		54.57	4.00	27.50	36.50
8		29.50	3.00	11.50	32.29
10		33.00	.50	4.50	13.16

Stored 3 weeks in the dark at 22.5°C: Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		2.25	21.25	15.25	94.19
4		19.25	6.75	15.75	53.89
6		25.50	2.00	12.25	35.85
8		31.75	.75	14.75	32.80
10		32.25	.75	7.75	20.86

Fresh: Hrs.				
2	4.50	33.25	8.50	89.30
4	16.50	22.00	16.25	70.31
6	26.25	4.75	22.50	57.94
8	28.25	2.75	15.50	39.24
10	31.00	2.00	9.50	27.05

Stored 3 weeks in the light at 22.5°C: (1) Hrs.				
2	5.75	36.25	16.25	90.00
4	13.67	24.33	15.00	74.20
6	26.00	6.33	30.67	58.73
8	21.33	6.67	29.67	63.01
10	32.00	1.50	10.75	27.68

Fresh: Hrs.				
2	7.00	27.60	6.00	82.75
4	22.66	13.00	18.00	58.28
6	22.14	9.28	24.57	69.38
8	21.16	7.83	21.00	57.67
10	27.33	1.50	18.50	42.25

Stored 3 weeks in the light at 22.5°C: (2) Hrs.				
2	3.28	25.42	8.71	91.23
4	16.83	14.17	20.16	67.10
6	-- --	-- --	-- --	-- --
8	32.00	.57	7.57	20.08
10	40.00	.20	3.40	.08

Fresh: Hrs.				
2	-- --	-- --	-- --	-- --
4	-- --	-- --	-- --	-- --
6	-- --	-- --	-- --	-- --
8	-- --	-- --	-- --	-- --
10	-- --	-- --	-- --	-- --

Stored 3 weeks in the light at 22.5°C: (3) Hrs.				
2	2.86	37.14	8.57	94.11
4	20.14	13.43	13,14	56.97
6	23.57	2.71	22.14	52.40
8	22.67	4.67	14.83	45.24
10	30.60	.80	7.00	.20

Table I. (COnt'd.).

Fresh:

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		7.00	27.50	6.00	82.75
4		22.66	13.00	13.00	58.28
6		22.14	9.28	24.57	69.38
8		21.16	7.83	21.00	57.67
10		27.33	1.50	18.50	42.25

Fresh:

Hrs.					
2	--	--	-- --	-- --	-- --
4	--	--	-- --	-- --	-- --
6	--	--	-- --	-- --	-- --
8	--	--	-- --	-- --	-- --
10	--	--	-- --	-- --	-- --

Stored 15 weeks in the light at 22.5°C: (1)

Hrs.	n.	post-proph.	clumps	scat.	% eff.
2		2.83	21.17	13.50	92.45
4		15.00	9.43	22.14	67.79
6		28.86	1.28	20.43	42.93
8		27.67	.17	6.33	19.02
10		33.28	.57	2.86	9.34

Stored 15 weeks in the light at 22.5°C: (2)

Hrs.				
2	3.67	20.83	13.00	90.21
4	18.40	8.40	13.00	53.76
6	31.14	.71	3.86	12.79
8				
10				

Table II. Mitotic Indices of the Untreated Controls, Fresh and "Altered" Colchicine Solutions.

Untreated Control Hrs.	Fresh		Untreated Control	Stored 12 hours in the dark at 5°C:
2	58.75	65.75	73.25	76.35
4	60.75	75.00	69.75	77.25
6	83.00	88.50	79.00	97.25
8	72.75	80.60	71.50	61.25
10	84.00	73.50	90.75	82.00

Untreated Control Hrs.	Fresh		Untraeted Control	Stored 12 hours in the dark at 22.5°C:
2	80.75	83.75	70.50	97.00
4	87.50	98.25	86.25	103.50
6	88.50	101.75	89.00	90.25
8	84.75	92.75	89.75	105.00
10	-- --	78.75	94.75	96.75

Untreated Control Hrs.	Fresh	Untreated Control	Stored 12 hours in the light at 22.5°C:
2	(same as above)	82.25	100.00
4		86.00	91.00
6		81.00	115.00
8		93.00	95.00
10		80.75	89.25

Untreated Control Hrs.	Fresh		Untreated Control	Stored 24 hours in the dark at 5°C:
2	63.75	72.50	76.75	33.75
4	74.75	87.50	80.25	70.75
6	74.25	81.50	97.50	76.50
8	73.00	80.75	84.75	90.00
10	82.00	87.00	91.25	74.50

Table II. (Cont'd.).

Untreated Control Hrs.		Fresh	Untreated Control	Stored 24 hours in the dark at 22.5°C:
2	70.40	70.00	98.56	84.36
4	77.28	115.00	106.67	114.16
6	84.00	107.20	95.99	129.40
8	77.66	111.80	97.99	116.40
10	94.33	86.00	84.71	117.71

Untreated Control Hrs.		Fresh	Untreated Control	Irrad. 24 hours with uv at 34°C:
2	(same as above)		(same as above)	91.99
4				101.25
6				95.49
8				117.83
10				96.60

Untreated Control Hrs.		Fresh	Untreated Control	Irrad. 24 hours with uv at 22.5°C: (1)
2	63.25	95.00	56.00	71.65
4	88.80	91.66	56.00	52.99
6	73.25	87.76	72.00	67.16
8	84.80	79.50	67.00	65.40
10	76.20	86.00	68.75	84.67

Untreated Control Hrs.		Fresh	Untreated Control	Irrad. 24 hours with uv at 22.5°C: (2)
2	52.80	61.80	52.75	56.00
4	78.20	72.20	53.50	71.60
6	90.40	97.01	76.50	94.00
8	89.80	81.00	71.25	-- --
10	87.00	82.60	78.25	-- --

Table II. (Cont'd.).

Untreated Control Hrs.		Fresh	Untreated Control	Irrad. 24 hours with uv at 22.5°C:(3)
2	50.51	87.40	81.34	49.50
4	77.33	102.32	103.25	74.67
6	84.00	85.00	95.50	91.00
8	100.99	89.25	77.00	92.33
10	96.34	92.00	103.50	73.67

Untreated Control Hrs.		Fresh	Untreated Control	Stored 3 weeks in the dark at 5°C:(1)
2	73.00	56.50	68.75	61.80
4	76.25	75.00	82.00	78.00
6	87.50	83.00	81.00	89.00
8	68.50	81.00	75.25	91.75
10	73.25	76.25	79.75	106.50

Untreated Control Hrs.		Fresh	Untreated Control	Stored 3 weeks in the dark at 5°C:(2)
2	66.25	55.50	67.25	52.75
4	81.75	74.00	77.50	60.50
6	74.50	136.75	99.00	91.25
8	101.25	95.75	100.75	90.00
10	90.50	91.75	91.50	79.00

Untreated Control Hrs.		Fresh	Untreated Control	Stored 3 weeks inthe dark at 22.5°C:
2	(same as above)		(same as above)	42.00
4				79.00
6				84.50
8				90.00
10				88.75

Table II. (Cont'd.).

Untreated Control		Fresh	Untreated Control	Stored 3 weeks in the light at 22.5°C; (1)
Hrs.				
2	80.75	83.75	82.25	100.00
4	87.50	98.25	86.00	91.67
6	88.50	101.75	81.00	115.00
8	84.75	92.75	93.00	95.00
10	-- --	78.75	80.75	89.25

Untreated Control		Fresh	Untreated Control	Stored 3 weeks in the light at 22.5°C; (2)
Hrs.				
2	74.33	72.80	72.00	68.69
4	77.13	92.82	74.71	93.99
6	74.28	98.56	70.00	-- --
8	82.57	98.82	84.60	86.42
10	90.28	92.66	88.32	96.20

Untreated Control		Fresh	Untreated Control	Stored 3 weeks in the light at 22.5°C: (3)
Hrs.				
2	-- --	-- --	(same as above)	98.00
4	-- --	-- --		93.99
6	-- --	-- --		95.70
8	-- --	-- --		88.84
10	-- --	-- --		78.60

Untreated Control		Fresh	Untreated Control	Stored 15 weeks in the light at 22.5°C; (1)
Hrs.				
2	(same as for stored 3 weeks 3)		54.33	70.50
4			58.33	85.00
6			59.71	102.99
8			67.49	73.17
10			75.00	83.57

Table II. (Cont'd.).

Untreated Control Hrs.	Fresh		Untreated Control	Stored 15 weeks in the light at 22.5°C: (2)
2	-- --	-- --	54.33	70.33
4	-- --	-- --	58.33	76.60
6	-- --	-- --	59.71	74.14
8	-- --	-- --	67.49	-- --
10	-- --	-- --	75.00	-- --

Table III. Data and Indices of Effect for the Fresh and "Altered" Colchicine Solutions.

Fresh Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
2	13.67	166.67	26.33	206.67	1.7
4	78.00	83.33	45.33	206.66	1.0
6	95.00	18.00	117.66	230.66	.6
Stored 12 hours in the dark at 5°C;					
Hrs.					
2	9.00	161.33	44.00	214.33	1.7
4	64.00	92.00	62.33	218.33	1.1
6	106.00	18.00	185.66	209.66	.5
Fresh					
Hrs.					
2	20.50	152.00	46.00	218 50	1.6
4	80.25	50.25	68.25	198.75	.8
6	124.00	17.75	86.25	228.00	.5
Stored 12 hours in the dark at 22.5°C:					
Hrs.					
2	8.67	165.00	39.33	213.00	1.7
4	70.00	102.00	54.25	226.25	1.1
6	125.00	11.75	74.75	212.05	.4
Stored 12 hours in the light at 22.5°C:					
Hrs.					
2	13.25	137.00	64.75	215.00	1.5
4	127.60	39.80	61.00	228.40	.6
6	121.00	11.00	111.00	243.67	.5

Table III. (Cont'd.).

Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
Fresh					
2	6.00	169.66	33.00	208.66	1.7
4	39.67	82.00	44.33	216.00	.9
6	99.33	34.33	77.67	211.33	.6
Stored 24 hours in the dark at 5°C:					
Hrs.					
2	12.33	168.67	35.67	216.67	1.7
4	71.33	83.33	63.00	237.66	.9
6	119.67	17.67	75.00	212.34	.5
Fresh					
Hrs.					
2	21.00	181.70	18.00	220.70	1.7
4	61.20	114.60	39.80	215.60	1.2
6	97.80	25.20	109.00	232.20	.6
Stored 24 hours in the dark at 22.5°C:					
Hrs.					
2	26.62	153.20	33.62	231.44	1.5
4	71.40	85.80	68.20	224.60	1.0
6	111.40	6.20	111.00	228.60	.5
Irrad. 24 hours with uv at 34°C:					
Hrs.					
2	106.71	22.71	95.23	224.70	.6
4	191.50	9.28	23.57	224.35	.1
6	191.50	3.83	14.00	209.33	.1

Table III. (Cont'd.).

Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
Fresh					
2	39.00	130.30	54.00	223.30	1.4
4	46.50	65.50	99.00	211.00	1.0
6	154.75	9.75	47.75	212.25	.3
Irrad. 24 hours with uv at 22.5°C: (1)					
Hrs.					
2	79.50	18.00	105.25	202.75	.6
4	153.67	1.00	24.33	179.00	.1
6	195.40	.60	8.40	204.40	.0
Fresh					
Hrs.					
2	39.75	73.50	66.00	179.25	.8
4	120.80	17.60	51.40	189.80	.4
6	126.00	15.50	68.00	209.50	.4
Irrad. 24 hours with uv at 22.5°C:					
Hrs.					
2	90.33	.67	19.33	110.33	.1
4	151.00	.40	5.80	157.20	.0
6	186.50	1.50	3.00	191.00	.0
Fresh					
Hrs.					
2	37.00	101.60	41.80	180.40	1.3
4	108.00	42.33	42.33	192.66	.6
6	147.75	4.25	4.25	170.50	.1

Table III. (Cont'd.).

Irrad. 24 hours with uv at 22.5°C: Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
2	98.25	22.25	78.75	198 .75	.6
4	119.67	3.00	20.67	143.34	.1
6	164.00	.67	13.00	177.67	.0
Fresh Hrs.					
2	8.00	155.00	47.50	210.50	1.7
4	75.25	91.00	53.25	219.50	1.0
6	134.25	16.75	54.50	205.50	.4
Stored 3 weeks in the dark at 5°C: Hrs.					
2	25.00	107.50	92.00	224.50	1.3
4	103.00	55.25	78.50	236.75	.8
6	177.00	9.50	41.00	227.50	.2
Fresh Hrs.					
2	10.75	164.00	34.25	209.00	1.7
4	85.75	89.25	52.75	227.75	1.0
6	113.75	14.50	82.25	210.50	.5
Stored 3 weeks in the dark at 5°C: Hrs.					
2	22.00	93.50	86.75	202.25	1.3
4	49.00	92.75	59.75	208.50	1.2
6	85.75	16.00	75.00	176.75	.6

Table III. (Cont'd.).

Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
Stored 3 weeks in the dark at 22.5°C:					
2	35.75	101.00	82.00	218.75	1.3
4	102.25	36.75	63.00	202.00	.6
6	145.25	13.00	80.25	239.50	.4
Fresh					
Hrs.					
2	20.50	152.00	46.00	218.15	1.6
4	80.25	36.75	68,25	198.75	.8
6	124.00	17.75	86.25	228.00	.5
Stored 3 weeks in the light at 22.5°C:(1)					
Hrs.					
2	16.75	130.25	60.75	207.75	1.5
4	59.67	113.33	53.99	226.99	1.2
6	85.33	28.33	103.67	217.33	.7
Fresh					
Hrs.					
2	36.60	151.14	28.60	216.34	1.5
4	92.83	61.16	67.16	221.15	.8
6	88.28	37.14	107.85	233.27	.7
Stored 3 weeks in the light at 22.5°C: (2)					
Hrs.					
2	51.14	136.00	51.14	209.85	1.5
4	73.34	57.83	82.83	214.99	.9
6	-- --	-- --	-- --	--- --	- -

Table III. (Cont'd.).

Hrs.	n. post-proph.	clumps	scatters	t. post-proph.	index of effect
Stored 3 weeks in the light at 22.5°C: (3)**					
2	19.14	144.71	46.57	210.42	1.5
4	99.57	55.42	59.86	214.85	.8
6	106.71	13.71	93.57	213.99	.7
Stored 15 weeks in the light at 22.5°C: (1)					
Hrs.					
2	35.00	123.00	51.00	209.00	1.6
4	73.11	45.14	87.14	205.99	.8
6	140.43	4.28	60.43	205.14	.3
Stored 15 weeks in the light at 22.5°C: (2)*=					
Hrs.					
2	26.17	119.16	56.33	201.66	1.4
4	125.90	20.90	54.20	201.00	.6
6	181.43	4.28	17.14	203.42	.1

** No fresh control for this solution, but it was prepared from the same sample of dry powder as # 2. This solution was also exposed to light at the same time as solution # 2.

*= Aliquot from #3, and also without a fresh control.

Table IV. Areas of the Curves of the Indices of Effect Obtained from the Fresh and "Altered" Colchicine Solutions.

Fresh	Stored 12 hours in the dark at 5°C:
11.3	11.1
Fresh	**Stored 12 hours in the dark at 22.5°C:**
10.2	11.5
	Stored 12 hours in the light at 22.5°C:
	9.2
Fresh	**Stored 24 hours in the dark at 5°C:**
10.8	10.5
Fresh	**Stored 24 hours in the dark at 22.5°C:**
12.0	10.2
	Irrad. 24 hours with uv at 3½°C:
	2.7
Fresh	**Irrad. 24 hours with uv at 22.5°C: (1)**
9.2	2.7
Fresh	**Irrad. 24 hours with uv at 22.5°C: (2)**
5.2	.4
Fresh	**Irrad. 24 hours with uv at 22.5°C: (3)**
7.3	2.6
Fresh	**Stored 3 weeks in the dark at 5°C: (1)**
10.9	8.0
Fresh	**Stored 3 weeks in the dark at 5°C; (2)**
11.0	10.4
	Stored 3 weeks in the dark at 22.5°C:
	7.8
Fresh	**Stored 3 weeks in the light at 22.5°C:(1)**
9.8	11.4
Fresh	**Stored 3 weeks in the light at 22.5°C:(2)**
9.5	-- -
Fresh	**Stored 3 weeks in the light at 22.5°C:(3)**
-- -	9.3
Fresh	**Stored 15 weeks in the light at 22.5°C:(1)**
9.5	9.8
Fresh	**Stored 15 weeks in the light at 22.5°C:(2)**
----	7.5