# Diurnal Mitotic Periodicity, Karyotype Analysis, Natural and Induced Chromosomal Aberrations In the Root Apices of EPHEDRA FOLIATA Boiss

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CONTENTS

## SUPERVISOR'S NOTE

The thesis entitled "Diurnal mitotic periodicity, Karyotype analysis, Natural and Induced chromosomal aberrations in the root apices of *Ephedra foliata* Boiss" submitted by Shri C.B.S.R. Sarma, M.Sc., for the degree of Doctor of Philosophy, embodies the results of the investigations done under my supervision and I certify that the work is original.

November 28th, 1968

B.D.Deshpande
( Supervisor)

# A C K N O W L E D G E M E N T S

I preface by recording my deep indebtedness to my former teacher and guide Late Prof.B.N. Mulay who initiated me into the present problem of research. I am very grateful to Dr.B.D. Deshpande for his keen interest, encouragement and inspiring guidance. My thanks are also due to Dr.S.K. Pillai for his constant attention and deep concern for my work.

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But for the timely help and assistance of Mr. A. Sankara Reddy, my brother and a few anonymous persons, this work could have never seen the light of the day.

C.B.S.R. Sarma

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*Ephedra foliata* growing on *Gymnosporia spinosa*

Cultivated bush of *Ephedra foliata*

# INTRODUCTION

Ephedra foliata is a lianous gymnosperm inhabiting arid regions like Rajasthan, belonging the order Ephedrales (Eames,1952). It is dioecious, flowering during winter. The male plants grow on Capparis aphylla and Prosopis spicegera and female plants on Salvadora oleracea and Gymnosporia spinosa in and around Pilani. This plant has been chosen for the present study owing to the general economic importance of the genus and prevalence of several variations, concerning the bisporangiate cones, flowering period in males and morphology and water relations of seeds.

The present study includes I. Diurnal mitotic periodicity, II. Karyotype analysis, III. Natural aberrations and IV. Induced aberrations.

Cell division, being an aspect of growth is influenced by the circadian fluctuations of environmental conditions, because growth on the whole is rhythmic. But there is dearth of information (Erickson,1964) in this line except for a few old references (Friesner,1920 and the references cited there).

Karyotype analysis forms the basis of any cytological study because of its general fixity and inevitability in explaining chromosomal aberrations. The only information known about

Ephedra foliata is that its haploid number is 7, consisting of 5 metacentrics and 2 acrocentrics (Mehra,1946).

While studying the karyotype many spontaneous aberrations have been encountered and hence are duely incorporated. Because spontaneous aberrations are supposed to be indices of karyotype instability which inturn is a concomitant of microevolution.

Induction of aberrations may point to possibilities for improving a plant for economic exploitation. It must be remembered that therapeutically important Ephedrine is present in several other species of Ephedra, but foliata. Aminoacids, which are nutritive, if deficient may hinder certain biosynthetic pathways. But what happens, if they are excessive, is not known from the cytological point of view. The second category of chemicals are the antineoplastic alkaloids of Vinca, used in the chemotherapy of cancer. These have earlier been employed only on animal tissues (Nuess et al,1964). A study of their effects on the normal plant tissues might assist in the cytological elucidation of tumour inhibition in some unknown way. Further, both types of chemicals, are being employed for the first time on any plant tissue. However, the main purpose of employing these "nutritive" and "toxic" chemicals is to probe newer avenues of information about their mutagenicpotency as observed cytologically.

## REVIEW OF LITERATURE

Investigations on the genus Ephedra initiated by Stapf (1889) have been followed by Jaccard (1894) who was the first to find out the chromosome number for any Ephedra spp, when he reported that the somatic number of E. helvetica is 8. Land (1904) has reported the somatic chromosome number of E. trifurca to be 12. The same number has been reported for E. distachya by Berridge and Sandey (1907). However, all these are of historical interest and now the chromosome numbers of about 20 species in an estimated total of 32, are known authentically.

Geitler (1929) reported the haploid and diploid numbers for E. major and E. campylopoda to be 7 and 14. The diploid numbers of E. americana, E. distachya, E. equisetina and E. nebrodensis were reported by Florin (1932) and of E. altissima, E. distachya and E. sinica by Resende (1937). Mehra (1934, 1946), Hunziker (1953,1955), Krapovickas (1955) and Vazquez(1959) are some important workers who reported the chromosome numbers for various other species of Ephedra, from Asia, South America, Europe etc. These include E. foliata, E. gerardiana, E. intermedia, E. sexatilis, E. likiagenesis E. altissima var algerica, E. sinica, E. americana, E. andina, E. rupestris, E. breana, E. frustillata, E. ochreata, E. triandra, E. tweediana E. nebrodensis etc. Mehra (1946) gave a summary of chromosome numbers based on

the earlier accounts and his own work. This was later modified by Khoshoo (1961) in the light of newer reports. A consolidated account of chromosome numbers is presented in the Table A.

Concerning E. foliata, Mehra (1934) was the first to report its haploid chromosome number as 7, which was supported by Maheshwari (1935). But Mulay (1941) felt that it is likely to be more than 7, perhaps 10 at least in the species growing in Karachi, Sind.

According to Mehra (1946) the basic chromosome number of the genus is 7, consisting of 5 long metacentrics and 2 short acrocentrics. Satellites were not found although secondary constrictions were observed. He feels that autopolyploidy is of common occurrence in the genus and the sizes of meiotic chromosomes is about half of the mitotic ones. Hunziker (1955) concurs with Mehra's account of the basic chromosome complement. In a tetraplod species (E. andina) he recorded 10 metacentric and 4 acrocentric chromosomes in the haploid complement. According to him, the karyotypes of only E. gerardiana and E. sinica are different from the aforementioned basic type prevalent among the rest of the species of Ephedra. He reported the presence of satellites in the species he worked. However, a detailed study of the karyotype has not been attempted.

Cytological work other than concerning the chromosome number is very meagre. Gifford (1941) worked on the cytohistology of shoot apices in E. altissima. Mehra (1934,1938) germinated the pollen grains of E. foliata and E. gerardiana in artificia

cultures and studied their cytology. Mehra (1946) further reported the occurrence of diploid pollen grains in some species and inequality in the size of the two male nuclei (1950) in E. altissima, E. intermedia and E. sexatilis. He also studied (1949) the effect of sulfanilamide on the pollen grains of E. foliata, E. sinica, E. intermedia, and E. sexatilis. Pathania (1961) studied the effects of Sodium nucleate on the root tips of E. foliata and E. gerardiana. Sarma (1968) induced chromosome breakages in the root apices of E. foliata by employing coumarin. Effects of low temperature on diurnal mitotic periodicity (in press) and effects of sulfanilamide on mitosis (in press) in the same material has also been studied by Sarma.

Table A

Chromosome numbers of Ephedra

| Sl.No. | Species | Chromosome number n | Chromosome number 2n | Reference |
|---|---|---|---|---|
| 1. | E. altissima | 14 | 28 | Resende 1937, Mehra 1946 |
| 2. | E. americana | 7 | 14 | Florin 1932, Resende 1937, Hunziker 1955 |
| | as andina | - | 14, 28, 30 | Resende 1937, Hunziker, 1953, 1955 |
| | as rupestris | - | 14 | Krapovickas 1954, Hunziker 1955 |
| 3. | E. breana | - | 14, 28 | Hunziker 1953, 1955, Krapovickas 1955 |
| 4. | E. distachya | - | 28 | Florin 1932, Resende 1937 |
| | as gerediana | 7 | - | Mehra 1934, 1946 |
| 5. | E. equisetina | - | 14 | Florin 1932 |
| 6. | E. foliata | 7 | - | Mehra 1934, 1946 |
| 7. | E. fragilis (compylopoda) | 7 | 14 | Geitler 1929 |
| 8. | E. frustillata | - | 14 | Krapovickas 1954, Hunziker 1955 |
| 9. | E. helvetica | 8 | - | Jaccard 1894 |
| 10. | E. intermedia | 14 | 28 | Mehra 1946 |

## MATERIALS AND METHODS

The seeds of *Ephedra foliata* were collected in the month of May from the neighbourhood of Pilani, Jhunjhunu district, Rajasthan. They were germinated and the experiments with root tips were carried out before October, because the seed viability is lost with the onset of winter.

I. Diurnal mitotic periodicty:- Presoaked seeds were placed for germination in soil-filled petridishes. The controls were kept under normal conditions of photoperiod and temperature. Normal photoperiod consists of 10 light hours and 14 dark hours. Continuous dark treatment involves the germination of seeds in a dark chamber, the temperature being the same as in controls. The low temperature treatment is given by germinating the seeds in refrigerator adjusted at $6^{\circ}C$. Continuous light treatment was given by germinating the seeds under a fluorescent tube light of 40 watts.

Root apices of about 5 mm length from controls and treatments were collected at hourly intervals throughout the 24 hours of day and night. They are then fixed in Carnoy's fluid for 15 minutes and stored in 70% alcohol. Conventional methods had been followed (Darlington and La Cour, 1963) for

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staining, squashing and preparing the slides. The squashing and mounting of the material have been done under a cover glass, which helps in avoiding squeezing of cells by ensuing large space. Yet enough care has been taken to prevent any loss of material from underneath the cover glass. Various mitotic phases have been scored from the temporary preparations following the sampling method of Brown and Rickless, (1949). The mitotic and phase indices have been calculated according to the following methods.

$$\text{Mitotic Index} : \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

$$\text{Phase Index:} \quad \frac{\text{Number of cells in a particular phase}}{\text{Number of dividing cells}} \times 100$$

II. Karyotype analysis: For the diploid karyotype analysis, root apices of appropriate size were pretreated in 0.05% colchicine for about an hour. After washing they were fixed in Carnoy's fluid for 15 minutes and stored in 70% alcohol. Temporary and permanent slides were prepared following the conventional schedule.

Camera Lucida diagrams of well-spread metaphases were drawn from roottip squashes for karyotype analysis. Photomicrographs of suitable metaphases were also taken. Total lengths of chromosomes, and of their individual arms were measured. Further analysis and fixing of the karyotype has been done following the practices of Battaglia,(1955) and Bansal et.al. (1965).

III. Natural aberrations: The natural aberrations were observed and scored from the root tips fixed in Carnoy's fluid as well as acetic alcohol, stained in Leuco Basic Fushsin and squashed in acetocarmine. Aceticacid-N-Butyl alcohol schedule has been followed in making the slides permanent. Percentage distribution of various aberrations was calculated.

IV. Induced aberrations: To study the induction of aberrations, root apices were treated with the following chemicals. 1. Arginine, 2. Glycine, 3. Methionine, 4. Threonine, 5.Valine (amino acids) 6. Lochnerinine, 7. Sitsirikine, 8. Vinblastine, 9.Vindoline, 10.Yohimbine (antineoplastic drugs). The amino acids used were the aqueous solutions of 0.01%, 0.05%, and 0.1% concentration and antineoplastic drugs 0.001%,0.002% and 0.004%. The latter are prepared from the stock solution of 0.01% strength made in benzene. The amino acids were of E. Merck's make and alkaloids were procured from Lily Research laboratories, Indiana, U.S.A.

Germinating seeds with healthy root apices were treated in the aforementioned solutions for different durations -- 1, 3, 6, 12, and 24 hours. After the treatment, the root apices were cut, washed, fixed in Carnoy's fluid for 15 minutes and stored in 70% alcohol. Temporary and permanent slides were made according to conventional techniques,(Darlington and La Cour, 1963).

## OBSERVATIONS

### Contents

# I. DIURNAL MITOTIC PERIODICITY

## Explanation of terms and abbreviations

1. Mitotic cycle: Duration of mitotic activity from its inception to the finish. In a cell population, high prophase index with a corresponding low telophase index is the beginning of a cycle. Similarly low prophase index and corresponding high telophase index marks the end of a cycle.
2. Preparatory period: Period of negligible mitotic indices.
3. Peak: State of high mitotic index with low mitotic indices preceding and following.
4. Fall: State of low mitotic index with high mitotic indices preceding and following.
5. Wallow: A sudden decrease or increase of prophase index or telophase index against the usual course of cycle.
   a. Prophase depress wallow: A sudden decrease in the prophase indices, when they should normally rise.
   b. Prophase spurt wallow: A sudden increase in the prophase indices, when they should normally decrease.
   c. Telophase depress wallow: A sudden decrease in the telophase indices, when they should normally rise.

d. Telophase spurt wallow: A sudden increase in telophase indices, when they should normally decrease.

6. Overlap: A state of relation between prophase and telophase when their indices run paralelly with each other or even one making the other, numerically insignificant instead of following the usual inverse relationship. The former is called parallel overlap, and the latter, the obliterate overlap. If the parallel overlap is due to increase of telophase (at the begining of a cycle) it is called the Parallel Ascending Type. But if it occurs owing to decrease in telophases (at the end of cycle), it is called the Parallel Descending Type.

7. Abbreviations used in various tables of this chapter:

| | | | | | |
|---|---|---|---|---|---|
| TC | : | Total Cells | pi | : | prophase index |
| TM | : | Total Mitoses | mi | : | metaphase index |
| Tp | : | Total prophase | ai | : | anaphase index |
| Tm | : | Total metaphases | ti | : | telophase index |
| Ta | : | Total anaphases | MI | : | Mitotic Index |
| Tt | : | Total telophase | | | |

## 1. Controls

General mitotic activity:

(Table I.1,Fig.I.1)

Under normal conditions of temperature and photo-period the diurnal mitotic activity proceeds steadily. Divisions are negligible during two periods - (i) 10-12 am, and (ii) 8-12 pm which are called "Preparatory periods". The former is the first preparatory period and the latter, the second. The duration of these periods totals to 6 hours.

The number of cells studied range between 1970 and 3214, of the dividing cells between 0.20 and 7.15. The means of all studied cells, observed mitoses and mitotic indices work out to be 2562.3,101.9 and 3.94, respectively. Similarly, the mean indices of prophase, metaphase, anaphase and telophase 31.95, 15.69, 18.17 and 34.20, respectively.

Mitotic cycles:

(Table I.2, Fig. I.2)

The division patterns are highly rhythmical. There are on the whole five cycles of mitoses, falling broadly into two waves. The first wave consists of 3 cycles (cycles 1, 2 and 3) and the second wave, 2 cycles (cycles 4 and 5). Two preparatory periods intercept the two waves. Within a wave the

cycles are continuous and successive.

The cycles are as follows:

1. 12 - 2 am
2. 2 - 5 am
3. 5 - 10 am
4. 12 - 3 pm
5. 3 - 8 pm

The first cycle takes only 2 hours, whereas the second and the third cycles take 3 and 5 hours respectively. Thus the duration of the first wave is 10 hours (12 pm - 10 am). Then the first preparatory period of 2 hours duration (10 - 12 am) sets in. The fourth cycle which follows is completed within 3 hours and the fifth cycle, within 5 hours. So the duration of the second wave is 8 hours (12 - 8 pm). Thus the range of duration of cycles lies between 2 - 5 hours. The mean duration works out to 3.6 hours. Now the second preparatory period of 4 hours duration (8 - 12 pm) sets in, which in turn is followed by the first cycle (of the first wave) of the next day. Thus the cycle continues.

In the first cycle, starting after the second preparatory period the mean mitotic index is 7.02. Among the phase indices that of prophase is more (35.30) than those of others. Metaphase index is the lowest (14.69). It is in this cycle that the highest mitotic peak has been recorded at 1 am

(7.15). The second cycle has a mean mitotic index of 5.96. The maximum phase index is of prophase (32.79) and minimum, of metaphase (19.03). In the third cycle the mean mitotic index is 4.73. The maximum phase index is of prophase (33.89) and minimum of metaphase (15.15). This cycle is followed by the first preparatory period. The average mitotic index in the whole first wave, thus works out to 5.97. In the fourth cycle, starting after the first preparatory period, the mean mitotic index is 5.39. The maximum phase index is of telophase (33.79) and minimum of metaphase (16.62). In the fifth cycle, the mean mitotic index is 4.08. The maximum phasic index is of telophase (33.81) and minimum of metaphase (15.01). This is followed by the second preparatory period. The average mitotic index in the whole second wave works out to 4.73.

The mean mitotic indices of the five cycles ranging between 4.08 and 7.02, when pooled together with the negligible mitotic indices of the preparatory periods result in an overall diurnal mean mitotic index of 3.94 for the root tips grown under normal conditions of temperature and photoperiod.

Phasic behaviour:

(Table I.2,Fig.I.2)

Through out the course of division, in all the cycles, an inverse relationship between prophases and telophases has been observed. In the first three cycles that run from 12 to 10 am, the prophase indices are always more and the metaphase

indices, the least. But the telophase indices, except in the second cycle, are almost nearer to those of prophase indices. In the last two cycles, running from 12 - 8 pm, telophase indices are more and metaphase indices, the least. But the prophase indices also run very close to those of telophase. So the maximum indices are always those of either prophase or telophase and minimum, those of metaphase.

Diurnal distribution of mitoses and mitotic phases in the root apices of *Ephedra foliata* grown under normal conditions of photoperiod and temperature

| | T | TC | TM | Tp | pi | Tm | mi | Ta | ai | Tt | ti | MI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM | 1. | 2463 | 176 | 94 | 53.41 | 29 | 16.48 | 27 | 15.34 | 26 | 14.77 | 7.15 |
| | 2. | 2700 | 186 | 32 | 17.20 | 24 | 12.90 | 34 | 18.28 | 96 | 51.61 | 6.89 |
| | 3. | 2851 | 166 | 76 | 45.78 | 28 | 16.87 | 24 | 14.46 | 38 | 22.89 | 5.82 |
| | 4. | 2608 | 170 | 24 | 14.12 | 36 | 21.18 | 58 | 34.12 | 52 | 30.59 | 6.52 |
| | 5. | 2750 | 152 | 23 | 15.13 | 20 | 13.16 | 21 | 13.82 | 88 | 57.89 | 5.53 |
| | 6. | 2310 | 154 | 110 | 71.43 | 14 | 9.09 | 10 | 6.49 | 20 | 12.99 | 6.67 |
| | 7. | 2436 | 147 | 56 | 38.10 | 28 | 19.05 | 25 | 17.01 | 38 | 25.85 | 6.03 |
| | 8. | 2144 | 136 | 20 | 14.71 | 30 | 22.06 | 48 | 35.29 | 38 | 27.94 | 6.34 |
| | 9. | 2473 | 106 | 12 | 11.32 | 11 | 10.38 | 13 | 12.26 | 70 | 66.04 | 4.29 |
| | 10. | 2347 | 8* | 2 | - | 1 | - | - | - | 5 | - | 0.34 |
| | 11. | 2461 | 9* | 4 | - | 1 | - | 1 | - | 3 | - | 0.37 |
| | 12. | 2600 | 6* | 2 | - | 1 | - | 1 | - | 2 | - | 0.23 |
| PM | 1. | 2801 | 142 | 72 | 50.70 | 24 | 16.90 | 21 | 14.79 | 25 | 17.61 | 5.07 |
| | 2. | 2895 | 159 | 45 | 28.30 | 40 | 25.16 | 42 | 26.42 | 32 | 20.13 | 5.49 |
| | 3. | 2745 | 154 | 26 | 16.88 | 12 | 7.79 | 18 | 11.68 | 98 | 63.64 | 5.61 |
| | 4. | 2601 | 142 | 80 | 56.34 | 20 | 14.08 | 20 | 14.08 | 22 | 15.49 | 5.46 |
| | 5. | 2533 | 149 | 75 | 50.34 | 20 | 13.42 | 22 | 14.77 | 32 | 21.47 | 5.88 |
| | 6. | 2830 | 144 | 28 | 19.44 | 34 | 23.61 | 46 | 31.94 | 36 | 25.00 | 5.09 |
| | 7. | 2362 | 101 | 8 | 7.92 | 9 | 8.91 | 10 | 9.90 | 74 | 73.27 | 4.28 |
| | 8. | 1970 | 4* | 1 | - | 1 | - | - | - | 2 | - | 0.20 |
| | 9. | 2646 | 9* | 4 | - | 1 | - | 1 | - | 3 | - | 0.34 |
| | 10. | 3214 | 10* | 3 | - | 2 | - | 1 | - | 4 | - | 0.31 |
| | 11. | 2413 | 11* | 3 | - | 2 | - | 1 | - | 5 | - | 0.46 |
| | 12. | 2342 | 5* | 2 | - | 1 | - | - | - | 2 | - | 0.21 |
| Total | | 61495 | 2446 | 802 | 511.12 | 389 | 251.04 | 444 | 290.66 | 811 | 547.18 | 94.57 |
| Mean | | 2562.3 | 101.9 | 33.4 | 31.95 ±5.02 | 16.2 | 15.69 ±1.35 | 18.8 | 18.17 ±2.21 | 33.79 | 34.20 ±5.17 | 3.94 ±0.55 |

* Regarded negligible and hence not considered for further analysis.

Table I.2

Nature of mitotic activity in the root apices of Ephedra foliata grown under normal conditions of photoperiod and temperature

| S.No. | Item of study | Number of cycle 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|
| 1. | Duration of the cycle | 12pm-2am | 2-5 am | 5-10 am | 12am-3pm | 3-8 pm |
| 2. | Time taken by the cycle | 2 hrs | 3 hrs | 5 hrs | 3 hrs | 5 hrs |
| 3. | Range of mitotic indices | 6.89-7.15 | 5.52-6.52 | 0.34-6.67 | 5.07-5.61 | 0.2-5.88 |
| 4. | Mean mitotic index | 7.02 | 5.96 | 4.73 | 5.39 | 4.08 |
| 5. | Mean prophase index | 35.30 | 32.67 | 33.89 | 31.96 | 33.51 |
| 6. | Mean metaphase index | 14.69 | 19.03 | 15.15 | 16.62 | 15.01 |
| 7. | Mean anaphase index | 16.81 | 21.86 | 17.74 | 17.63 | 17.67 |
| 8. | Mean telophase index | 33.19 | 25.44 | 33.21 | 33.79 | 33.81 |
| 9. | Number of mitotic peaks | | | N I L | | |
| 10. | Time of maximum mitoses | 1 am | | | | |
| 11. | Number of mitotic falls | | | N I L | | |
| 12. | Time of minimum fall | | | N I L | | |
| 13. | Number of preparatory-periods | | | 2 | | |
| 14. | Duration of preparatoryperiods | | | | 10-12 am | 8-12 pm |
| 15. | Time taken by preparatoryperiods | | | | 6 hrs (2 + 4) | |
| 16. | Number of wallows | | | N I L | | |
| a. | prophase depress wallow | | | | | |
| b. | prophase spurt wallow | | | | | |
| c. | telophase depress wallow | | | | | |
| d. | telophase spurt wallow | | | | | |
| 17. | Number of overlaps | | | N I L | | |
| a. | obliterate | | | | | |
| b. | parallel ascencing | | | | | |
| c. | parallel descending | | | | | |

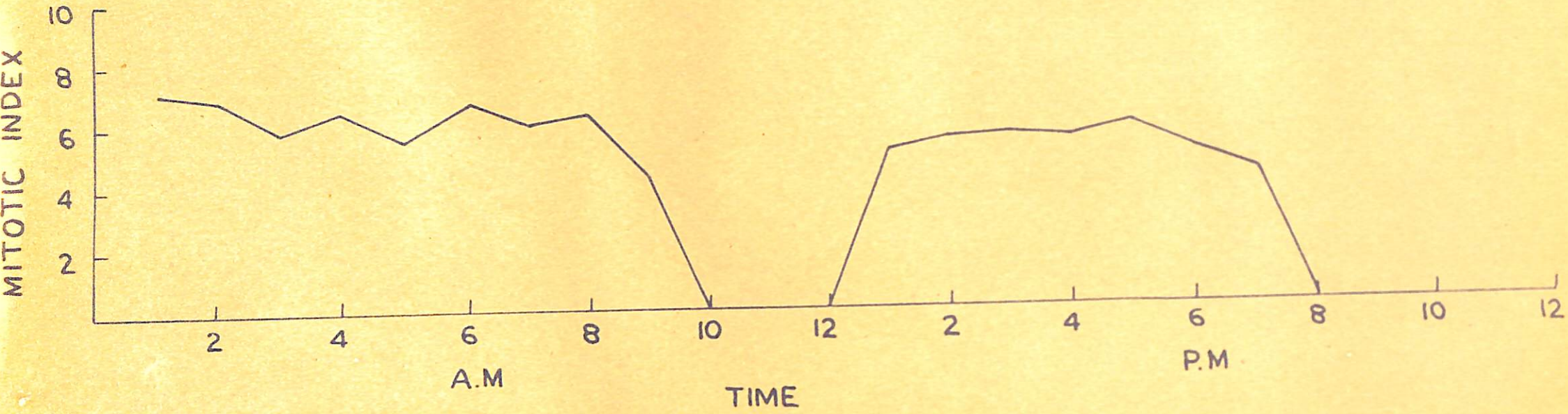


FIG. I·1 DIURNAL DISTRIBUTION OF MITOTIC INDICES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTROL CONDITIONS.

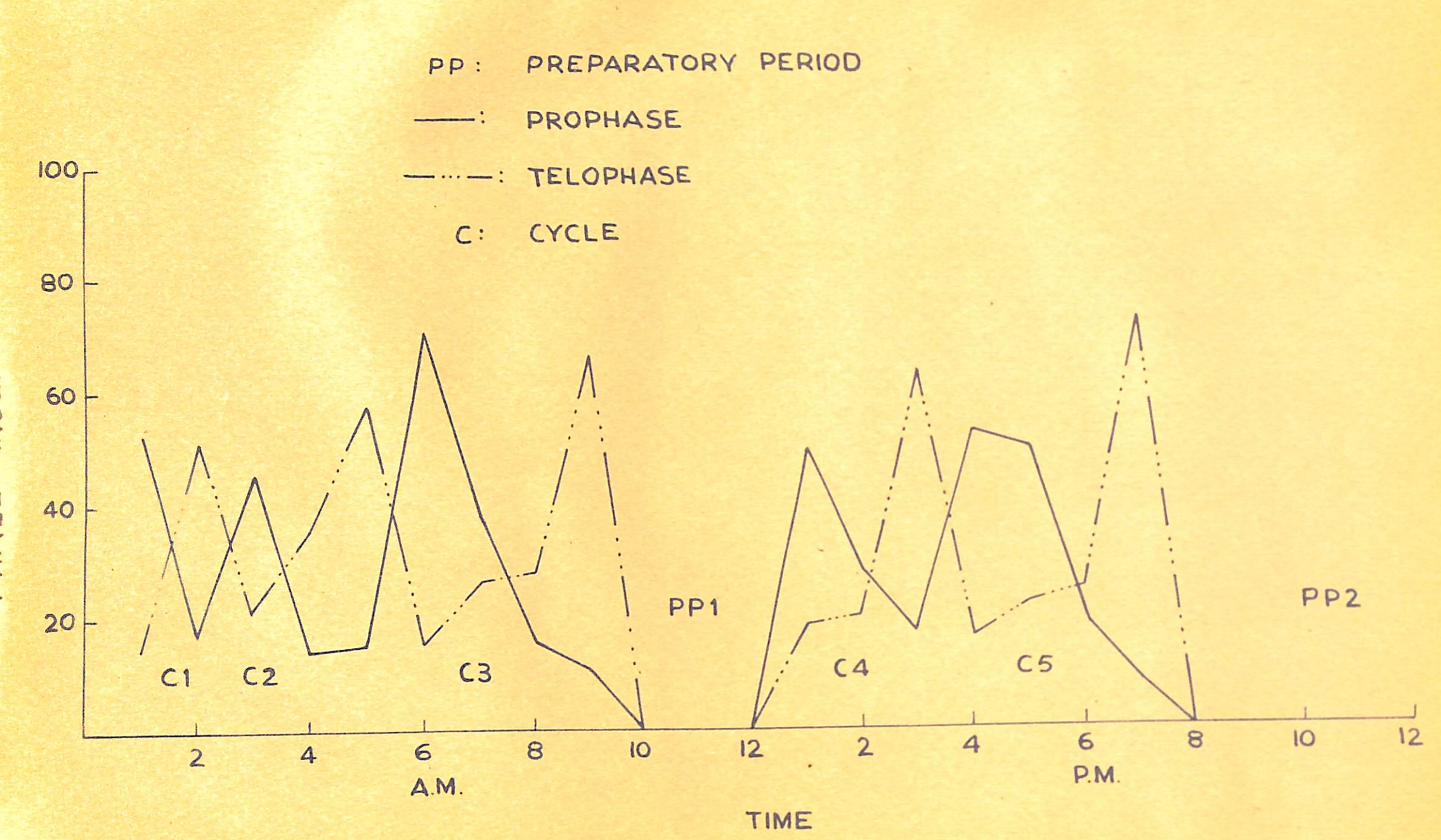


FIG. I 2 DIURNAL DISTRIBUTION OF MITOTIC CYCLES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTROL CONDITIONS.

## 2. Low temperature

General mitotic activity:

(Table I.3, Fig. I.3)

The roottips from low temperature treatment diurnally manifest an ebb-tide pattern of divisions. There are peaks and falls in the mitotic indices which are spread over somewhat evenly throughout the day and night. There are altogether 6 peaks of which 4 are prominent (8 am, 2 pm, 7 pm and 12 ) and 2 less so (10 am and 4 pm). There are 6 falls 3 prominent (5 am, 2 pm and 8 pm) and the rest 3 less so (9 am, 1 pm and 5 pm). The maximum peak has been recorded at 7 pm when the mitotic index was only 21.32. Further, 3 pm is the only time of negligible mitotic activity.

The number of cells studied range between 1606 and 3034, of dividing cells between 4 and 470 and of the mitotic indices between 0.24 and 21.32. The means of total cells studied observed mitoses and mitotic indices work out to be 2333.4, 148.89 and 6.45, respectively. Similarly, the mean indices of prophase, metaphase, anaphase and telophase are 38.84, 30.48, 10.19 and 20.49 respectively.

Mitotic cycles:

(Table I.4, Fig. I.4)

The division patterns in the root tips get disturbed

slightly. There are on the whole 5 mitotic cycles. Unlike in the controls they do not fall into any categorised waves. Generally the cycles are continuous occurring one after another. The period between 8 and 9 pm is regarded as a preparatory period owing to the negligibility of prophase indices and remnancy of telophases, although definite preparatory periods comparable to those in controls are absent.

The five cycles are as follows:

1. 2 - 5 am
2. 5 - 11 am
3. 11 am - 3 pm
4. 3 - 8 pm
5. 9 pm - 2 am.

The first cycle takes only 3 hours, the second, 6 hours, the third 4 hours, the fourth 5 hours and the fifth also 5 hours. In between the fourth and the fifth cycles there is a brief preparatory period of one hour duration. Thus the range of duration of cycles lies between 3 and 6 hours. The mean duration works out to 4.6 hours.

In the first cycle the mean mitotic index is 1.49. Among the phase indices, that of metaphase (47.29) is maximum and of telophase (6.48), the minimum. In this cycle there is only one mitotic fall. In the second cycle, the mean mitotic index is 7.71. The maximum phase index is that of prophase

(42.48) and minimum that of anaphase (11.55). In this cycle, there are two mitotic peaks and one mitotic fall. In the third cycle the mean mitotic index is 6.14. Among the phase indices that of prophase is maximum (59.37) and that of anaphase minimum (5.50). In this cycle there is one mitotic peak and the fall at 3 pm is the lowest in this treatment when the mitotic index is merely 0.24. In the fourth cycle, the mean mitotic index is 6.48. The prophase index is the maximum (46.12) and anaphase index (7.66), the minimum, among the phase indices. In this cycle there are two mitotic peaks at 4 pm and 7 pm, and two falls at 5 pm and 8 pm. The peak occurring at 7 pm is the maximum in this treatment when the mitotic index is 21.32. Now a preparatory period of one hour duration sets in (8-9 pm), followed by the fifth cycle. In this last cycle, the mean mitotic index is 8.23. Maximum phasic index is that of prophase (34.72) and minimum that of anaphase (12.70). There is only one mitotic peak.

Phasic behaviour:

(Table I.4, Fig. I.4)

The relative dominance of various phase indices is slightly different in different cycles. In the first, metaphase indices are maximum and telophase indices, minimum. In the rest of the cycles, prophase indices are the maximum and anaphase indices, the minimum. But in case of the third and fourth cycles the anaphase indices are extremely low and in the

fifth cycle the prophase and metaphase indices are very close. So the maximum indices are always those of prophase or metaphase and minimum of anaphase or telophase, under low temperature.

During the course of the second cycle, the prophase index exhibits a "wallow" at 8 am when it suddenly falls as against the normal course. Hence, this is called the "prophase depress wallow". In the same cycle, at the same time (8 am), the telophase index suddenly shows an increase which hence is called the "telophase spurt wallow."

In the third, fourth and the fifth cycles, the inverse relationship between prophases and telophases is disturbed and hence "overlaps" occur, one in each cycle. These are of the "parallel" type during 3-4 pm and 8-9 pm because indices of prophase and telophase simultaneously increase without masking either. These are called the "ascending" type because the telophases rise instead of falling. During 1 to 2 am, the overlap is of the "parallel descending" type as the telophases fall simultaneously with prophases when they should normally rise.

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Table I.3

Diurnal distribution of mitoses and mitotic phases
in the root apices of *Ephedra foliata* grown under low temperature

| T | | TC | TM | Tp | pi | Tm | mi | Ta | ai | Tt | ti | MI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM | 1. | 3012 | 195 | 20 | 10.26 | 65 | 33.33 | 33 | 16.92 | 77 | 39.49 | 6.47 |
| | 2. | 2886 | 79 | 2 | 2.53 | 50 | 63.29 | 17 | 21.52 | 10 | 12.66 | 2.74 |
| | 3. | 2100 | 42 | 8 | 19.05 | 22 | 52.38 | 5 | 11.90 | 7 | 16.67 | 2.00 |
| | 4. | 2658 | 40 | 12 | 30.00 | 18 | 45.00 | 3 | 7.50 | 7 | 17.50 | 1.50 |
| | 5. | 2367 | 23 | 3 | 13.04 | 10 | 43.48 | 3 | 13.04 | 7 | 30.43 | 0.97 |
| | 6. | 1606 | 80 | 30 | 37.50 | 20 | 25.00 | 10 | 12.50 | 20 | 25.00 | 4.98 |
| | 7. | 1726 | 202 | 124 | 61.39 | 29 | 14.36 | 17 | 8.42 | 32 | 15.84 | 11.70 |
| | 8. | 2550 | 379 | 151 | 39.84 | 75 | 19.79 | 37 | 9.76 | 116 | 30.61 | 14.86 |
| | 9. | 1913 | 60 | 35 | 58.33 | 10 | 16.67 | 6 | 10.00 | 9 | 15.00 | 3.14 |
| | 10. | 2807 | 226 | 117 | 50.00 | 35 | 15.49 | 14 | 6.19 | 64 | 28.32 | 8.05 |
| | 11. | 3034 | 107 | 2 | 1.87 | 46 | 42.99 | 24 | 22.43 | 35 | 32.71 | 3.53 |
| | 12. | 2253 | 75 | 31 | 41.33 | 16 | 21.33 | 7 | 9.33 | 21 | 28.00 | 3.33 |
| PM | 1. | 1918 | 21 | 9 | 42.86 | 6 | 28.57 | 1 | 4.75 | 5 | 23.81 | 1.09 |
| | 2. | 2071 | 412 | 387 | 93.93 | 6 | 1.46 | 10 | 2.43 | 9 | 2.18 | 19.89 |
| | 3. | 1641 | 4* | 2 | - | - | - | - | - | 2 | - | 0.24 |
| | 4. | 2456 | 93 | 33 | 35.48 | 22 | 23.66 | 10 | 10.75 | 28 | 30.11 | 3.79 |
| | 5. | 2439 | 44 | 24 | 54.55 | 10 | 22.73 | 3 | 6.82 | 7 | 15.91 | 1.80 |
| | 6. | 2357 | 100 | 61 | 61.00 | 12 | 12.00 | 10 | 10.00 | 17 | 17.00 | 4.24 |
| | 7. | 2205 | 470 | 374 | 79.57 | 36 | 7.66 | 21 | 4.47 | 39 | 8.30 | 21.32 |
| | 8. | 2612 | 32 | - | - | 30 | 93.75 | 2 | 6.25 | - | - | 1.23 |
| | 9. | 2381 | 140 | - | - | 69 | 49.29 | 20 | 14.29 | 51 | 36.43 | 5.88 |
| | 10. | 2081 | 143 | 76 | 53.15 | 26 | 18.18 | 13 | 9.09 | 28 | 19.58 | 6.87 |
| | 11. | 2650 | 229 | 158 | 69.00 | 47 | 20.52 | 14 | 6.11 | 10 | 4.37 | 8.64 |
| | 12. | 2280 | 375 | 145 | 38.67 | 113 | 30.13 | 37 | 9.87 | 80 | 21.33 | 16.45 |
| Total | | 56003 | 3571 | 1800 | 893.34 | 773 | 701.05 | 317 | 234.35 | 681 | 471.24 | 154.72 |
| Mean | | 2333.4 | 148.8 | 75.0 | 38.84 ±5.48 | 32.2 | 30.48 +4.31 | 13.2 | 10.19 +1.05 | 28.4 | 20.49 +2.23 | 6.45 +0.91 |

* Regarded negligible and hence not considered for further analysis

Nature of mitotic activity in the root apices of *Ephedra foliata* grown under continuous low temperature

| S.No | Item of study | N-umber of cycle | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1. | Duration of the cycle | 2-5 am | 5-11 am | 11am-3pm | 3-8 pm | 9pm-2am |
| 2. | Time taken by the cycle | 3 hrs | 6 hrs | 4 hrs | 5 hrs | 5 hrs |
| 3. | Range of mitotic indices | 0.97-2.74 | 0.97-14.86 | 0.24-19.89 | 0.24-21.32 | 2.74-16.45 |
| 4. | Mean mitotic index | 1.49 | 7.71 | 6.14 | 6.48 | 8.23 |
| 5. | Mean prophase index | 20.69 | 42.48 | 59.37 | 46.12 | 34.72 |
| 6. | Mean metaphase index | 47.29 | 22.38 | 17.12 | 31.96 | 33.09 |
| 7. | Mean anaphase index | 10.81 | 11.55 | 5.50 | 7.66 | 12.70 |
| 8. | Mean telophase index | 6.48 | 24.58 | 17.99 | 14.26 | 19.48 |
| 9. | Number of mitotic peaks | - | 2 | 1 | 2 | 1 |
| 10. | Time of maximum peak | | | | 7 pm | |
| 11. | Number of mitotic falls | 1 | 1 | 2 | 2 | - |
| 12. | Time of minimum fall | | | 3 pm | | |
| 13. | Number of preparatory periods | | 1 | | | |
| 14. | Duration of preparatory periods | | | | 8-9 pm | |
| 15. | Time taken by preparatory periods | | 1 hr. | | | |
| 16. | Number of wallows | | 2 | | | |
| a. | prophase depress wallow | - | 1 | - | - | - |
| b. | prophase spurt wallow | - | - | - | - | - |
| c. | telophase depress wallow | - | - | - | - | - |
| d. | telophase spurt wallow | - | 1 | - | - | - |
| 17. | Number of overlaps | | | 3 | | |
| a. | obliterate | - | - | - | - | - |
| b. | parallel ascending | - | - | 1 | 1 | - |
| c. | parallel descending | - | - | - | - | 1 |

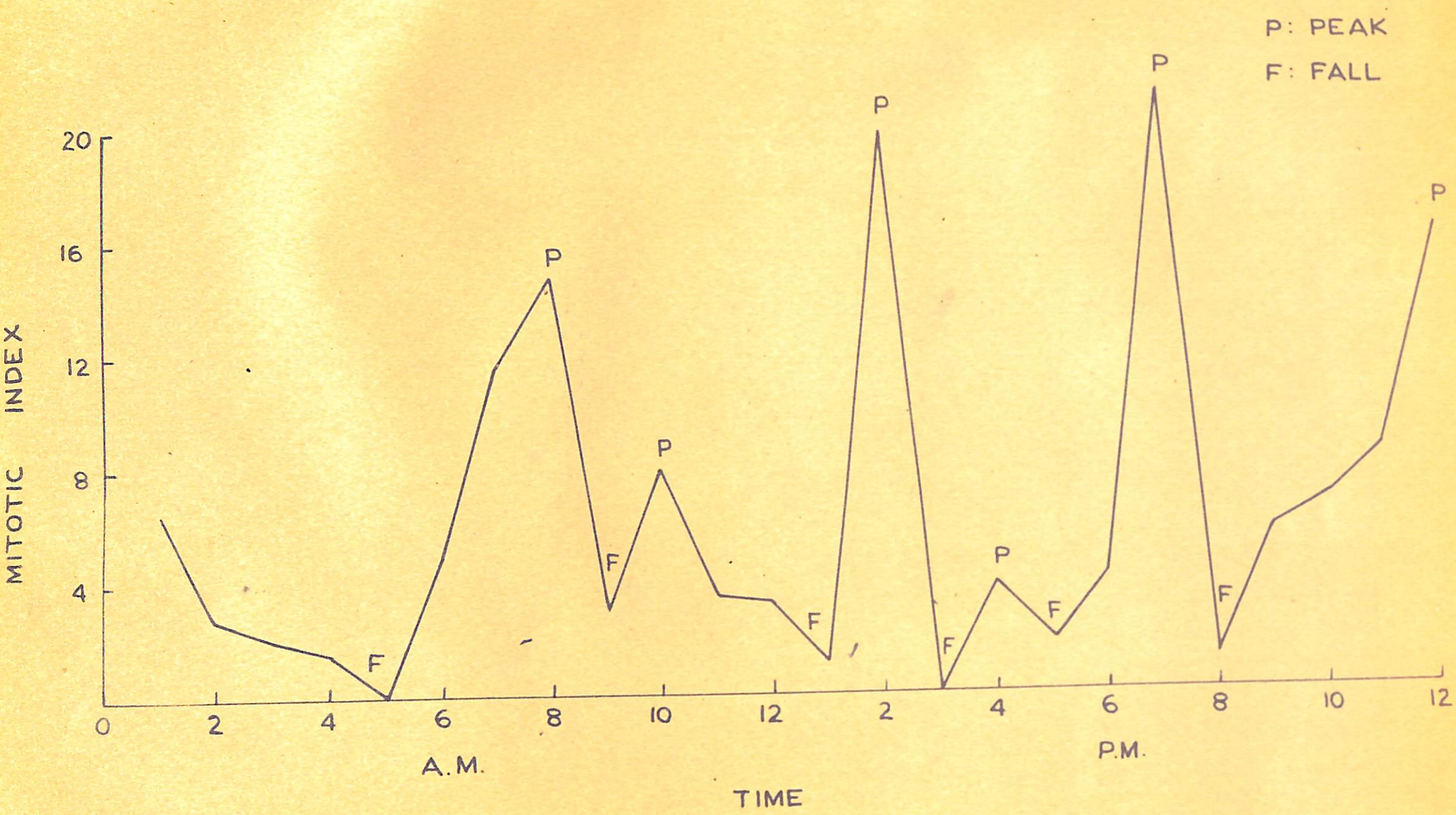


FIG 1·3 DIURNAL DISTRIBUTION OF MITOTIC INDICES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER LOW TEMPERATURE.

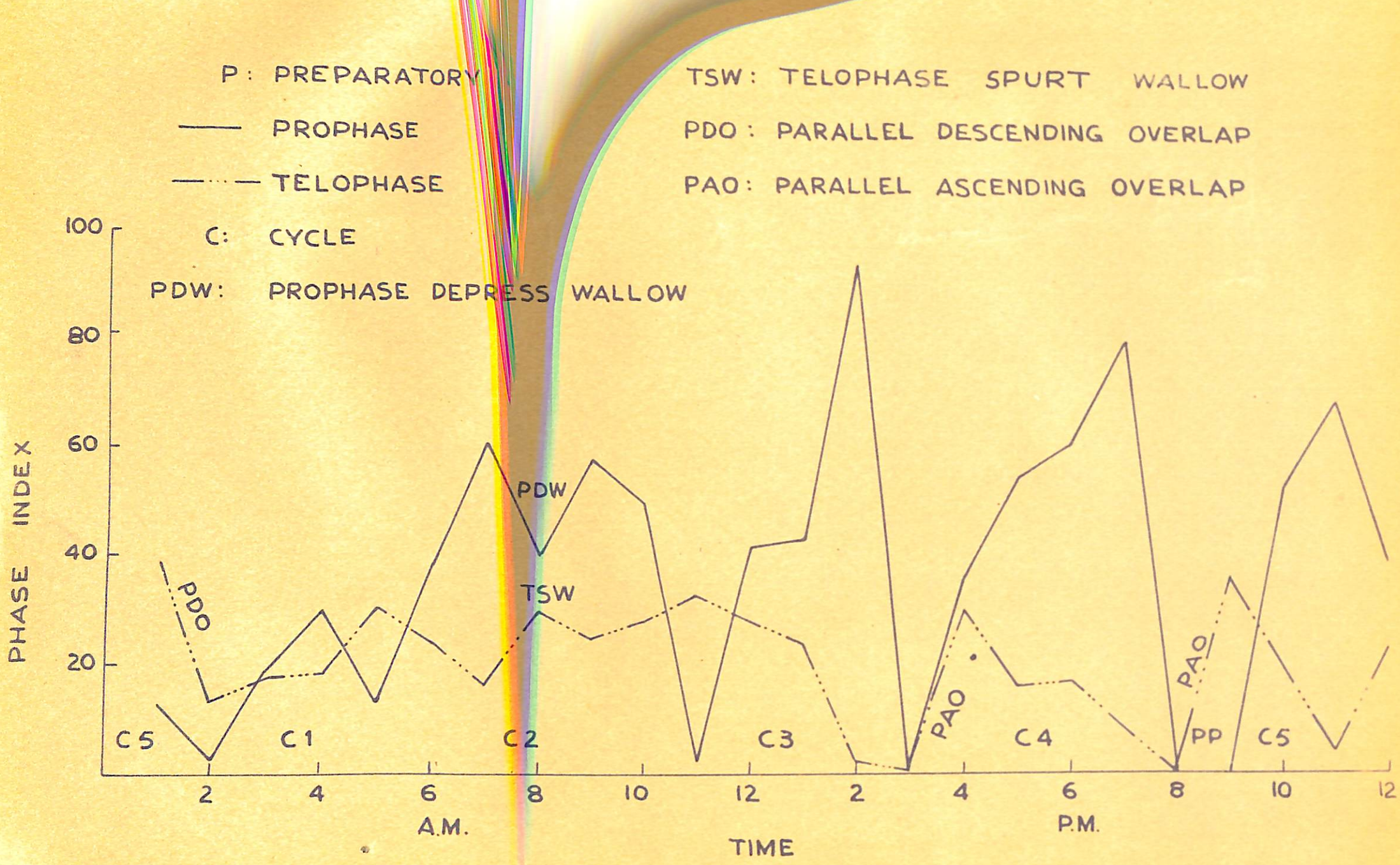


FIG. I·4 DIURNAL DISTRIBUTION OF MITOTIC CYCLES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER LOW TEMPERATURE.

## 3. Darkness

General mitotic activity:

(Table I.5, Fig. I.5)

Mitotic indices in root tips maintained under total darkness exhibit a wavy pattern. However, 4 peaks are recognisable. These occur at 2 am, 8 am, 2 pm and 9 pm, the one occurring at 8 am being the only prominent one. Similarly there are three falls occurring at 7 am, 1 pm and 7 pm. The maximum mitotic peak has been recorded at 8 am when the mitotic index was 13.12 and the minimum fall, at 7 am when the mitotic index was 0.90. There is not a single time when the mitotic activity is negligible, nor are there any preparatory periods.

The number of cells studied range between 2120 and 3372, mitoses between 22 and 359, and mitotic indices between 0.90 and 13.12. The means of total studied cells, mitoses and mitotic indices work out to 2682.3, 86.21 and 3.21 respectively. Similarly, the mean indices of prophase, metaphase, anaphase and telophase are 30.23, 29.77, 15.61 and 24.39.

Mitotic cycles:

(Table I.6, Fig. I.6)

The mitotic activity is impaired to a considerable extent. There are on the whole five mitotic cycles. Unlike in

the control, they do not fall into any categorised waves. Generally, the cycles are continuous occurring one after the other.

The five mitotic cycles are as follows:

1. 2 - 6 am
2. 6 - 11 am
3. 11 am - 6 pm
4. 6 - 10 pm
5. 10 pm - 2 am

The first cycle takes only 4 hours, the second 5 hours, the third 7 hours, the fourth 4 hours and the fifth 4 hours. Thus the range of duration of cycles lie between 4 - 7 hours and the mean duration works out to 4 - 8 hours.

In the first cycle, the mean mitotic index is 3.11. The maximum phasic index is that of metaphase (34.15) and minimum that of telophase (11.23). There are no mitotic peaks and falls. The mean mitotic index in the second cycle is 4.74. Among the phase indices, metaphase index is the maximum (32.61) and telophase index (18.25) minimum. In this cycle, there is one mitotic peak and one mitotic fall which are the maximum and minimum respectively in this treatment. In the third cycle, the mean mitotic index is 2.66. Maximum phase index is that of telophase (33.58) and minimum that of prophase (17.78). There is only one peak and one fall. The mean mitotic index in the

fourth cycle is 2.63. The maximum phase index is that of prophase (47.82) and minimum that of anaphase (7.08). There is only one peak and one fall. In the fifth cycle the mean mitotic index is 2.92. Among the phase indices, the maximum is that of prophase (35.48) and minimum that of anaphase (15.41). There is only one mitotic peak. No falls are observed.

Phasic behaviour:

(Table I.6, Fig.I.6)

The relative dominance of different phase indices in different cycles is variable. In the first and the second cycles the metaphase indices are maximum and the telophase indices, minimum. In the third cycle the telophase indices are maximum and the prophase indices, the least. In the fourth and fifth cycles the prophases are maximum and anaphases, minimum. But in the latter, the prophase indices and metaphase indices run closely.

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Under this treatment there are five wallows - three of prophase and two of telophase. The prophase wallows are of depression type occurring at 8 am (2nd cycle), 1 pm (3rd cycle) and 8 pm (4th cycle) and the telophase ones are of spurt type occurring at 8 am (2nd cycle) and 3 pm (3rd cycle).

There is a single overlap from 11 am to 1 pm (3rd cycle) when telophase indices run very high instead of coursing down, and completely make insignificant the rising prophase indices of the ensuing cycle. This is referred to as "Obliterate overlap".

Table I.5

Diurnal distribution of mitoses and mitotic phases in the root apices of Ephedra foliata grown under continuous darkness

| T | | TC | TM | Tp | pi | Tm | mi | Ta | ai | Tt | ti | MI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM | 1. | 2703 | 74 | 72 | 97.30 | 2 | 2.70 | - | - | - | - | 2.74 |
| | 2. | 2930 | 126 | 26 | 20.63 | 65 | 51.59 | 13 | 10.32 | 22 | 17.46 | 4.30 |
| | 3. | 2892 | 121 | 64 | 52.89 | 34 | 28.10 | 7 | 5.79 | 16 | 13.22 | 4.18 |
| | 4. | 3030 | 109 | 43 | 39.45 | 30 | 27.52 | 12 | 11.01 | 24 | 22.02 | 3.60 |
| | 5. | 2480 | 48 | 16 | 33.33 | 14 | 29.17 | 6 | 12.50 | 12 | 25.00 | 1.94 |
| | 6. | 2348 | 64 | 7 | 10.94 | 14 | 21.88 | 10 | 15.63 | 33 | 51.56 | 2.73 |
| | 7. | 2435 | 22 | 12 | 54.55 | 6 | 27.27 | 2 | 9.09 | 2 | 9.09 | 0.90 |
| | 8. | 2736 | 359 | 76 | 21.17 | 106 | 29.53 | 97 | 27.02 | 80 | 22.28 | 13.12 |
| | 9. | 2275 | 126 | 53 | 42.06 | 38 | 30.16 | 20 | 15.87 | 15 | 11.90 | 5.54 |
| | 10. | 2237 | 51 | 7 | 13.73 | 15 | 29.41 | 17 | 33.33 | 12 | 23.53 | 2.28 |
| | 11. | 2411 | 45 | - | - | 21 | 46.67 | 13 | 28.89 | 11 | 24.44 | 1.87 |
| | 12. | 3028 | 67 | 7 | 10.45 | 19 | 28.36 | 15 | 22.39 | 26 | 38.81 | 2.21 |
| PM | 1. | 3372 | 51 | 3 | 5.88 | 17 | 33.33 | 10 | 19.61 | 21 | 41.18 | 1.41 |
| | 2. | 2120 | 98 | 22 | 22.45 | 37 | 37.76 | 17 | 17.35 | 22 | 22.45 | 4.62 |
| | 3. | 3010 | 120 | 39 | 32.50 | 28 | 23.33 | 20 | 16.67 | 33 | 27.50 | 3.99 |
| | 4. | 2729 | 63 | 23 | 36.51 | 16 | 25.40 | 16 | 25.40 | 8 | 12.70 | 2.31 |
| | 5. | 2630 | 56 | 7 | 12.50 | 17 | 30.36 | 10 | 17.86 | 22 | 39.29 | 2.13 |
| | 6. | 2570 | 48 | 2 | 4.17 | 17 | 35.42 | 3 | 6.25 | 26 | 54.17 | 1.87 |
| | 7. | 3050 | 37 | 23 | 62.16 | 11 | 29.73 | 1 | 2.70 | 2 | 5.41 | 1.11 |
| | 8. | 2301 | 60 | 36 | 60.00 | 10 | 16.67 | 4 | 6.67 | 10 | 16.67 | 2.61 |
| | 9. | 2883 | 113 | 74 | 65.49 | 16 | 14.16 | 1 | 0.88 | 22 | 19.47 | 3.92 |
| | 10. | 2704 | 83 | 3 | 3.61 | 35 | 42.17 | 15 | 18.07 | 30 | 36.14 | 3.07 |
| | 11. | 2833 | 70 | 7 | 10.00 | 30 | 42.86 | 13 | 18.57 | 20 | 28.57 | 2.47 |
| | 12. | 2668 | 58 | 8 | 13.79 | 18 | 31.03 | 19 | 32.76 | 13 | 22.41 | 2.17 |
| Total | | 64375 | 2069 | 630 | 725.56 | 616 | 714.56 | 341 | 374.61 | 482 | 585.27 | 77.08 |
| Mean | | 2682.3 | 86.2 | 26.3 | 30.23 ±5.04 | 25.7 | 29.77 ±2.12 | 14.2 | 15.61 ±1.94 | 20.08 | 24.39 ±2.77 | 3.21 ±0.49 |

Table I.6

Nature of mitotic activity in the root apices of *Ephedra foliata* grown under continuous darkness

| S.No. | Item of study | Number of cycle | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| 1. | Duration of the cycle | 2-6 am | 6-11 am | 11am-6pm | 6-10 pm | 10pm-2am |
| 2. | Time taken by the cycle | 4 hrs | 5 hrs | 7 hrs | 4 hrs | 4 hrs |
| 3. | Range of mitotic indices | 1.94-4.30 | 0.9-13.12 | 1.41-4.62 | 1.11-3.92 | 2.17-4.30 |
| 4. | Mean mitotic index | 3.11 | 4.74 | 2.65 | 2.63 | 2.92 |
| 5. | Mean prophase index | 27.95 | 26.30 | 17.78 | 47.82 | 35.48 |
| 6. | Mean metaphase index | 34.15 | 32.61 | 3057 | 25.66 | 32.05 |
| 7. | Mean anaphase index | 26.67 | 22.84 | 17.93 | 7.08 | 15.41 |
| 8. | Mean telophase index | 11.23 | 18.25 | 33.58 | 19.42 | 17.11 |
| 9. | Number of mitotic peaks | - | 1 | 1 | 1 | 1 |
| 10. | Time of maximum peak | - | 8 am | - | - | - |
| 11. | Number of mitotic falls | - | 1 | 1 | 1 | - |
| 12. | Time of minimum fall | - | 7 am | - | - | - |
| 13. | Number of preparatory periods | - | - | - | - | - |
| 14. | Duration of preparatory periods | - | - | - | - | - |
| 15. | Time taken by preparatory periods | - | - | - | - | - |
| 16. | Number of wallows | | | 5 | | |
| a. | prophase depress wallow | - | 1 | 1 | 1 | - |
| b. | prophase spurt wallow | - | - | - | - | - |
| c. | telophase depress wallow | - | - | - | - | - |
| d. | telophase spurt wallow | - | 1 | 1 | - | - |
| 17. | Number of overlaps | | | 1 | | |
| a. | obliterate | - | - | 1(11am-1pm) | - | - |
| b. | parallel ascending | - | - | - | - | - |
| c. | parallel descending | - | - | - | - | - |

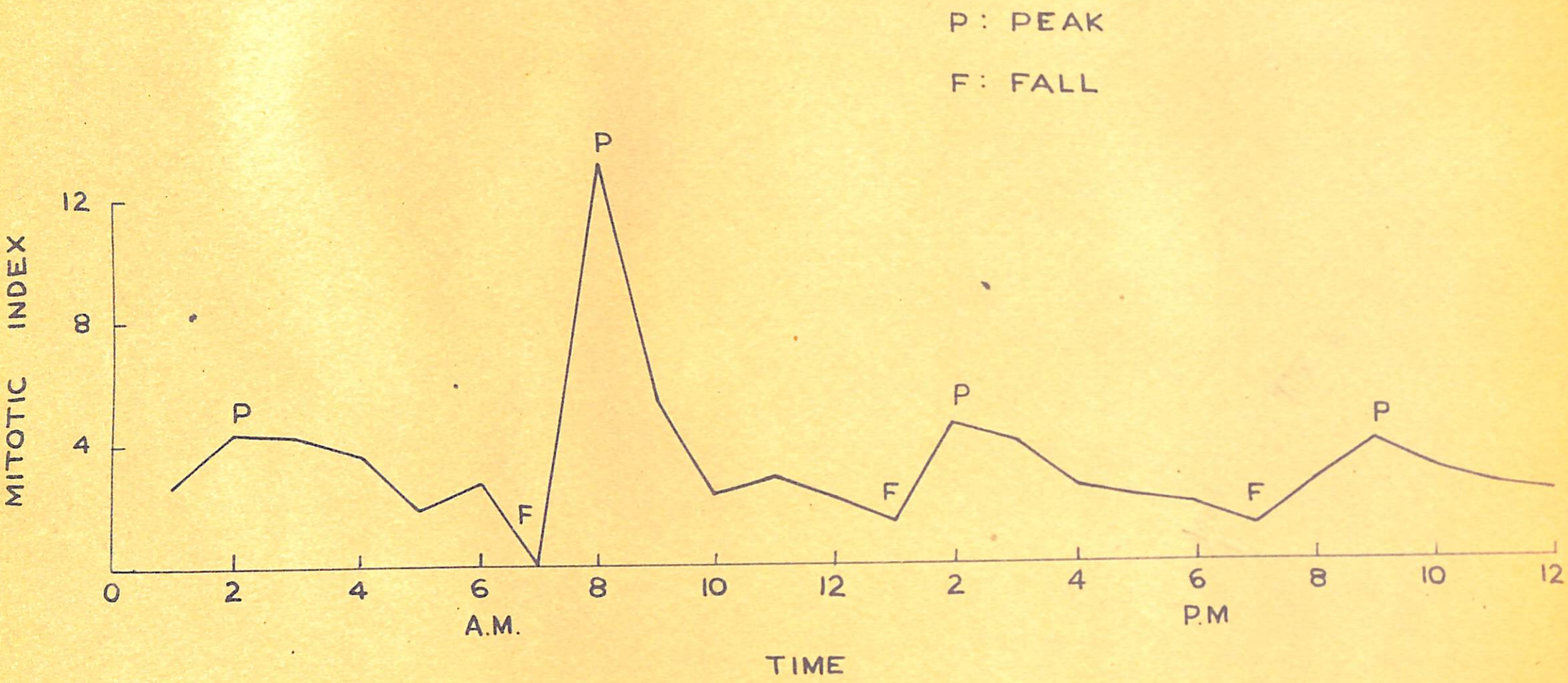


FIG. I·5 DIURNAL DISTRIBUTION OF MITOTIC INDICES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTINUOUS DARKNESS.

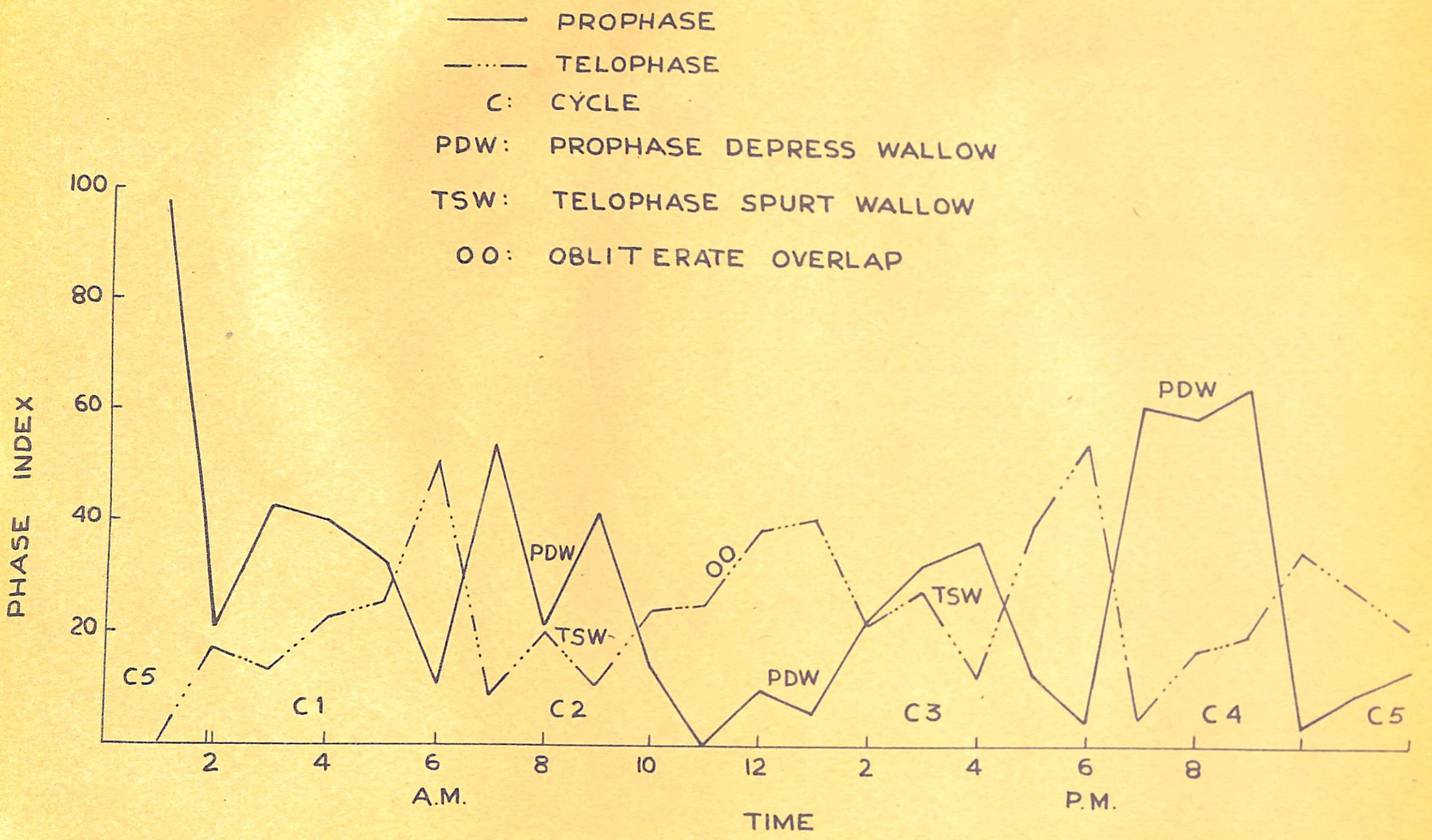


FIG. I·6 DIURNAL DISTRIBUTION OF MITOTIC CYCLES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTINUOUS DARKNESS.

## 4. Light

General mitotic activity:

(Table I.7, Fig. I.7)

The root tips maintained under continuous light manifest a wavy pattern of cell divisions. However about 7 peaks can be recognised at 3 am, 8 am, 12 am, 2 pm, 6 pm, 9 pm and 12 pm. Similarly, there are 7 falls at 1 am, 7 am, 9 am, 12 am, 5 pm, 7 pm and 10 pm, the last being the only steep one when the mitotic index was 1.6. The maximum mitotic peak has been recorded at 6 pm, when the mitotic index was 10.45. The mitotic activity is never negligible and hence there are no preparatory periods.

The number of cells studied range between 972 and 3180, mitoses between 24 and 256, and mitotic indices between 1.6 and 10.45. The means of total studied, cells observed mitoses and mitotic indices work out to 1955.9, 140.7 and 7.36 respectively. Similarly the mean indices of prophase, metaphase, anaphase and telophase are 47.68, 21.59, 7.56 and 23.17, respectively.

Mitotic cycles:

(Table I.8, Fig. I.8)

The rhythm of mitotic cycles is disturbed to the maximum extent. The inverse relationship between the prophase

and telophase indices has been greatly impaired. There appears to be on the whole only three cycles:

1. 4 am - 1 pm
2. 1 - 10 pm
3. 10 pm - 4 am

The first, and the second cycle take 9 hours each, and the third 6 hours. Thus the range of duration of cycles lies between 6 and 9 hours and the mean duration works out to 8 hours.

In the first cycle, the mean mitotic index is 7.25. Maximum phase index is that of prophase (49.19) and the minimum that of anaphase (6.59). There are two mitotic peaks and three falls. In the second cycle, the mean mitotic index is 6.95. Among the phase indices, prophase index is the maximum (48.44) and the anaphase index minimum (8.66). There are three peaks. The one at 6 pm being the maximum under this treatment. Similarly, there are three falls the one at 10 pm (1.6) being the minimum under this treatment. In the third cycle, the mean mitotic index is 8.13. Among the phase indices, prophase index is the maximum (44.29) and the anaphase index, (7.37), the minimum. There are two peaks and one fall.

Phasic behaviour:

(Table I.8, Fig.I.8)

The relative dominance of different phase indices is

same in all the three cycles i.e., prophase index is the most dominant and that of anaphase, the least.

There are six wallows in total, three of prophase and three of prophase. Of the prophase ones, the first is of the "spurt" type occurring at 12 am (1st cycle) and the other two occurring at 3 pm and 5 pm (2nd cycle) are of "depression" type. All the telophase wallows are of "spurt" type. They occur at 9 am (1st cycle), 3 pm and 5 pm (2nd cycle).

There is no overlap either of obliterate or of parallel type. A noteworthy feature is the dominance of prophases throughout while at the same time maintaining an inverse relationship with the telophases.

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Table I.7

Diurnal distribution of mitoses and mitotic phases in the root apices of Ephedra foliata grown under continuous light

| T | | TC | TM | Tp | pi | Tm | mi | Ta | ai | Tt | ti | MI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM | 1. | 2240 | 140 | 68 | 48.57 | 20 | 14.29 | 8 | 5.71 | 44 | 31.43 | 6.25 |
| | 2. | 2700 | 200 | 90 | 45.00 | 31 | 15.50 | 15 | 7.50 | 64 | 32.00 | 7.41 |
| | 3. | 2740 | 256 | 112 | 43.75 | 40 | 15.63 | 20 | 7.81 | 84 | 32.81 | 9.34 |
| | 4. | 2464 | 212 | 76 | 35.85 | 60 | 28.30 | 12 | 5.66 | 64 | 30.19 | 8.60 |
| | 5. | 1900 | 132 | 60 | 45.45 | 24 | 18.18 | 8 | 6.06 | 40 | 30.30 | 6.95 |
| | 6. | 1892 | 112 | 56 | 50.00 | 24 | 21.43 | 8 | 7.14 | 24 | 21.43 | 5.92 |
| | 7. | 2036 | 116 | 68 | 58.62 | 28 | 24.14 | 4 | 3.45 | 16 | 13.79 | 5.70 |
| | 8. | 2916 | 252 | 140 | 55.56 | 52 | 20.63 | 8 | 3.17 | 52 | 20.63 | 8.64 |
| | 9. | 2160 | 128 | 68 | 53.13 | 12 | 9.38 | 12 | 9.38 | 36 | 28.13 | 5.93 |
| | 10. | 1632 | 156 | 72 | 46.15 | 32 | 20.51 | 16 | 10.26 | 36 | 23.08 | 9.56 |
| | 11. | 2320 | 176 | 68 | 38.64 | 60 | 34.09 | 16 | 9.09 | 32 | 18.18 | 7.59 |
| | 12. | 2004 | 132 | 80 | 60.60 | 24 | 18.18 | 4 | 3.03 | 24 | 18.18 | 6.59 |
| PM | 1. | 1240 | 104 | 36 | 34.62 | 28 | 36.92 | 8 | 7.69 | 32 | 30.77 | 8.39 |
| | 2. | 1100 | 104 | 52 | 50.00 | 24 | 23.08 | 12 | 11.54 | 16 | 15.38 | 9.45 |
| | 3. | 1240 | 108 | 44 | 40.74 | 32 | 29.63 | 12 | 11.11 | 20 | 18.52 | 8.71 |
| | 4. | 1132 | 88 | 60 | 68.18 | 16 | 18.18 | 4 | 4.55 | 8 | 9.09 | 7.77 |
| | 5. | 2140 | 136 | 64 | 47.06 | 24 | 17.65 | 12 | 8.82 | 36 | 26.47 | 6.36 |
| | 6. | 1416 | 148 | 76 | 51.35 | 28 | 18.92 | 8 | 5.41 | 36 | 24.32 | 10.45 |
| | 7. | 3042 | 136 | 88 | 64.71 | 32 | 23.53 | - | - | 16 | 11.76 | 4.47 |
| | 8. | 3180 | 196 | 96 | 48.98 | 60 | 30.61 | 8 | 4.08 | 32 | 16.33 | 6.16 |
| | 9. | 1000 | 76 | 24 | 31.58 | 24 | 31.58 | 12 | 15.79 | 16 | 21.05 | 7.60 |
| | 10. | 1500 | 24 | 8 | 33.33 | 4 | 16.67 | 4 | 16.67 | 8 | 33.33 | 1.60 |
| | 11. | 1976 | 152 | 68 | 44.74 | 36 | 23.68 | 20 | 13.16 | 28 | 18.42 | 7.69 |
| | 12. | 972 | 92 | 44 | 47.83 | 16 | 17.39 | 4 | 4.35 | 28 | 30.43 | 9.47 |
| Total | | 46942 | 3376 | 1618 | 1144.43 | 731 | 518.12 | 235 | 181.43 | 792 | 556.05 | 176.59 |
| Mean | | 1955.9 | 140.7 | 67.4 | 47.68 ±1.92 | 30.5 | 21.59 ±1.25 | 9.8 | 7.56 ±0.81 | 33.0 | 23.17 ±1.48 | 7.36 ±1.89 |

Table I.8

Nature of mitotic activity in the root apices of *Ephedra foliata* grown under continuous light

| S.No | Item of study | Number of cycle 1 | 2 | 3 |
|---|---|---|---|---|
| 1. | Duration of the cycle | 4am-1pm | 1 pm-10pm | 10pm-4am |
| 2. | Time taken by the cycle | 9 hrs | 9 hrs | 6 hrs |
| 3. | Range of mitotic indices | 5.70-9.56 | 1.6-10.45 | 1.6-9.47 |
| 4. | Mean mitotic index | 7.25 | 6.95 | 8.13 |
| 5. | Mean prophase index | 49.19 | 48.44 | 44.29 |
| 6. | Mean metaphase index | 21.49 | 23.32 | 19.13 |
| 7. | Mean anaphase index | 6.59 | 8.66 | 7.37 |
| 8. | Mean telophase index | 22.72 | 19.58 | 29.31 |
| 9. | Number of mitotic peaks | 2 | 3 | 2 |
| 10. | Time of maximum peak | - | 6 pm | - |
| 11. | Number of mitotic falls | 3 | 3 | 1 |
| 12. | Time of minimum fall | - | 10pm | - |
| 13. | Number of preparatory periods | - | - | - |
| 14. | Duration of preparatory periods | - | - | - |
| 15. | Time taken by preparatory periods | - | - | - |
| 16. | Number of wallows | 6 | | |
| a. | prophase depress wallow | - | 2 | - |
| b. | prophase spurt wallow | 1 | - | - |
| c. | telophase depress wallow | - | - | - |
| d. | telophase spurt wallow | 1 | 2 | - |
| 17. | Number of overlaps | NIL | | |
| a. | obliterate | - | - | - |
| b. | parallel ascending | - | - | - |
| c. | parallel descending | - | - | - |

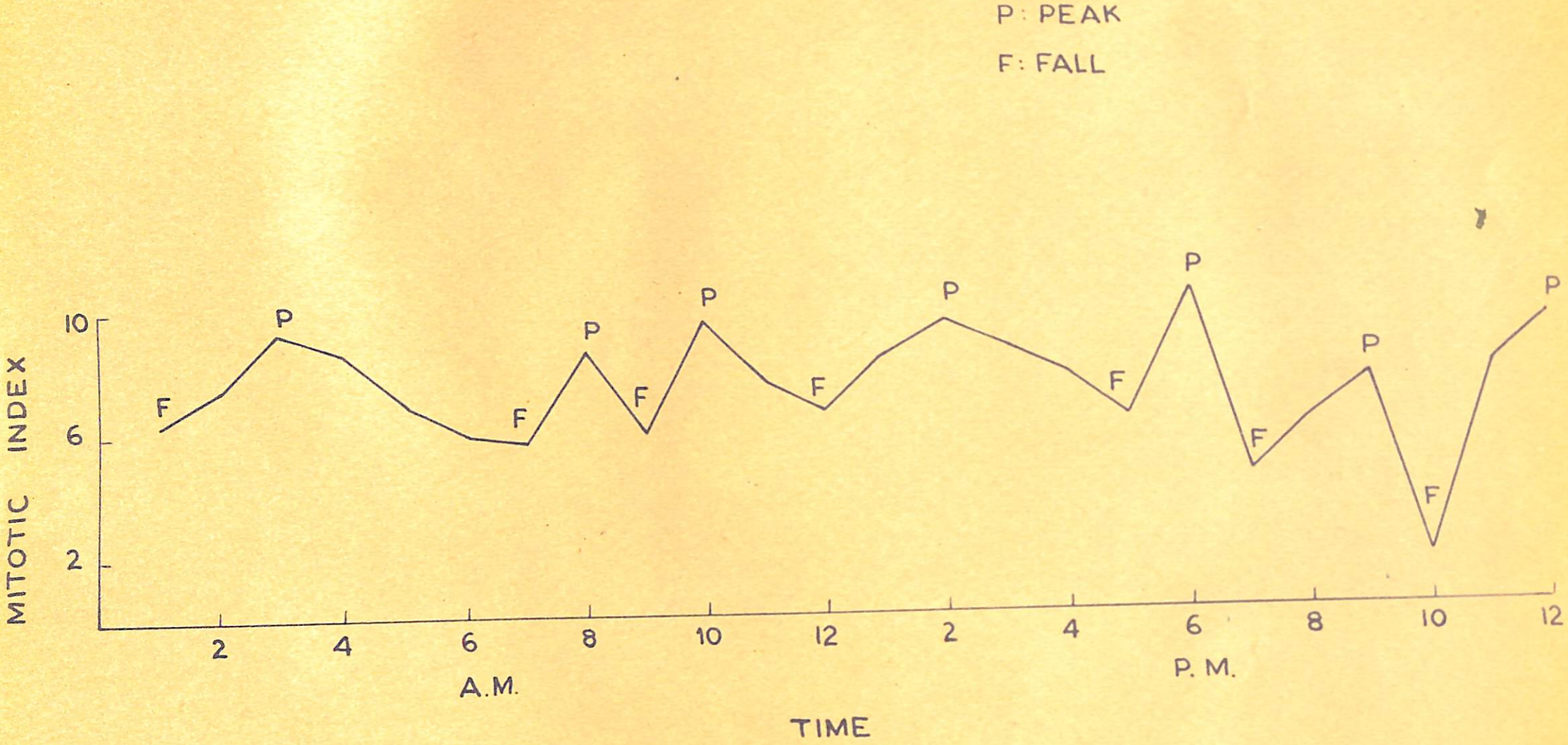



FIG. I·7 DIURNAL DISTRIBUTION OF MITOTIC INDICES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTINUOUS LIGHT.

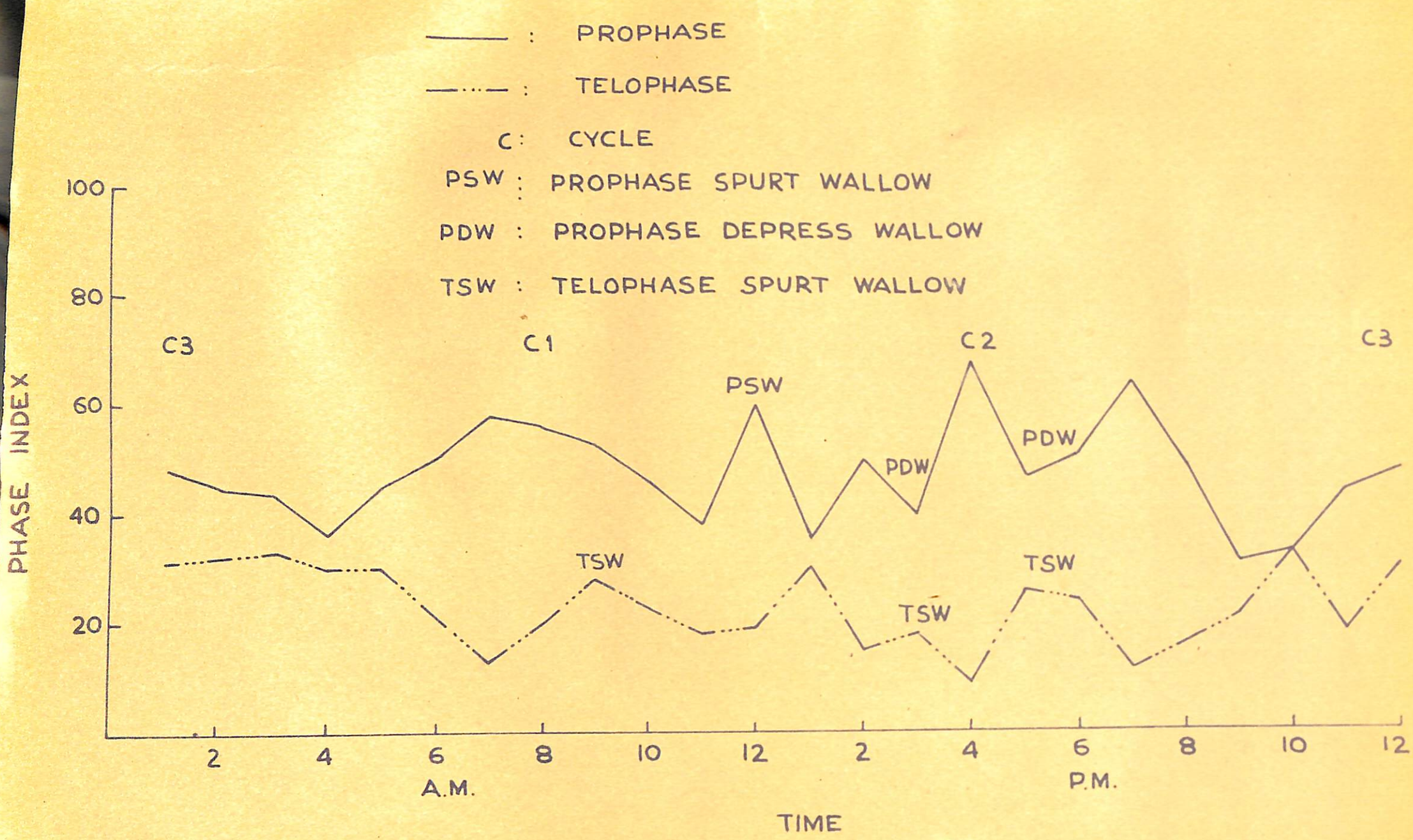



FIG. I·8 DIURNAL DISTRIBUTION OF MITOTIC CYCLES IN THE ROOT APICES OF EPHEDRA FOLIATA GROWN UNDER CONTINUOUS LIGHT.

## Table I.9

Diurnal variability of mitotic indices in the root apices of *Ephedra foliata* grown under different environmental conditions

| Time | | Control | Low temperature | Darkness | Light |
|---|---|---|---|---|---|
| A.M. | 1 | 7.15 | 6.47 | 2.73 | 6.25 |
| | 2 | 6.89 | 2.74 | 4.30 | 7.41 |
| | 3 | 5.82 | 2.0 | 4.18 | 9.34 |
| | 4 | 6.52 | 1.50 | 3.60 | 8.60 |
| | 5 | 5.53 | 0.97 | 1.94 | 6.95 |
| | 6 | 6.67 | 4.98 | 2.73 | 5.92 |
| | 7 | 6.03 | 11.70 | 0.90 | 5.70 |
| | 8 | 6.34 | 14.86 | 13.12 | 8.64 |
| | 9 | 4.29 | 3.14 | 5.54 | 5.93 |
| | 10 | 0.34 | 8.05 | 2.28 | 9.56 |
| | 11 | 0.37 | 3.53 | 1.87 | 7.59 |
| | 12 | 0.23 | 3.33 | 2.21 | 6.59 |
| P.M. | 1 | 5.07 | 1.09 | 1.41 | 8.39 |
| | 2 | 5.49 | 19.89 | 4.62 | 9.45 |
| | 3 | 5.61 | 0.24 | 3.99 | 8.71 |
| | 4 | 5.46 | 3.79 | 2.31 | 7.77 |
| | 5 | 5.88 | 1.80 | 2.13 | 6.36 |
| | 6 | 5.09 | 4.24 | 1.87 | 10.45 |
| | 7 | 4.28 | 21.32 | 1.11 | 4.47 |
| | 8 | 0.20 | 1.23 | 2.61 | 6.16 |
| | 9 | 0.34 | 5.88 | 3.92 | 7.6 |
| | 10 | 0.31 | 6.87 | 3.07 | 1.6 |
| | 11 | 0.46 | 8.64 | 2.47 | 7.69 |
| | 12 | 0.21 | 16.47 | 2.17 | 9.47 |
| Total | | 94.57 | 154.72 | 77.08 | 176.59 |
| Mean | | 3.94 ±0.55 | 6.45 ±0.91 | 3.21 ±0.49 | 7.36 ±1.89 |

Table I.10

** Mean mitotic and phase indices in root apices of *Ephedra foliata* grown under different environmental conditions

| Environment | Total cells studied | Total mitoses observed | Mitotic index | Phase indices prophase | metaphase | anaphase | telophase |
|---|---|---|---|---|---|---|---|
| Control | 2562.29 | 101.91 | 3.94 ± 0.55 | 31.95 ±5.02 | 15.69 ±1.35 | 18.17 ±2.21 | 34.20 ±5.17 |
| Low temperature | 2333.45 | 148.79 | 6.45 ± 0.91 | 38.84 ±5.48 | 30.48 ±4.31 | 10.19 ±1.05 | 20.49 ±2.23 |
| Darkness | 2682.29 | 86.21 | 3.21 ± 0.49 | 30.23 ±5.04 | 29.77 ±2.12 | 15.61 ±1.94 | 24.39 ±2.77 |
| Light | 1955.91 | 140.66 | 7.36 ± 1.89 | 47.68 ±1.92 | 21.59 ±1.25 | 7 .56 ±0.81 | 23.17 ±1.48 |

* Readings are means of 24 averages corresponding to the 24 hours of the day and night

Table I.11

Resume of the mitotic nature in the root apices of *Ephedra foliata* grown under different environmental conditions

| No | Item of study | Conditions of germination | | | |
|---|---|---|---|---|---|
| | | Control | Low temperature | Darkness | Light |
| 1. | Number of cycles | 5 | 5 | 5 | 3 |
| 2. | Range of duration of cycle | 2-5 hrs. | 3-6 hrs. | 4-7 hrs | 6-9 hrs |
| 3. | Mean duration of cycle | 3.6 hrs | 4.6 hrs | 4.8 hrs | 8.0 hrs |
| 4. | Range of mitotic indices | 0.2-7.15 | 0.24-21.32 | 0.9-13.12 | 1.6-10.45 |
| 5. | Mean mitotic index | 3.94 | 6.45 | 3.21 | 7.36 |
| 6. | Mean prophase index | 31.95 | 38.84 | 30.23 | 47.68 |
| 7. | Mean metaphase index | 15.69 | 30.48 | 29.77 | 21.59 |
| 8. | Mean anaphase index | 18.17 | 10.19 | 15.61 | 7.56 |
| 9. | Mean telophase index | 34.20 | 20.49 | 24.39 | 23.17 |
| 10. | Number of mitotic peaks | - | 6 | 4 | 7 |
| 11. | Time of maximum peak | - | 7 pm | 8 am | 6 pm |
| 12. | Number of mitotic falls | - | 6 | 3 | 7 |
| 13. | Time of minimum fall | - | 3 pm | 7 am | 10 pm |
| 14. | Number of preparatory periods | 2 | 1 | - | - |
| 15. | Duration of preparatory periods | 10-12 am & 8-12 pm | 8-9 pm | - | - |
| 16. | Time taken by preparatory period(s) | 6 hrs | 1 hr | - | - |
| 17. | Number of Wallows | - | 2 | 5 | 6 |
| a. | prophase depress wallow | - | 1 | 3 | 2 |
| b. | prophase spurt wallow | - | - | - | 1 |
| c. | telophase depress wallow | - | - | - | - |
| d. | telophase spurt wallow | - | 1 | 2 | 3 |
| 18. | Number of overlaps | - | 3 | 1 | - |
| a. | obliterate | - | - | 1 | - |
| b. | parallel ascending | - | 2 | - | - |
| c. | parallel descending | - | 1 | - | - |

Table I.12

Approximate durations of mitotic phases in the root apices of Ephedra foliata under different environment conditions determined by Index Method

| | Control | Low temperature | Darkness | Light |
|---|---|---|---|---|
| Prophase | 69.01 | 107.2 | 87.06 | 228.86 |
| Metaphase | 33.89 | 84.12 | 85.74 | 103.63 |
| Anaphase | 39.24 | 28.12 | 44.95 | 36.29 |
| Telophase | 73.87 | 56.55 | 70.24 | 111.21 |
| Total | 216 | 276 | 288 | 480 |

Readings are in minutes

1. Phase duration : $\frac{\text{Phase index}}{100}$ X / Duration of mitotic cycle

## II. KARYOTYPE ANALYSIS

Root apices of Ephedra foliata show somatic chromosome numbers of 13,14,15 and 16, the commonst being 14. Even in the 2n = 14 cells four types, - a,b,c, and d have been recognised based on the total lengths and arm ratios of chromosomes the 'a' type being more frequent. Variations in the lengths of chromosomes in various diploid (14a,14b,14c and 14d) and aneu ploid karyotypes (13,15 and 16) are given in Table II.1.

In the haploid complement of 14a, there are 4 metacentrics, 1 submetacentric and 2 acrocentrics. Average chromosome length is 14.96 microns and arm ratio, 1.68. In 14b, there are 4 metacentrics, 2 submetacentrics and 1 acrocentric. Average chromosome length is 13.30 and arm ratio 1.44. In 14c, there are 3 metacentrics, 2 submetacentrics and 2 acrocentrics. Average chromosome length is 17.66 microns and arem ratio, 1.87. In 14d, there are 2 metacentrics, 3 submetacentrics and 2 acrocentrics. Average chromosome length is 16.24 microns and arm ratio,1.68 (Tables II.2,II.4).

Among the aneuploids 13 type is essentially like 14a. But one of the submetacentric pair (chromosome No.5) or of the last acrocentric pair (chromosome No.7) is lost. In case of the

former, the average chromosome length works out to 12.87 microns with an arm ratio of 1.44 and in case of the latter, 13.07 and 1.69 respectively. On the other hand, the 15 - type is just like the 14b type with an extra acrocentric (chromosome 7). The average chromosome length is 11.78 and arm ratio 1.42. In 16 type there are 3 metacentrics 3 submetacentrics and 1 acrocentric, unlike any of the 14-types. The two extrachromosomes are the submetacentric, (chromosome 6) and acrocentric, (Tables II.2, II.4 and Photos II.1,3,4).

The total, and hence the average chromosome lengths of 14-types are more than in aneuploids (Fig.II.2). Further, the average chromosome length showed direct relationship with arm ratios (Table II.2).

Neither satellites, nor secondary constrictions had been observed except in one case (Photo II.2) where it is noticed at the terminus of a long thread on the short arm of the submetacentric.

Various types of diploid complements have been illustrated in Fig.II.1. The chromosomes of the haploid complements (derived from the diplord sets) had been systematically numbered and karyotype formulae also had been laid for each type (Table II.4).

Table II.1

Analysis of chromosome lengths in various kinds of karyotypes in the root apices of *Ephedra foliata*

| Chromosome number | SOMATIC 14a La | 14a Sa | 14b La | 14b Sa | 14c La | 14c Sa | KARYOTYPE 14d La | 14d Sa | 13 La | 13 Sa | 15 La | 15 Sa | 16 La | 16 Sa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 10.06 | 9.24 | 9.15 | 8.15 | 12.5 | 10.16 | 9.83 | 9.0 | 8.75 | 8.0 | 7.5 | 6.5 | 7.0 | 6.0 |
| 2. | 9.54 | 8.66 | 8.64 | 7.66 | 11.66 | 10.0 | 10.36 | 9.36 | 8.75 | 7.25 | 7.0 | 7.0 | 6.0 | 6.0 |
| 3. | 9.34 | 8.26 | 8.5 | 7.16 | 12.25 | 9.08 | 9.3 | 8.0 | 8.25 | 7.75 | 7.0 | 6.0 | 6.0 | 5.0 |
| 4. | 8.76 | 7.84 | 8.0 | 6.8 | 10.58 | 8.08 | 10.0 | 8.66 | 8.25 | 7.75 | 7.0 | 6.0 | 6.0 | 5.0 |
| 5. | 8.9 | 7.9 | 7.55 | 6.75 | 10.33 | 8.83 | 11.65 | 7.65 | 8.25 | 7.0 | 6.0 | 5.5 | 5.5 | 5.5 |
| 6. | 8.0 | 7.0 | 7.67 | 6.83 | 9.5 | 8.75 | 11.33 | 6.83 | 7.5 | 6.5 | 6.0 | 5.5 | 5.5 | 4.5 |
| 7. | 8.0 | 7.08 | 7.0 | 6.66 | 12.33 | 8. 0 | 10.5 | 7.16 | 7.0 | 6.0 | 6.0 | 6.0 | 5.0 | 4.5 |
| 8. | 7.33 | 6.57 | 6.48 | 6.02 | 10.5 | 7.83 | 11.0 | 7.83 | 6.75 | 5.5 | 3.5 | 3.5 | 4.5 | 4.5 |
| 9. | 8.58 | 6.22 | 6.87 | 5.33 | 9.58 | 7.66 | 8.15 | 5.65 | 9.5 | 6.0 | 9.0 | 6.0 | 7.0 | 5.0 |
| 10. | 8.44 | 5.76 | 6.5 | 4.5 | 9.25 | 7.08 | 6.84 | 4.66 | 8.5 * | 4.5 | 5.75 | 4.5 | 7.0 | 5.0 |
| 11. | 10.58 | 3.82 | 6.25 | 5.0 | 12.0 | 3.33 | 11.83 | 5.5 | 9.0 | 3.5 | 8.0 | 4.25 | 7.0 | 4.0 |
| 12. | 9.22 | 3.58 | 7.0 | 4.42 | 9.67 | 3.83 | 10.5 | 4.33 | 9.0 | 3.0 | 7.0 | 4.0 | 6.5<br>6.5 | 4.0<br>4.0 |
| 13. | 7.74 | 2.92 | 8.25 | 3.58 | 1033 | 3.5 | 8.66 | 3.5 | 8.0 | 2.75 | 8.0 | 3.0 | 7<br>7 | 3<br>3 |
| 14. | 7.75 | 2.5 | 8.5 | 3.0 | 7.66 | 3.0 | 9.67 | 4.33 | 7.0 * | 3.0 | 7.0<br>6.75 | 3.0<br>2.75 | 7 | 3 |
| | 122.24 | 86.35 | 108.36 | 78.86 | 148.14 | 98.63 | 139.62 | 88.46 | 106/<br>107.5 | 74/<br>75.5 | 101.5 | 73.5 | 100.5 | 72.0 |
| | | | | | | | | | | | 175 | | 172.5 | |
| | 208.59 | | 187.22 | | 246.77 | | 228.00 | | 180/183 | | | | | |

Readings are in microns.

* Either may become deficient.

| Sl. No. | Type of karyotype | Chromosome 1 TL | Chromosome 1 AR | Chromosome 2 TL | Chromosome 2 AR | Chromosome 3 TL | Chromosome 3 AR | Chromosome 4 TL | Chromosome 4 AR | Chromosome 5 TL | Chromosome 5 AR | Chromosome 6 TL | Chromosome 6 AR | Chromosome 7 TL | Chromosome 7 AR | Average TL | Average AR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | E.foliata (4M1SM2A) 14a. | 18.75 (9.8+8.95) | 1.1 | 17.1 (9.05+8.05) | 1.12 | 15.9 (8.45+7.45) | 1.15 | 14.49 (7.66+6.83) | 1.13 | 14.5 (8.51+5.99) | 1.42 | 13.6 (9.9+3.7) | 2.79 | 10.45 (7.75+2.7) | 3.07 | 14.96 | 1.68 |
| 2. | E.foliata (4M2SM1A) 14b. | 16.8 (8.9+7.9) | 1.1 | 15.23 (8.25+6.98) | 1.2 | 14.4 (7.61+679) | 1.16 | 13.08 (6.74+6.34) | 1.06 | 11.6 (6.69+4.91) | 1.38 | 12.33 (7.62+4.71) | 1.55 | 11.66 (8.38+3.28) | 2.62 | 13.30 | 1.44 |
| 3. | E.foliata (3M2SM2A) 14c. | 22.16 (12.08+10.08) | 1.18 | 19.99 (11.41+8.58) | 1.15 | 18.7 (9.91+8.79) | 1.1 | 19.33 (11.42+7.91) | 1.58 | 16.7 (9.4+7.3) | 1.4 | 14.42 3.33 (10.83+3.59) | | 12.25 3.33 (8.99+3.26) | | 17.66 | 1.87 |
| 4. | E.foliata (2M3SM2A) 14d. | (19.25 (10.06+9.19) | 1.1 | 17.98 (9.65+8.33) | 1.14 | 18.73 (11.49+7.24) | 1.55 | 18.25 (10.75+7.5) | 1.3 | 12.68 (7.49+5.19) | 1.48 | 16.07 2.28 (11.16+4.91) | | 13.08 2.94 (9.16+3.92) | | 16.24 | 1.68 |
| 5. | E.foliata(2n:13) (4M1SM(-)2A(-)) | 16.38 (8.75+7.63) | 1.14 | 16.0 (8.25+7.75) | 1.05 | 14.63 (7.87+6.76) | 1.13 | 12.62 (6.87+5.75) | 1.2 | *14.25 (9+5.25) | 1.73 | 12.25 (9+3.25) | 2.8 | *10.38 (7.5+2.88) | 2.75 | 12.87/ 13.07 | 1.44/ 1.69 |
| 6. | E.foliata(2n:15) (4M2SM1A(+)) | 14.0 (7.25+6.75) | 1.05 | 13.0 (7+6) | 1.17 | 11.5 (6+5.5) | 1.1 | 9.5 (5.75+4.75) | 1.0 | 12.5 (7.38+5.12) | 1.4 | 11.63 (7.5+4.13) | 1.83 | **10.17 (7.25+2.92) | 2.42 | 11.78 | 1.42 |
| 7. | E.foliata(2n=16) (3M3SM(+)1A(+)) | 12.5 (6.5+6.0) | 1.08 | 11.0 (6+5) | 1.2 | 10.5 (5.5+5) | 1.1 | 9.25 (4.75+4.5) | 1.05 | 12 (7+5) | 1.4 | **10.67 (6.67+4) | 1.66 | ** 10 (7+3) | 2.33 | 10.85 | 1.4 |

TL = Total length.
AR = Arm ratio
( ) = Long arm + short arm.

Readings are in microns.

* may be deficient by one homologue(i.e.,either may be monosomic)
** may be extra by one homologue(i.e.,trisomic)

Table II.3

Karyotype analysis of haploid complements of various species of Ephedra

| S.No. | Name of species | Chromosome 1 TL | AR | Chromosome 2 TL | AR | Chromosome 3 TL | AR | Chromosome 4 TL | AR | Chromosome 5 TL | AR | Chromosome 6 TL | AR | Chromosome 7 TL | AR | Average TL | Average AR | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Ephedra ochreata | 12.0 (6.5+5.5) | 1.2 | 12 (6.5+5.5) | 1.2 | 12.5 (7.0+5.5) | 1.3 | 12.5 (6.5+6.0) | 1.1 | 12 (6.5+6.5) | 1.2 | 8.5 (6.5+2.0) | 3.3 | 8.0 (6.5+1.5) | 4.3 | 11.1 | 1.94 | Hunziker 1955 |
| 2. | E.breana | 16.0 (8.0+8.0) | 1.0 | 16.5 (9.0+7.5) | 1.2 | 16 (8.5+7.5) | 1.1 | 15 (7.5+7.5) | 1.0 | 14 (7.5+6.5) | 1.1 | 10 (8+2) | 4.0 | 9.0 (7+2) | 3.5 | 13.8 | 1.84 | -do- |
| 3. | E.frustillata | 18.0 (9+9) | 1.0 | 17 (9.0+8.0) | 1.1 | 16 (8.0+8.0) | 1.0 | 15 (8+7) | 1.1 | 16 (9+7) | 1.3 | 14 (11.5+2.5) | 4.6 | 12.5 (10+2.5) | 4.0 | 15.5 | 2.01 | -do- |
| 4. | E.rupestris | 18.0 (9.5+8.5) | 1.1 | 18 (9.5+8.5) | 1.1 | 18 (10+8) | 1.3 | 15 (9+6) | 1.5 | 14 (8+6) | 1.3 | 14 (11.5+2.5) | 4.6 | 12 (9.5+2.5) | 3.8 | 15.6 | 2.1 | -do- |
| 5. | E.tweediana | 20 (13+7) | 1.9 | 21 (11.5+9.5) | 1.2 | 18.5 (10+8.5) | 1.2 | 19 (11.5+8.5) | 1.4 | 17 (10+7) | 1.4 | 13 (9+4) | 2.2 | 11.5 (9+2.5) | 3.6 | 17.1 | 1.84 | -do- |
| 6. | E.triandra | 24 (12+12) | 1.0 | 21 (10.5+10.5) | 1.0 | 17 (9+8) | 1.1 | 20.5 (11.5+9) | 1.3 | 20.5 (11.5+9) | 1.3 | 14.5 (11.5+3) | 3.8 | 13.5 (11+2.5) | 4.4 | 19 | 1.99 | -do- |
| 7. | E.multiflora | 26.5 (13.3+13.2) | 1.0 | 26.5 (15.5+11) | 1.4 | 23.5 (14+9.5) | 1.5 | 21.5 (12+9.5) | 1.3 | 21.5 (13.5+8) | 1.7 | 15.5 (12.5+3) | 4.2 | 14.5 (12+2.5) | 4.8 | 21.3 | 2.27 | -do- |
| 8. | E.americana | 31 (17+14) | 1.2 | 28 (15+13) | 1.2 | 32 (17+15) | 1.1 | 29.5 (15.5+14) | 1.1 | 26.5 (15+11.5) | 1.3 | 19.5 (15+4.5) | 3.3 | 20 (16.5+3.5) | 4.7 | 26.6 | 1.99 | -do- |
| 9. | E.foliata | 18.75 (9.8+8.95) | 1.1 | 17.1 (9.05+8.05) | 1.12 | 15.9 (8.45+7.45) | 1.15 | 14.49 (7.66+6.83) | 1.13 | 14.5 (8.51+5.99) | 1.42 | 13.6 (9.9+3.7) | 2.79 | 10.45 (7.75+2.7) | 3.07 | 14.96 | 1.68 | Present Author |

TL = Total Length.
AR = Arm ratio
( ) = Longarm + Short arm
Readings are in microns.

TABLE II.4

Nature of karyotypes in various species of Ephedra

| Sl. No. | Species | Haploid Numbers | Metacentrics | Submetacentrics | Acrocentrics | Chromosomes with sateth tes | | Karyotype Formula | Average Chromosome length |
|---|---|---|---|---|---|---|---|---|---|
| 1. | E.ochreata | 7 | 4 | 1 | 2 | 3 | - | $4M_{ss}1SM_s2A$ | 11.1 |
| 2. | E.breana | 7 | 4 | 1 | 2 | 2 | - | $4M_s1SM_s2A$ | 13.8 |
| 3. | E.frustillata | 7 | 4 | 1 | 2 | 1 | - | $4M_{ss}1SM2A$ | 15.5 |
| 4. | E.rupestris | 7 | 2 | 3 | 2 | 2 | - | $2M_{ss}3SM2A$ | 15.6 |
| 5. | E.tweediana | 7 | 0 | 5 | 2 | 1 | - | $5SM_s2A$ | 17.1 |
| 6. | E.triandra | 7 | 3 | 2 | 2 | 3 | - | $3M_{sss}2SM\ 2A$ | 19.0 |
| 7. | E.multiflora | 7 | 1 | 4 | 2 | 3 | 3 | $1M_s4SM_{3sc}2A$ | 21.3 |
| 8. | E.americana | 7 | 2 | 3 | 2 | 2 | - | $2M3SM_{ss}2A$ | 26.6 |
| 9.a. | E.foliata(14a) | 7 | 4 | 1 | 2 | - | - | 4M,1SM,2A | 14.96 |
| b. | E.foliata(14b) | 7 | 4 | 2 | 1 | - | - | 4M,2SM,1A | 13.30 |
| c. | E.foliata(14c) | 7 | 3 | 2 | 2 | - | - | 3M,2SM,2A | 17.66 |
| d. | E.foliata(14d) | 7 | 2 | 3 | 2 | - | - | 2M,3SM,2A | 16.24 |
| 10. | E.foliata(13) | 7(-) | 4 | 1(-) | 2(-) | - | - | 4M,1SM(-),2A(-) | 12.87/ 13.07 |
| 11. | E.foliata(15) | 7(+) | 4 | 2 | 1(+) | - | - | 4M,2SM,1A(+) | 11.78 |
| 12. | E.foliata(16) | 7(++) | 3 | 3(+) | 1(+) | - | - | 3M,3SM(+),1A(+) | 10.85 |

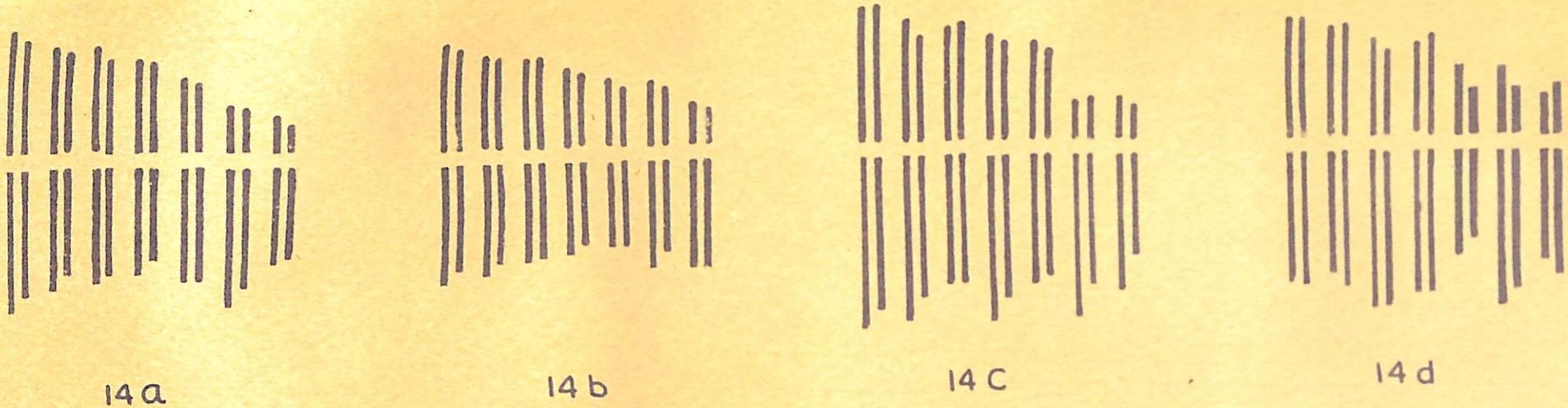



FIG. II·1 VARIOUS TYPES OF DIPLOID CHROMOSOME COMPLEMENTS IN THE ROOT APICES OF EPHEDRA FOLIATA (X 2000)

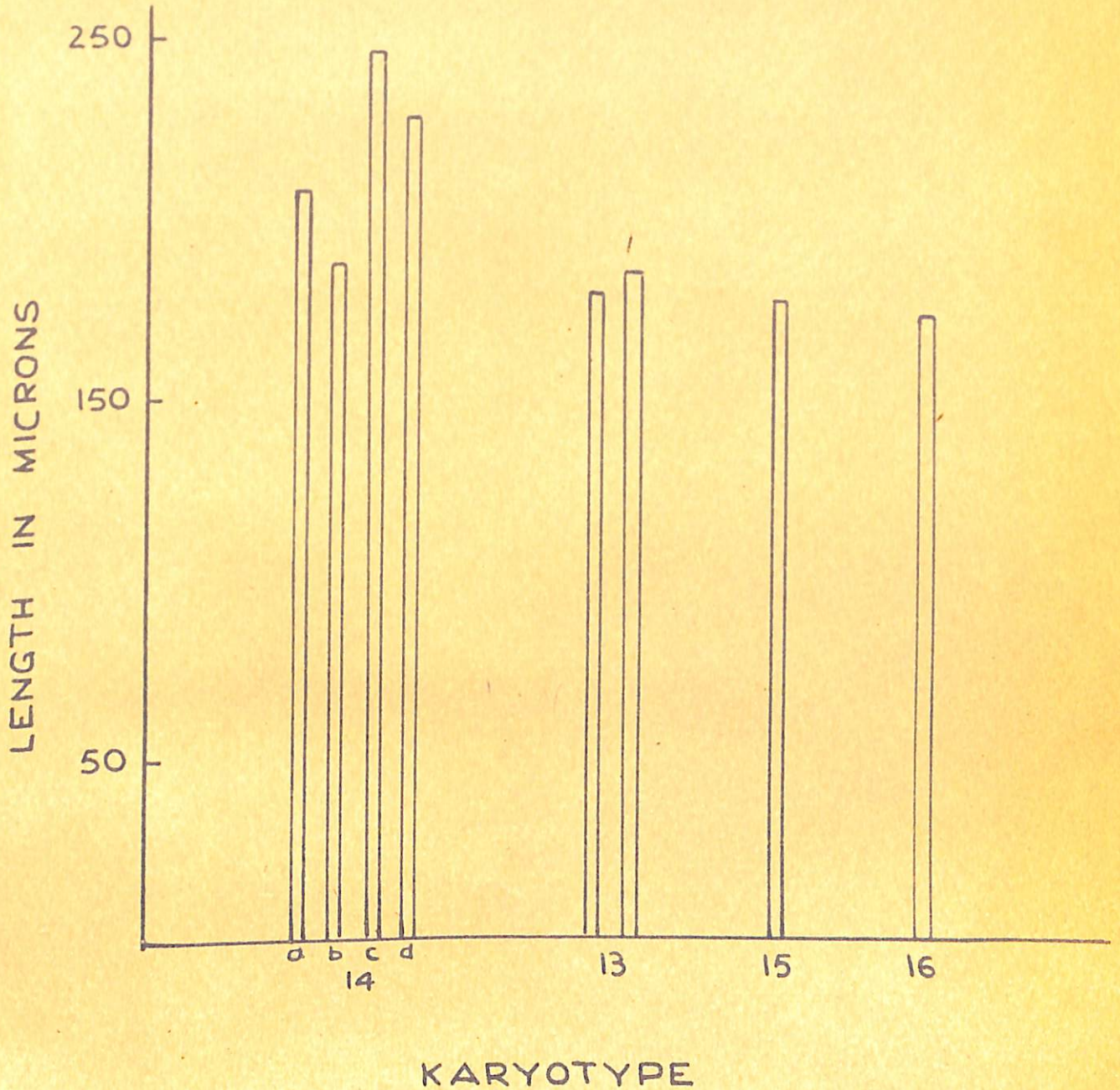
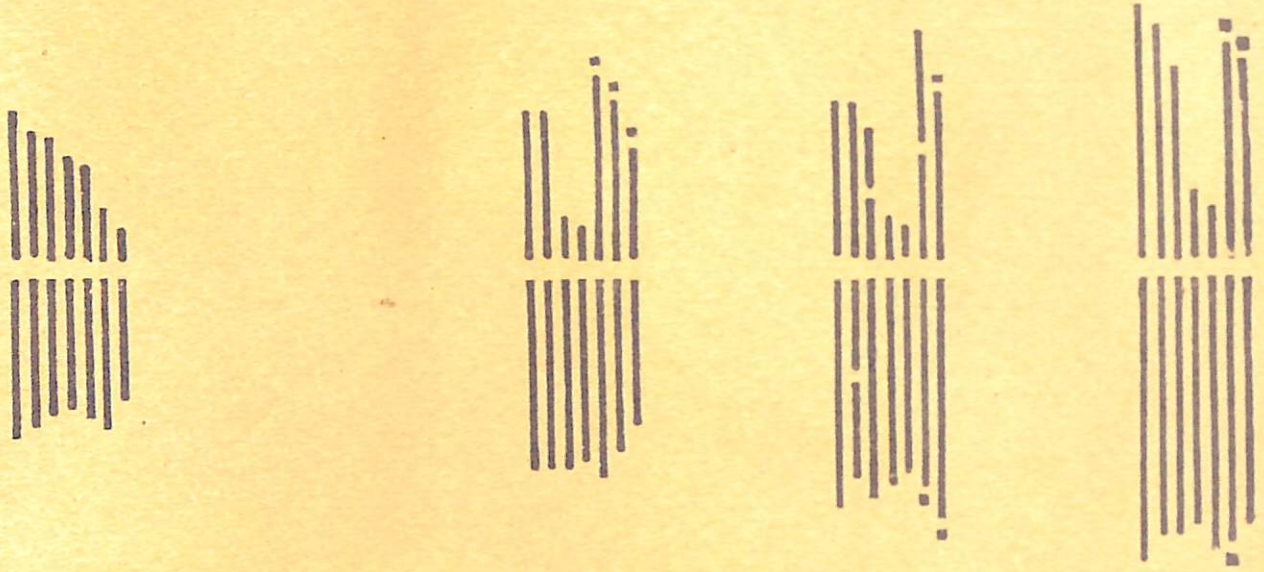



FIG. II. 2 TOTAL CHROMOSOME LENGTHS OF DIFFERENT KARYOTYPES IN THE ROOTAPICES OF EPHEDRA FOLIATA

E. OCHREATA E. BREANA E. FRUSTILLTA E. RUPESTRIS E. TWEEDIANA



E. FOLIATA E. TRIANDRA E. MULTIFLORA E. AMERICANA

FIG. II·3 KARYOTYPES OF DIFFERENT SPECIES OF EPHEDRA *(X2000).

Photo II.1 A
13 chromosomes
X 600

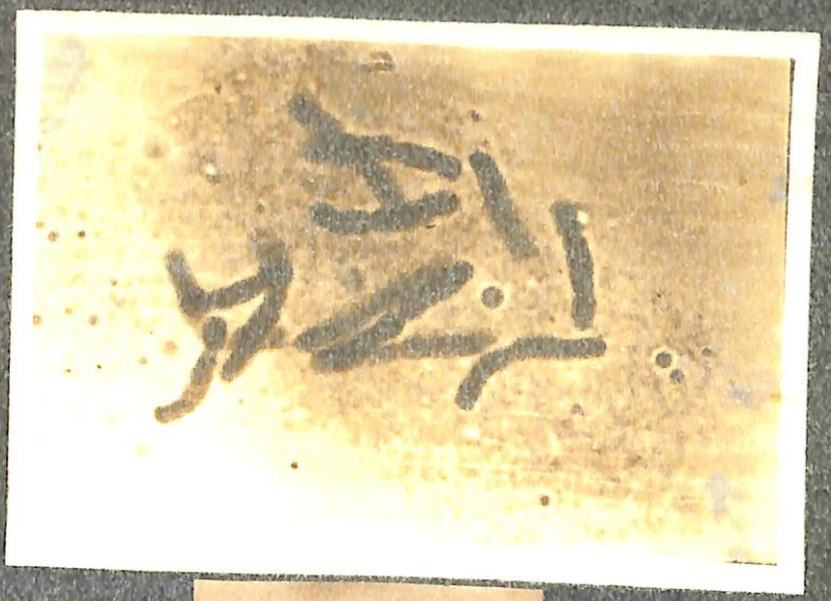


Photo II. 1 B
13 chromosomes
X 1024

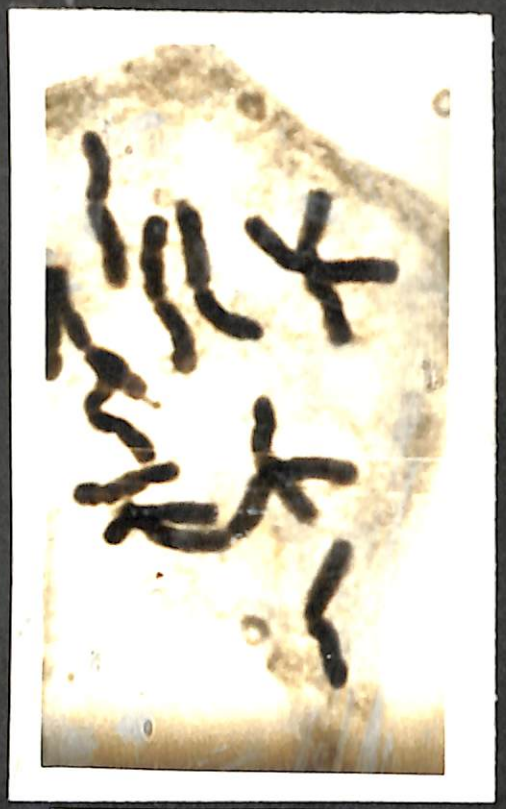


Photo II. 2 A:
14 Chromosomes
showing satellite
X 1280

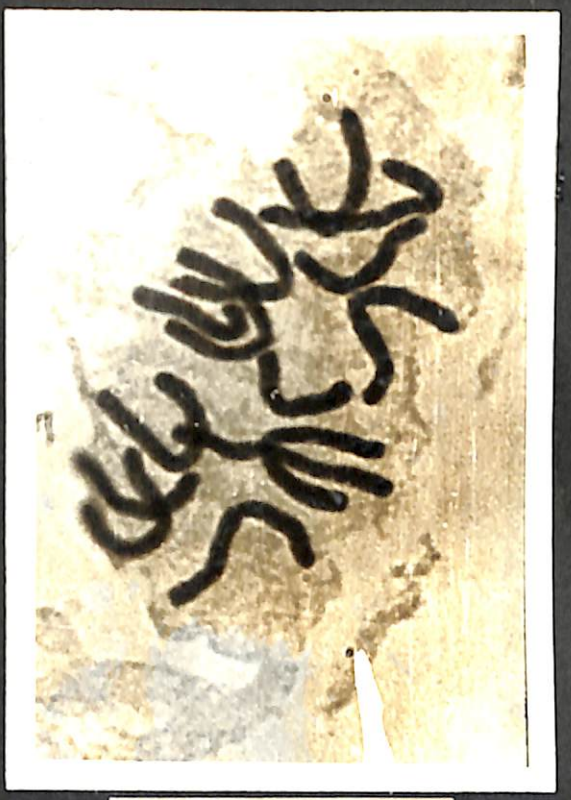


Photo II. 2 B:
14 Chromosomes
X 1280

Photo II. 2 C:
14 chromosomes
X 1024

Photo II. 2 D:
14 Chromosomes
X 1024

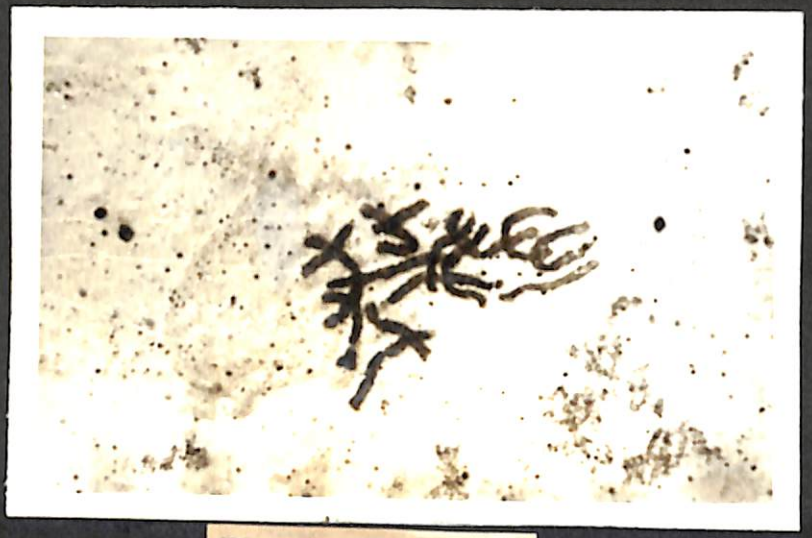


Photo II. 3 A:
15 Chromosomes
X 1024

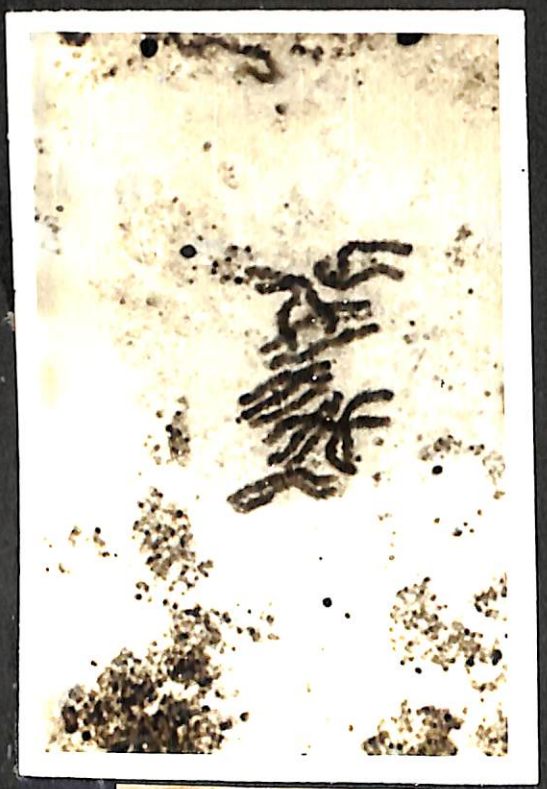


Photo II. 4 B:
16 Chromosomes
X 1024

Photo II. 4A:
16 Chromosomes
X 1024

## III. NATURAL ABERRATIONS

### 1. Micronuclei

Occurrence and number:

(Table III.1 and Photos III.1A-1C)

Micronuclei occur in the columella and body cells of the root apices of Ephedra foliata. They may be present in a nondividing cell or in a cell of late telophase. In the former they are present on one or both sides of the main nucleus. But they are always present on the longitudinal axis of the cell, and mostly near the main nucleus. In cells of late telophase, they may be present at the equatorial region or at one or both the polar regions.

In columella 2178 cells were studied of which 11 had micronuclei giving a frequency of 0.085. In the body 10728 cells were studied of which 33 showed micronuclei giving a frequency of 0.255. Of these 33 aberrent cells, 21 showed a single micronucleus; 7 cells, 2 micronuclei; 3 cells 3 micronuclie; and 2 cells, 5 micronuclei; besides the usual main nucleus. In columella cells the micronuclear number was invariably one, and in body cells from 1-3 and 5. Thus, out of 12906 cells, 44 are recorded as aberrent giving a frequency of 0.34.

Size and micronuclear index:

(Table III.1)

The columella cells have larger nuclei than body cells, in relation to their cell sizes. The readings following, concerning the sizes are in microns. Sizes of micronuclei varied from 8.8 to 38.8 in columella cells, with a mean of 23.0. The range of micronuclear index lies between 0.2 and 9.0 with a mean of 3.3.

In the body cells, when micronucleus is one its size varies from 5.2 to 52 giving a mean of 24. The micronuclear indices vary from 3.3 to 6.0, with a mean of 5.3.

When the micronuclei are two, the range of their individual sizes lies between 2.4 and 15.2 and that of total size between 11.2 and 30.4. The mean individual size is 10.35 and mean total size, 20.7. The range of micronuclar indices is 3.4 to 12.5, giving a mean of 5.6.

In cells with three micronuclei, the range of their individual sizes lies between 4 and 19, and of total size, between 16 and 32. The mean individual size is 8.0 and mean total size, 24.0. The range of micronuclear indices is from 3.7 to 7.0, giving a mean of 5.7.

When the micronuclear number is five, the range of their individual sizes vary form 3.0to 36, and of total size from 13 to 43. The mean individual sizes works out to 5.6 and mean total size, 28. The range of micronuclear indices is between 5.5 and 14.5, giving a mean of 9.0.

## 2. Bridges

Nature:

(Photos III.2A-2D)

Bridges were observed in columella as well as body cells, occurring both in anaphase and telophase. They may be 1,2, or more. When single, they were either thin or thick (equivalent to chromosome breadth.) When 2 or more, they were generally thick, running parallelly or crossing with each other. But, when thin, they ran invariably parallel and very close. When more than 2, they were always thick. All these are continuous, running from pole to pole. Sometimes, they were slightly discontinuous at places and appeared as chromatin streaming from one pole to the other. They were rare and are referred to as sticky bridges. Quite often bridges have been observed along with other aberrations like forwards and laggards.

Frequency:

(Table III.2)

The total percentage of bridges work out to 0.24 - 0.06 in columella, and 0.18 in body cells. Frequencies of single, double and multiple bridges were 0.054, 0.162 and 0.024 respectively. Most bridges persisted till telophase, where their frequency, hence was more.

Table III.1

Variations in the number and size of micronuclei found in the root apices of Ephedra foliata

| Sl. No. | Type of cells | Cells studied | Aberrent cells | Number of micronuclei | % | Mean size of Cell | Mean size of Nucleus | Range of micronuclear size individual | Range of micronuclear size total | Mean micronuclear size individual | Mean micronuclear size Total | Range of micronuclear Index | Mean micronuclear Index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Columella cells | 2178 | 11 | 1 | 0.055 | 591.2 | 228 | 8.8 - 38.8 | 8.8 - 38.8 | 23.0 | 23.0 | 0.2 - 9.0 | 3.3 |
| 2. | Body cells | 10728 | 33 | | 0.255 | | | | | | | | |
| | | | 21 | 1 | | 1760 | 320.5 | 5.2 - 52 | 5.2 - 52 | 24.0 | 24.0 | 3.3 - 6.0 | 5.3 |
| | | | 7 | 2 | | 1060 | 243 | 2.4 -15.2 | 11.2-30.4 | 10.35 | 20.7 | 3.4 -12.5 | 5.6 |
| | | | 3 | 3 | | 1800 | 404 | 4 - 19 | 16 - 32 | 8.0 | 24.0 | 3.7 - 7.0 | 5.7 |
| | | | 2 | 5 | | 1240 | 240 | 3 - 36 | 13 - 43 | 5.6 | 28 | 5.5 -14.5 | 9.0 |
| | Total | 12906 | 44 | | 0.34 | | | | | | | | |

Sizes are in microns.

$$\text{Micronuclear index} = \frac{Vmn}{Vmn+Vn} \times 100$$

Vmn = Volume of micronuclear

Vn = Volume of nucleus.

## 2. Bridges

Nature:

(Photos III.2A-2D)

Bridges were observed in columella as well as body cells, occurring both in anaphase and telophase. They may be 1,2, or more. When single, they were either thin or thick (equivalent to chromosome breadth.) When 2 or more, they were generally thick, running parallelly or crossing with each other. But, when thin, they ran invariably parallel and very close. When more than 2, they were always thick. All these are continuous, running from pole to pole. Sometimes, they were slightly discontinuous at places and appeared as chromatin streaming from one pole to the other. They were rare and are referred to as sticky bridges. Quite often bridges have been observed along with other aberrations like forwards and laggards.

Frequency:

(Table III.2)

The total percentage of bridges work out to 0.24 - 0.06 in columella, and 0.18 in body cells. Frequencies of single, double and multiple bridges were 0.054, 0.162 and 0.024 respectively. Most bridges persisted till telophase, where their frequency, hence was more.

Table III.2

Nature and frequency of bridges
in the root apices of *Ephedra foliata*

| | Type of cells | Cells studied | Phase of occurrence | Number of bridges 1 | 2 | more than 2 | Total aberrent cells | % |
|---|---|---|---|---|---|---|---|---|
| 1. | Columella cells | 6976 | Anaphase | 1 | 2 | - | 3 | 0.06 |
| | | | Telophase | 2 | 5 | - | 7 | |
| 2. | Body cells | 9667 | Anaphase | 2 | 7 | 2 | 11 | 0.18 |
| | | | Telophase | 4 | 13 | 2 | 19 | |
| | Total | 16643 | | 9 | 27 | 4 | 40 | |
| | % | | | 0.054 | 0.162 | 0.024 | | 0.24 |

## 3. Forwards

Nature:

(Photos III. 4A-4B)

Forwards, the precociously moving chromosomes, varied in number, nature and occurrence. Generally they were 1,2 or more. They were long or short, and occurred in metaphase, anaphase and telophase of both columella and body cells. When more than 1, they may be present only at one pole or at both. Rarely, diplochromosomes, with their undivided centromeres have been observed to behave as forwards. In several cells, they occur alongside other aberrations like bridges.

Frequency:

(Table III.3)

The total percentage of forwards in the root apices is 0.262 - 0.024 in columella, and 0.237 in body cells. Cells with 2 forwards were more, giving a frequency of 0.139.

Table III.3

Nature and frequency of forwards in the root apices of *Ephedra foliata*

| Type of cells | Cells studied | Phase of occurrence | Number of forwards 1 | 2 | more than 2 | Total cells aberrent | % |
|---|---|---|---|---|---|---|---|
| Columella Cells | 5678 | Metaphase | 1 | - | - | 1 | |
| | | Anaphase | 1 | - | - | 1 | 0.024 |
| | | Telophase | - | 1 | - | 1 | |
| Body Cells | 6534 | Metaphase | 4 | 2 | 1 | 7 | |
| | | Anaphase | 3 | 2 | 4 | 9 | 0.237 |
| | | Telophase | 1 | 12 | - | 13 | |
| Total | 12,212 | | 10 | 17 | 5 | 32 | |
| % | | | 0.082 | 0.139 | 0.041 | | 0.262 |

## 4. Laggards

Nature:

(Photos III.3A-3B)

Chromosomes, lagging during anaphase separation varied in their number and nature. They were present in columella as well as body cells. They were long or short, the latter being more frequent. When more than two, they may be similar or dissimilar occurring at the same or different sides of the equatorial region. Other aberrations like bridges occurred alongside laggards.

Frequency:

(Table III.4)

The total percentage of laggards in the material worked out to 0.15 - 0.03 in columella and 0.12 in body cells. Cells with 2 laggards were more frequent, occurring with a frequency of 0.09%.

Table III.4

Nature and frequency of laggards in the root apices of *Ephedra foliata*

| Type of Cells | Cells studied | Phase of occurrence | Number of laggards 1 | 2 | more than 2 | Total aberrent cells | % |
|---|---|---|---|---|---|---|---|
| Columella cells | 4273 | Anaphase | - | - | - | - | 0.03 |
| | | Telophase | - | 3 | - | 3 | |
| Body cells | 5725 | Anaphase | 1 | 2 | 1 | 4 | 0.12 |
| | | Telophase | 4 | 4 | - | 8 | |
| Total | 9998 | | 5 | 9 | 1 | 15 | |
| % | | | 0.05 | 0.09 | 0.01 | | 0.15 |

## 5. Nucleoli

Occurrence and number:

(Table III.5 and Photos III.5A - 5F)

Feulgen-stained root cells, hydrolysed normally, show nucleoli as unstained gaps if squashed in acetic acid (10%) and as dark-stained bodies if squashed in acetocarmine. But if hydrolysis is more, they appear as gaps in both. The stained nucleoli are either spherical or oval and rarely bizarre in their shapes. Nucleoli being universal, are present in columella as well as body cells of the root apex. Only one cell has been observed where a micronucleus possessed a nucleolus.

There were some variations concerning the number of nucleoli. In columella cells they varied from 1 to 5. The percentages of cells which showed 1,2,3,4 and 5 nucleoli are 12.3, 29.3, 14.6, 28.1 and 15.8 respectively. In body cells they varied from 1 to 4. The percentages of cells which showed, 1,2,3 and 4 nucleoli are 30, 40, 26.7 and 3.3 respectively. Thus in both tissues binucleolar condition was more frequent, but the least frequency was of uninucleolar condition in columella, and of tetranuclealar condition in body cells. On the whole binucleolate cells (34.65%) were most frequent.

Dimensions and ratios:

(Table III.6)

The individual as well as total sizes of nucleoli have been measured in microns. In columella cells, when there was one nucleolus its size ranged between 9 and 54, with a mean of 31. If the nucleoli were 2, the ranges of individual and total sizes were 8-45 and 18-75, the mean being 40. When they were 3, individual nucleolar range was 8-27 and total 28-63, the mean being 26.6. When nucleoli were 4, the individual (4-16) and total (26-58) nucleolar ranges have a mean of 33. In the infrequent case of 5 nucleoli, the individual range was 5-10 and the total 30-44, giving a mean of 31. Thus, the mean total nucleolar size in columella cells varied from 26.6 to 40.0. The nuclear - nucleolar ratios, similarly ranged from 6.62 to 8.877, and the cell nucleolar ratios, from 2.96 to 4.25. The total nucleolar size, nuclear - nucleolar ratio and cell-nucleolar ratio were more in cells with 2 nucleoli and least in cells with 3 nucleoli. The cell sizes were comparatively smaller and of nuclei, larger.

In body cells, when the nucleolus was one, the range of size was 12-88 with a mean of 28.8. When 2, the individual size varied from 4-48 and total size from 10-92. But the mean was only 20.4. When 3, the individual range was 3.27 and total 12-78, the mean being 46.6. In the infrequent 4 nucleolar cells, the individual and total ranges were 5-23 and 22-84 respectively. The mean was 37.2. Thus, the mean total nucleolar size varied

from 20.4 to 46.6. The nuclear - nucleolar ratios ranged from 1.59 to 3.93. The total nucleolar size, the nuclear-nucleolar ratio and cell-nucleolar ratio were always more in cells with 3 nucleoli and least in cells with 2 nucleoli, unlike in the columella cells.

Table III.5

Numerical variations of nucleoli
in the root apices of Ephedra foliata

| Number of Nucleoli | Columella cells | | | Body cells | | | Mean |
|---|---|---|---|---|---|---|---|
| | cells studied | cells observed | % | cells studied | cells observed | % | |
| | 82 | | | 90 | | | |
| 1. | | 10 | 12.3 | | 27 | 30 | 21.5 |
| 2. | | 24 | 29.3 | | 36 | 40 | 34.65 |
| 3. | | 12 | 14.6 | | 24 | 26.7 | 20.6 |
| 4. | | 23 | 28.1 | | 3 | 3.3 | 15.7 |
| 5. | | 13 | 15.8 | | - | - | 7.9 |

## Table III.6

Dimensional variations of nucleoli in the root apices of *Ephedra foliata*

| Type of cells | Number of nucleoli | Average size of Cell | Average size of Nucleus | Range of size of nucleoli individual | Range of size of nucleoli Total | Total average size of nucleoli | N - n ratio | C - n ratio |
|---|---|---|---|---|---|---|---|---|
| Columella cells | 1 | 953.7 | 353.0 | 9-54 | | 31 | 8.782 | 3.25 |
| | 2 | 941.8 | 450.6 | 8-45 | 18-75 | 40 | 8.877 | 4.25 |
| | 3 | 900 | 399.3 | 8-27 | 28-63 | 26.6 | 6.62 | 2.96 |
| | 4 | 884.5 | 378.0 | 4-16 | 26-58 | 33 | 8.73 | 3.84 |
| | 5 | 770 | 396.0 | 5-10 | 30-44 | 31 | 7.828 | 4.03 |
| Body cells | 1 | 1361 | 411.7 | 12-88 | | 28.8 | 6.993 | 2.12 |
| | 2 | 1278 | 373.2 | 4-48 | 10-92 | 20.4 | 5.493 | 1.59 |
| | 3 | 1186.3 | 479.1 | 3-27 | 12-78 | 46.6 | 9.727 | 3.93 |
| | 4 | 1074 | 483.0 | 5-23 | 22.84 | 37.2 | 7.702 | 3.46 |

Readings are in microns.

N - n ratio = Nuclear - nucleolar ratio

C - n ratio = Cell - nucleolar ratio

Table III.7

Frequency of various aberrations occurring naturally in the root apices of *Ephedra foliata*

| Type of cells | Type of aberration | | | | |
|---|---|---|---|---|---|
| | Micronucleus | Bridges | Forwards | Laggards | Total |
| Columella cells | 0.085 | 0.060 | 0.024 | 0.030 | 0.199 |
| Body cells | 0.255 | 0.180 | 0.237 | 0.120 | 0.792 |
| Total | 0.340 | 0.240 | 0.261 | 0.150 | 0.991 |

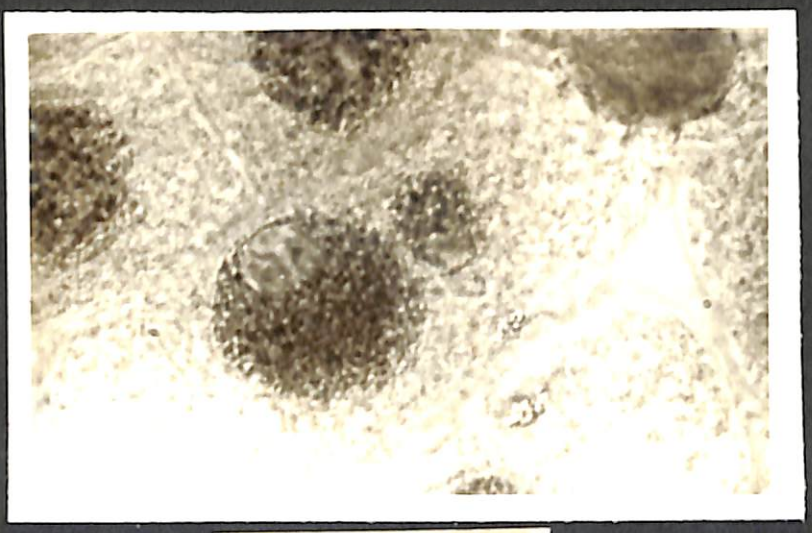


Photo III.1 A:
1 micronucleus
X 1024

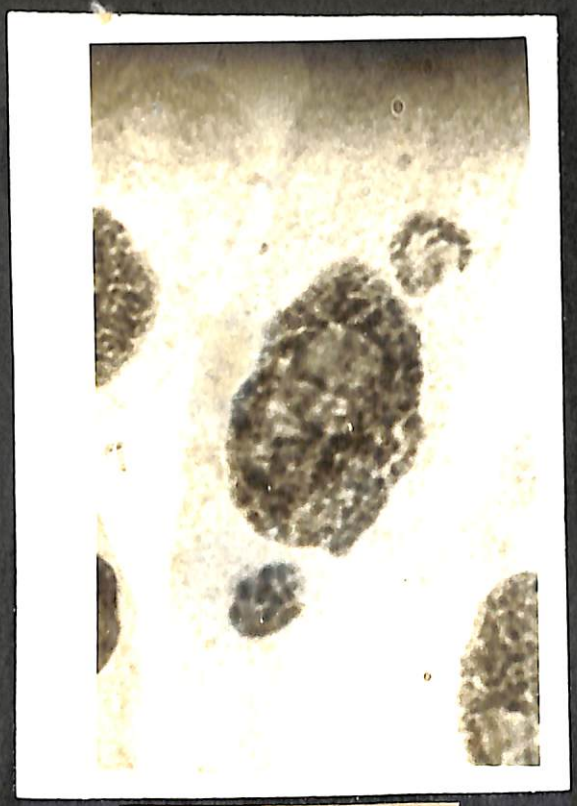


Photo III.1B :
2 micronuclei
X 1024

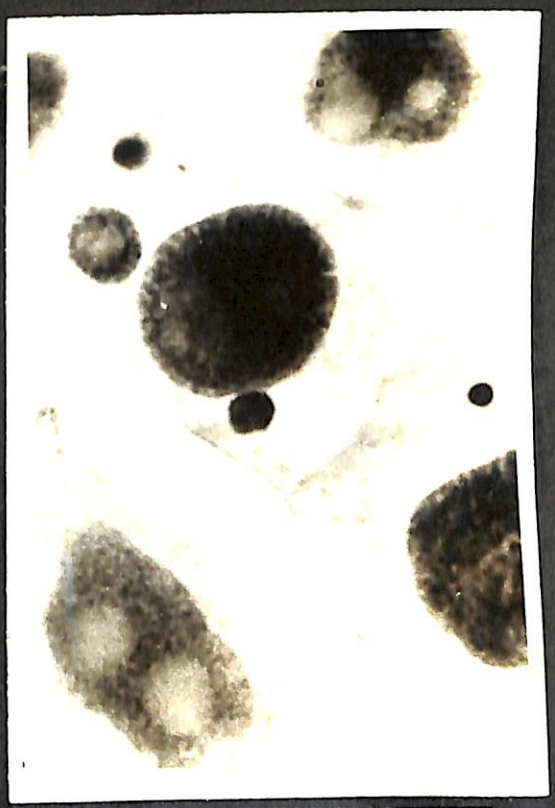


Photo III.1 C:
3 & 1 micronuclei
in adjascent cells
X 1024

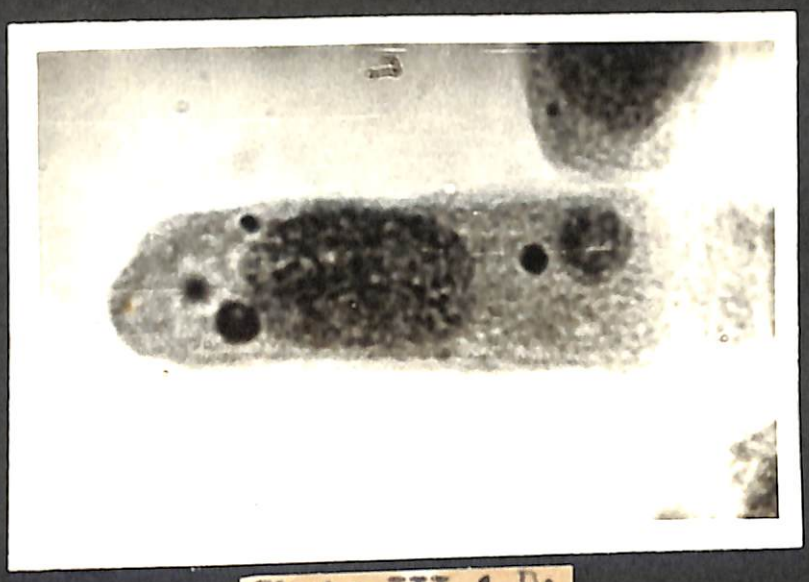


Photo III.1 D:
5 micronuclei
X 1024

Photo III.1 E:
Budding of micronuclei
X 800

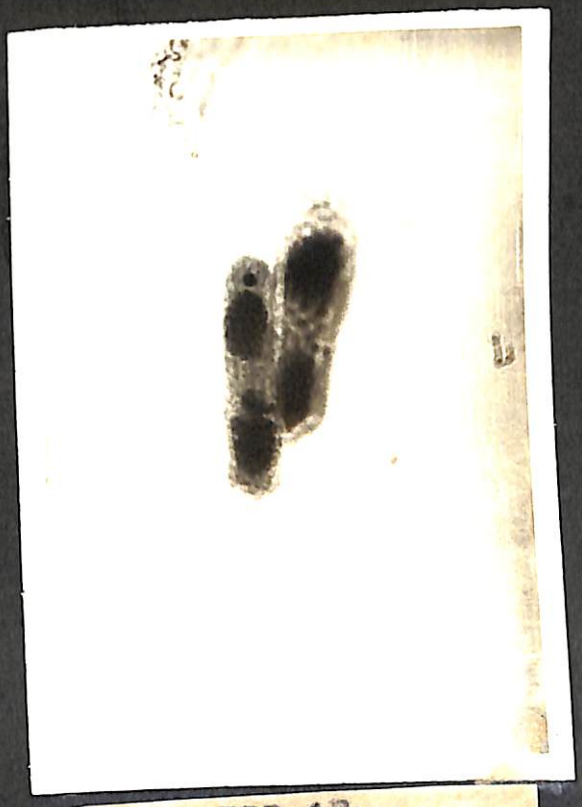


Photo III.1F:
1 micronucleus and micronuclear budding in adjascent cells.
X 576

Photo III.2 A:
Chromosome bridge
X 800

Photo III.2B:
Chromatid bridge
and a fragment
X 1024

Photo III.2 C:
2 bridges
X 800

Photo III.2 D:
3 bridges
X 1280

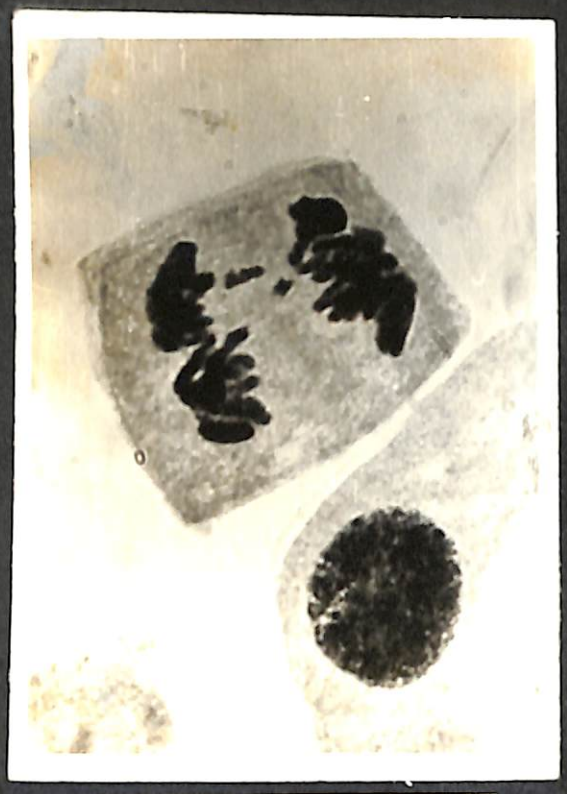


Photo III.3 A:
Lagging fragments
X 1024

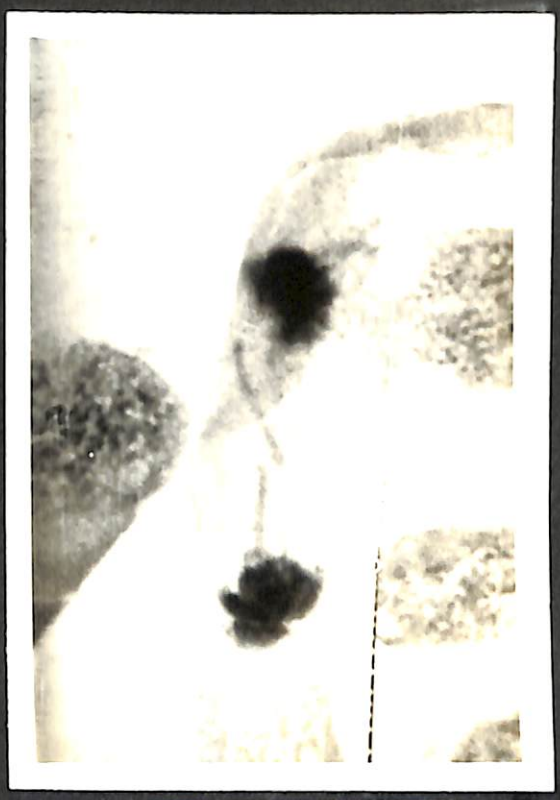


Photo III.3 B:
A laggard
X 1024

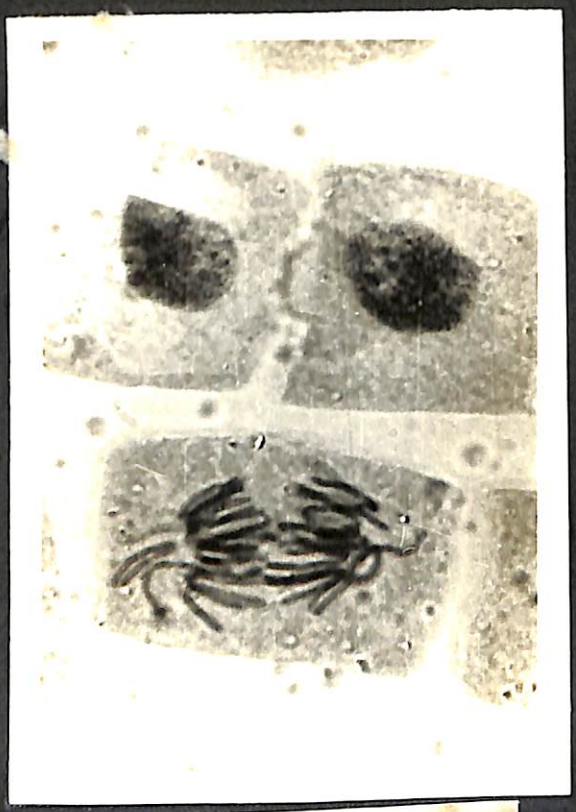


Photo III.4 A:
A metacentric forward
X 1024

Photo III.4 B:
An acrocentric forward
X 1024

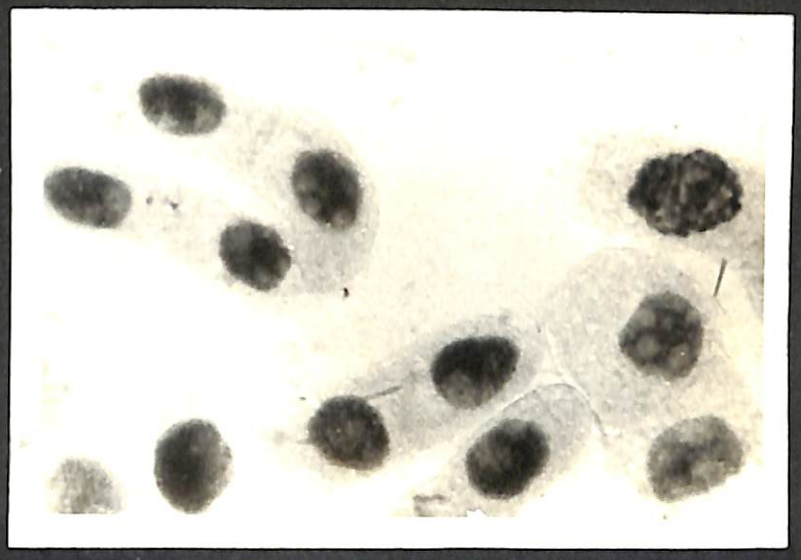


Photo III.5 A:
3-6 nuclear gaps
X 576

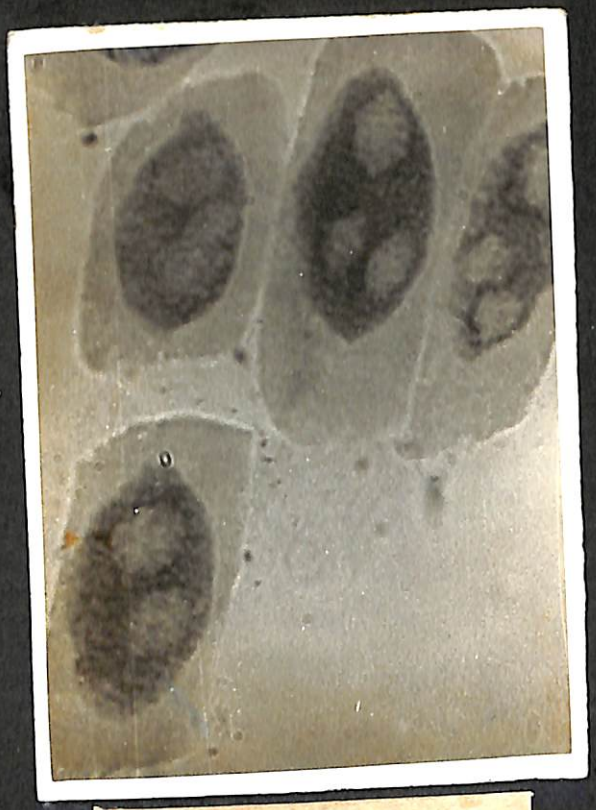


Photo III.5 B:
2-3 nucleolar gaps
X 800

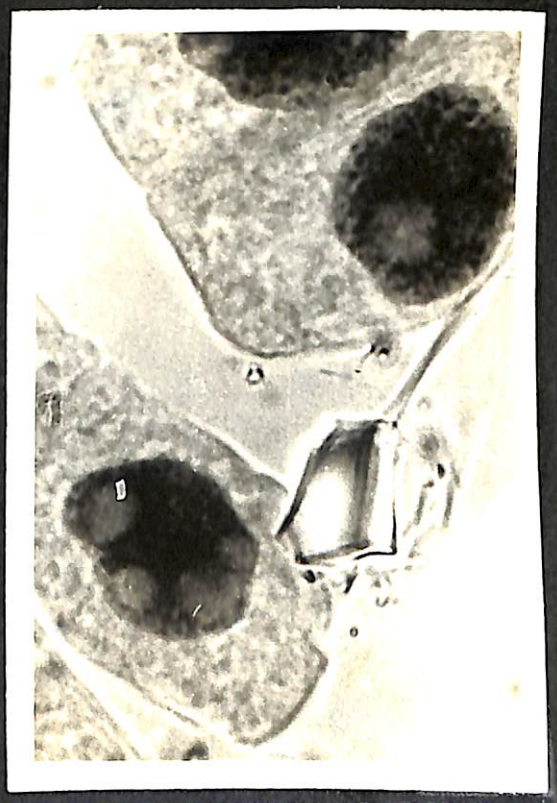


Photo III.5 C:
2-4 nucleolar gaps
X 800

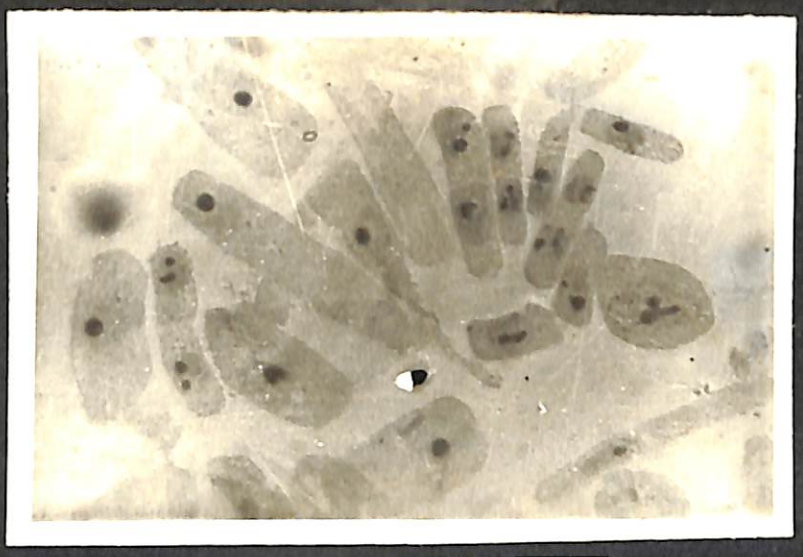


Photo III.5 D:
1-2 nucleoli,
nucleolar fusion.
X 426

Photo III.5 E:
1-6 nucleoli
X 576

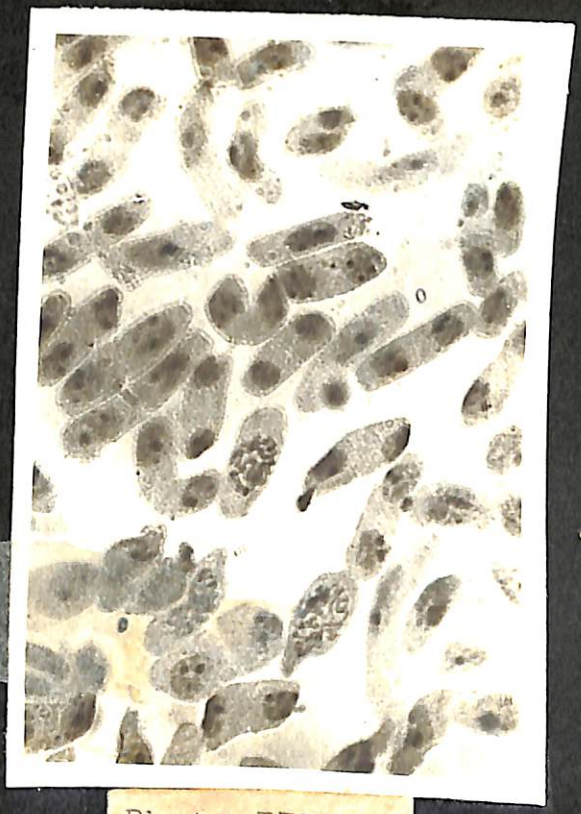


Photo III.5 F
1-4 Nucleoli
X 205

## IV. INDUCED ABERRATIONS

### Explanation of terms and abbreviations

I. KARYOKINETIC: Deviations in the morphology and behaviour of either chromosomal or spindle apparatus during the course of karyokinesis.

A. Chromosomal: Deviations in the morphology and behaviour of chromosomes.

1. Somatic reduction (Sr): Grouping of chromosomes into two approximately haploid sets in pro- or metaphase.
2. Chromosome diminution (Cd): Appearance of chromosomes in pro- or metaphase much shorter than is usual.
3. Chromosome clumping (Cc): Close adpression and clumping of chromosomes with each other during mitosis.
4. Molten metaphase (Mm): Wooly appearance and disfiguring of clarity in metaphase.
5. Chromosome dots (Chd): Dot-like feulgen positive chromatin material present when the cell is in meta- or anaphase.
6. Breakages (Bk): Breaking of chromosomes in prophase, or metaphase or anaphase. In metaphase, they may be single or double.

7. Bridges (Br): Feulgen positive connections between the two distal chromosomal groups occurring in ana-or telophase. They may be chromatid, or sticky type, varying in number.

8. Forwards (Fr): Chromosomes moving to pole precociously, i.e., earlier than the rest of the complement. They may be present during meta-, ana- or even telophase occurring at one or both poles. Their number is variable.

9. Laggards (Lg): Chromosomes remaining at the equatorial region of cell while the rest move poleward. They may occur only in anaphase or may persist till telophase. Their number is variable.

B. Spindle: Deviations in the morphology and behaviour of the spindle mechanism.

10. Spindle shifting (Ss): Alterations from its longitudinal orientation. It may be slight resulting in the oblique orientation of the chromosome groups, or it may be more, giving a diagonal grouping of chromosomes. Sometimes it may rotate by 90, becoming situated on the transverse axis of the cell as indicated by the situation of chromosome groups. This might occur even in metaphase, or anaphase or telophase.

11. Spindle disruption (Sd): Disturbing of spindle resulting in a helter-skelter of chromosomes or it may even be furcated at one or both ends.

12. Spindle inhibition (Si): Inhibition or destruction of the spindle resulting in the well spread metaphases or diplochromosomes or even tetraploid mitosis.

II. CYTOKINETIC: Deviations concerning the formation of cell plate following karyokinesis.

13. Phragmoplast shifting (Ps): Formation of phragmoplast away from the customary equatorial region of the cell, resulting in the formation of unequal daughter cells.

14. Phragmoplast inhibition (Pi): Inhibition of phragmoplast fromation resulting in binucleate cells.

III. NONKINETIC: Aberrations concerning the morphology and behaviour of the nucleus or nucleolus, unconnected with division.

15. Persistent nucleolus (Pn): Occurrence of nucleolus (ei) during karyokinesis.

16. Micronucleus (Mn): Accessory nucleus (ei) adjascent to the large main nucleus.

17. Nucleic acid starvation (Ns): The disorganisation of nuclear material as indicated by the differential response of nuclei to Feulgen reaction.

18. Nuclear atrophy (Na): Assumption of extravagent shapes by nuclei.

19. Nuclear ejection (Ne): Extrusion of nuclei from the disorganised cytoplasm.

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These abbreviations are followed in several tables of this chapter.

## 1. Arginine

Types and distribution of aberrations:

(Table IV.1A - 1D and Photos IV.1-4; 6-11;14-16; 18,19)

In the root apices of Ephedra foliata treated with 0.01%,0.05% and 0.1% concentrations of Arginine, for 1-24 hours, 15 aberrations are observed (IV.1A).

In 0.01%, 11 types of aberrations are present. Chromosome clumping is caused in 1,6 and 12 hr. treatments resulting in a frequency of 0.247. Molten metaphase, and phagmoplast inhibition occur after 12 hrs. treatment with respective mean frequencies of 0.180, and 0.053. Spindle disruption, spindle shifting, persistent nucleolus, and micronuclei occur only after 6 hrs. treatment with respective mean frequencies of 0.112, 0.202, 0.030 and 0.045. Forwards are observed after 1 and 3 hr treatments and bridges after 6,12 and 24 hours, with respective frequencies of 0.135 and 0.375. Nuclear atrophy is caused after 6 and 12 hrs treatment and nuclear ejection after 12 and 24 hours. Their respective frequencies are 0.150 and 0.188. The total percentage of aberration in this concentration is 1.717 (Table IV.1B).

In 0.05%, 8 types of aberrations are present. Of

these, some are present only in one duration: Somatic reduction (12 hrs), laggards (12 hrs), spindle disruption ( 6 hrs), phragmoplast inhibition (24 hrs) and micronucleus (24 hrs) with respective frequencies of 0.018, 0.024, 0.031, 0.103 and 0.031. Chromosome clumping is noticed in two treatments ( 1 and 24 hrs), forwards in three (1, 3 and 6 hrs) and spindle shifting also in three (3,6 and 12 hrs) giving mean frequencies of 0.213, 0.177 and 0.164 respectively. The total percentage of aberrations in this concentration is 0.761 (Table IV.1C).

In 0.1% 12 types of aberration are present. Of these, 9 are noticed only in one duration. They are : Chromosome diminution (12 hrs), breakages (3 hrs), forwards (3 hrs), spindle disruption (3 hrs), phragmoplast inhibition (24 hrs), persistent nucleolus (1 hrs) micronucleus (3 hrs), nuclear atrophy (24 hrs) and nuclear ejection (24 hrs) with respective mean frequencies of 0.065, 0.043, 0.152, 0.141, 0.141, 0.043, 0.098, 0.206, and 0.315. Among the rest, spindle shifting occurs in two durations (6 and 12 hrs), bridges in three (1,3 and 6 hrs) and chromosome clumping also in three (1,3 and 24) with mean frequencies of 0.163, 0.185 and 0.761 respectively. The total percentage of aberrations in this concentration is 2.313 (Table IV. 1D).

Frequency:

(Table IV.1E,1F)

Considering all the aberrations together, the mean aberration frequencies in 0.01%, 0.05% and 0.1% concentrations,

are 1.666, 0.812 and 2.194 respectively. In all the concentrations aberrations occur in all durations. Taking only the durations into account the total aberration frequencies in 1,3,6,12 and 24 hours of treatments, are 4.41, 3.41, 4.38, 4.79 and 6.37 respectively amounting to a total of 23.36 (Table IV 1E).

Now the individual aberrations are considered after pooling together their readings in different durations. Molten metaphase (0.01%), somatic reduction (0.05%), laggards (0.05%) chromosome diminution (0.1%) and breakages (0.1%) are present only in one concentration with respective frequencies of 0.18, 0.018, 0.024, 0.065, and 0.043. Bridges, persistent nucleolus, nuclear atrophy and nuclear ejection are noticed both in 0.01% and 0.1% with total frequencies of 0.56, 0.073, 0.356 and 0.503. The rest of the aberrations, i.e. chromosome clumping, forwards, spindle disruption, spindle shifting, phragmoplast inhibition and micronuclei are recorded in all the concentrations with respective frequencies of 1.221, 0.464, 0.284, 0.529, 0.297 and 0.174. The total percentage of aberrations produced by Arginine is 4.791 (Table IV.1F).

Mitotic indices:

(Table IV.1G)

In 0.01%, the maximum mitotic index is after 3 hrs (9.16) and minimum after 12 hrs (2.76) treatment. In 0.05% the maximum and minimum indices are after 6 hrs (12.47) and 24 hrs (7.03). In 0.1%, the maximum index is after 3 hrs (10.33) and

minimum after 24 hrs (1.64) treatment. The means of mitotic indices in 0.01%, 0.05% and 0.1% concentrations are calculated to be 5.84, 9.24 and 7.36 respectively.

On the whole, the range of mitotic indices in the material lies between 1.64 and 10.33. Among the concentrations, 0.05% gives the higher indices and 0.01%, lower. Among the durations, maximum index has been recorded after 6 hrs (10.10) and minimum after 24 hours (4.30). The mean mitotic index of the material works out to 7.48.

*Table IV.1A*

Presence of various aberrations in the root apices treated in different concentrations of Arginine for different durations

| Duration of treatment in hours | Concentration of the Chemical 0.01% | 0.05% | 0.1% |
|---|---|---|---|
| 1 | Fr,Cc | Cc,Fr | Cc,Br,Pn |
| 3 | Fr | Fr,Ss | Cc,Bk,Br,Fr,Sd,Mn |
| 6 | Cc,Br,Sd,Ss, Pn,Mn,Na | Fr,Sd,Ss | Br,Ss |
| 12 | Cc,Mm,Br,Pi,Na,Ne | Sr,Lg,Ss | Cd,Ss |
| 24 | Br,Ne | Cc,Pi,Mn | Cc,Pi,Na,Ne |

Total number of aberrations : 15

*Table IV.1B*

*Distribution of various aberrations in the root apices treated in 0.01% Arginine, for different durations*

| | | Duration of treatment in hours | | | | | | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 2884 | 2674 | 3241 | 3047 | 1484 | 13330 | |
| | Cells showing: | | | | | | | |
| 1. | Molten metaphase | - | - | - | 24 | - | 24 | 0.180 |
| 2. | Chromosome clumping | 7 | - | 5 | 21 | - | 33 | 0.247 |
| 3. | Bridges | - | - | 7 | 32 | 11 | 50 | 0.375 |
| 4. | Forwards | 8 | 10 | - | - | - | 18 | 0.135 |
| 5. | Spindle disruption | - | - | 15 | - | - | 15 | 0.112 |
| 6. | Spindle shifting | - | - | 27 | - | - | 27 | 0.202 |
| 7. | Phragmoplast inhibition | - | - | - | 7 | - | 7 | 0.053 |
| 8. | Persistent nucleolus | - | - | 4 | - | - | 4 | 0.030 |
| 9. | Micronucleus | - | - | 6 | - | - | 6 | 0.045 |
| 10. | Nuclear atrophy | - | - | 4 | 16 | - | 20 | 0.150 |
| 11. | Nuclear ejection | - | - | - | 11 | 14 | 25 | 0.188 |
| | Total aberrent cells | 15 | 10 | 68 | 111 | 25 | 229 | |
| | % | 0.52 | 0.37 | 2.09 | 3.67 | 1.68 | | 1.717 |

Table IV.1C

Distribution of various aberrations in the root apices treated in 0.05% Arginine for different durations

| | | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 2261 | 3563 | 3199 | 3388 | 3983 | 16394 | |
| | Cells showing: | | | | | | | |
| 1. | Somatic reduc-tion | - | - | - | 3 | - | 3 | 0.018 |
| 2. | Chromosome clumping | 22 | - | - | - | 13 | 35 | 0.213 |
| 3. | Forwards | 18 | 5 | 6 | - | - | 29 | 0.177 |
| 4. | Laggards | - | - | - | 4 | - | 4 | 0.024 |
| 5. | Spindle disruption | - | - | 5 | - | - | 5 | 0.031 |
| 6. | Spindle shifting | - | 6 | 14 | 7 | - | 27 | 0.164 |
| 7. | Phragmoplast inhibition | - | - | - | - | 17 | 17 | 0.103 |
| 8. | Micronucleus | - | - | - | - | 5 | 5 | 0.031 |
| | Total aberrent cells | 40 | 11 | 25 | 14 | 35 | 125 | |
| | % | 1.68 | 0.31 | 0.78 | 0.41 | 0.88 | | 0.761 |

Table IV.1D

Distribution of various aberrations in the root apices treated in 0.1% Arginine, for different durations

| | | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 1806 | 2303 | 994 | 1967 | 2128 | 9198 | |
| | Cells showing: | | | | | | | |
| 1. | Chromosome clumping | 32 | 18 | - | - | 20 | 70 | 0.761 |
| 2. | Chromosome diminution | - | - | - | 6 | - | 6 | 0.065 |
| 3. | Breakages | - | 4 | - | - | - | 4 | 0.043 |
| 4. | Bridges | 4 | 5 | 8 | - | - | 17 | 0.185 |
| 5. | Forwards | - | 14 | - | - | - | 14 | 0.152 |
| 6. | Spindle disruption | - | 13 | - | - | - | 13 | 0.141 |
| 7. | Spindle shifting | - | - | 7 | 8 | - | 15 | 0.163 |
| 8. | Phragmoplast inhibition | - | - | - | - | 13 | 13 | 0.141 |
| 9. | Persistent nucleolus | 4 | - | - | - | - | 4 | 0.043 |
| 10. | Micronucleus | - | 9 | - | - | - | 9 | 0.098 |
| 11. | Nuclear atrophy | - | - | - | - | 19 | 19 | 0.206 |
| 12. | Nuclear ejection | - | - | - | - | 29 | 29 | 0.315 |
| | Total aberrent cells | 40 | 63 | 15 | 14 | 81 | 213 | |
| | % | 2.21 | 2.73 | 1.51 | 0.71 | 3.81 | | 2.313 |

Table IV.1E

Frequency of total aberrations in the root apices treated in different concentrations of Arginine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Total | Mean |
|---|---|---|---|---|---|---|---|
| 0.01 | 0.52 | 0.37 | 2.09 | 3.67 | 1.68 | 8.33 | 1.666 |
| 0.05 | 1.68 | 0.31 | 0.78 | 0.41 | 0.88 | 4.06 | 0.812 |
| 0.1 | 2.21 | 2.73 | 1.51 | 0.71 | 3.81 | 10.97 | 2.194 |
| Total | 4.41 | 3.41 | 4.38 | 4.79 | 6.37 | 23.36 | 4.672 |

*Table IV.1F*

*Frequency of individual aberrations in the root apices treated in different concentrations of Argine*

| | Aberration | concentration of chemical 0.01% | 0.05% | 0.1% | Total |
|---|---|---|---|---|---|
| 1. | Somatic reduction | - | 0.018 | - | 0.018 |
| 2. | Chromosome clumping | 0.247 | 0.213 | 0.761 | 1.221 |
| 3. | Molten metaphase | 0.180 | - | - | 0.221 |
| 4. | Chromosome diminution | - | - | 0.065 | 0.065 |
| 5. | Bridges | 0.375 | - | 0.185 | 0.560 |
| 6. | Breakages | - | - | 0.043 | 0.043 |
| 7. | Forwards | 0.135 | 0.177 | 0.152 | 0.464 |
| 8. | Laggards | - | 0.024 | - | 0.024 |
| 9. | Spindle disruption | 0.112 | 0.031 | 0.141 | 0.284 |
| 10. | Spindle shifting | 0.202 | 0.164 | 0.163 | 0.529 |
| 11. | Phragmoplast inhibition | 0.053 | 0.103 | 0.141 | 0.297 |
| 12. | Persistent nucleolus | 0.030 | - | 0.043 | 0.073 |
| 13. | Micronucleus | 0.045 | 0.031 | 0.098 | 0.174 |
| 14. | Nuclear atrophy | 0.150 | - | 0.206 | 0.356 |
| 15. | Nuclear ejection | 0.188 | - | 0.315 | 0.503 |
| | Total | 1.717 | 0.761 | 2.313 | 4.791 |

*Table IV.1G*

Mitotic indices in the root apices treated in different concentrations of Arginine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.01 | 5.09 | 9.16 | 7.97 | 2.76 | 4.24 | 5.84 |
| 0.05 | 8.98 | 8.84 | 12.47 | 8.88 | 7.03 | 9.24 |
| 0.1 | 9.65 | 10.33 | 9.86 | 5.30 | 1.64 | 7.36 |
| Mean | 7.91 | 9.44 | 10.10 | 5.65 | 4.30 | 7.48 |

## 2. Glycine

Types and distribution of aberrations:
(Tables IV. 2A-2D and Photos IV.1,2,5-7; 9,10,13-16)

In the root apices of Ephedra foliata treated with 0.01%, 0.05% and 0.1% concentrations of glycine, for 1-24 hours, 11 aberrations are observed (Table IV.2A).

In 0.01%, 8 aberrations are observed. Somatic reduction and breakages occur after 1 hr treatment, spindle shifting and persistent nucleolus after 3 hours and forwards and phragmoplast inhibition after 6 hours with respective frequencies of 0.065, 0.055, 0.065, 0.046, 0.160 and 0.223 Bridges and micronuclei are found after 6 and 12 hrs of treatment with frequencies of 0.334 and 0.232. The total percentage of aberration is 1.180 (Table IV.2B).

In 0.05%, 5 aberrations are observed. Of these, forwards (12 hrs), phragmoplast shifting (1 hr) and phragmoplast inhibition occurs in one duration (24 hrs) only with mean frequencies of 0.083, 0.131 and 0.202. Bridges occur in two durations (12 and 24 hrs) and spindle shifting in three (1,3 and 12 hrs) giving respective frequencies of 0.131 and 0.617. The total percentage of aberrations in this concentration is 1.164 (Table IV.2C).

In 0.1%, 8 aberrations are observed. Chromosome

clumping, forwards, and breakages are present only in treatments of 24 hrs, 12 hrs and 1 hr respectively with frequencies of 0.243, 0.149 and 0.176. Bridges and persistent nucleolus occur only after 3 hours with respective frequencies of 0.176 and 0.68. On the other hand, chromosome dots occur after 3 and 6 hours, phragmoplast inhibition after 3 and 24 hours and spindle shifting after 1,3 and 6 hours of treatment. Their respective frequencies are 0.122, 0.541 and 0.622. The total percentage of aberrations in this concentration is 2.097 (Table IV.2D).

Frequency:

(Table IV.2E,2F)

Considering all the aberrations together, the mean aberration frequencies in 0.01%, 0.05% and 0.1% concentrations, are 1.326, 1.238 and 2.274. In 0.01%, no aberrations are observed in 24 hr treatment. Similar is the case in 0.05% also, but after 6 hrs. In 0.1% aberrations are observed in all durations. Taking the durations only into account, the total aberration frequencies in 1,3,6, 12 and 24 hrs are 3.28, 5.67,5.48,5.26 and 4.50, respectively amounting to a total of 24.19 (Table IV.2E).

Now the individual aberrations are considered after pooling together their readings in different durations. Somatic reduction (0.01%), micronuclei (0.01%) phragmoplast shifting (0.05%), chromosome dots and chromosome clumping (0.1%) occur only in one concentration with respective mean frequencies of 0.065,0.232, 0.131, 0.122 and 0.243. Breakages and persistent

nucleoli are present both in 0.01% and 0.1% while bridges, forwards, spindle shifting, and phragmoplast inhibition are noticed in all concentrations with frequencies of 0.231, 0.[illegible]4, 0.64,0.392, 1.304 and 0.966 respectively. The total percentage of aberrations caused by glycine sums up to 4.441(Table IV.2F).

Mitotic indices:

(Table IV.2G)

In 0.01%, the maximum mitotic index is after 6 hrs (11.40) and minimum, after 24 hours (0.3). In 0.05% and 0.1%, maximum indices are noticed after 1 hr (12.5 and 13.4) but minimum after 24 hrs (4.5) and 12 hrs (9.7) respectively. The means of mitotic indices in 0.01%, 0.05% and 0.1% concentrations are calculated to be 6.3, 9.0 and 11.5 respectively.

On the whole, the range of mitotic indices in the material lies between 0.3 and 13.4. Among concentrations, 0.1% gives higher indices and 0.01%, lower. Among the durations, maximum index has been observed after 1 hr (12.3) and minimum after 24 hrs (5.3). The mean mitotic index of the material works out to 8,9.

Table IV.2A

Presence of various aberrations in the root apices treated in different concentrations of Glycine for different durations

| Duration of treatment in hours | Concentration of the chemical 0.01% | 0.05% | 0.1% |
|---|---|---|---|
| 1 | Sr,Bk | Ss, Ps | Bk,Ss |
| 3 | Ss,Pn | Ss | Chd,Br,Ss,Pi,Pn |
| 6 | Br,Fr,Pi,Mn | - | Chd, Ss |
| 12 | Br,Mn | Br,Fr,Ss | Fr |
| 24 | - | Br,Pi | Cc,Pi |

Total number of aberrations : 11

Table IV.2B

Distribution of various aberrations in the root apices treated in 0.01% Glycine for different durations

| | | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 1976 | 2674 | 1779 | 1989 | 2421 | 10769 | |
| | Cells showing: | | | | | | | |
| 1. | Somatic reduction | 7 | - | - | - | - | 7 | 0.065 |
| 2. | Breakages | 6 | - | - | - | - | 6 | 0.055 |
| 3. | Bridges | - | - | 15 | 21 | - | 6 | 0.334 |
| 4. | Forwards | - | - | 17 | - | - | 17 | 0.160 |
| 5. | Spindle shifting | - | 7 | - | - | - | 7 | 0.065 |
| 6. | Phragmoplast inhibition | - | - | 24 | - | - | 24 | 0.223 |
| 7. | Persistent nucleolus | - | 5 | - | - | - | 5 | 0.046 |
| 8. | Micronucleus | - | - | 11 | 14 | - | 25 | 0.232 |
| | Total aberrent cells | 13 | 12 | 67 | 35 | - | 127 | |
| | % | 0.66 | 0.45 | 3.76 | 1.76 | - | | 1.180 |

Table IV.2C

Distribution of various aberrations in the root apices treated in 0.05% Glycine, for different durations

| | | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 1724 | 2021 | 1243 | 1024 | 2412 | 8424 | |
| 1. | Cells showing: Bridges | - | - | - | 4 | 7 | 11 | 0.131 |
| 2. | Forwards | - | - | - | 7 | - | 7 | 0.083 |
| 3. | Spindle shifting | 13 | 22 | - | 17 | - | 52 | 0.617 |
| 4. | Phragmoplast shifting | 11 | - | - | - | - | 11 | 0.131 |
| 5. | Phragmoplast inhibition | - | - | - | - | 17 | 17 | 0.202 |
| | Total aberrent cells | 24 | 22 | - | 28 | 24 | 98 | |
| | % | 1.39 | 1.08 | - | 2.73 | 0.99 | | 1.164 |

Table IV.2D

Distribution of various aberrations in the root apices treated in 0.1% Glycine for different duration

| | | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 6 | 12 | 24 | | |
| | Cells observed | 2422 | 1324 | 1094 | 1425 | 1129 | 7394 | |
| 1. | Cells showing: Chromosome dots | - | 4 | 5 | - | - | 9 | 0.122 |
| 2. | Breakages | 13 | - | - | - | - | 13 | 0.176 |
| 3. | Bridges | - | 13 | - | - | - | 13 | 0.176 |
| 4. | Forwards | - | - | - | 11 | - | 11 | 0.149 |
| 5. | Chromosome clumping | - | - | - | - | 18 | 18 | 0.243 |
| 6. | Spindle shifting | 17 | 15 | 14 | - | - | 46 | 0.622 |
| 7. | Phragmoplast inhibition | - | 18 | - | - | 22 | 40 | 0.541 |
| 8. | Persistent nucleolus | - | 5 | - | - | - | 5 | 0.068 |
| | Total aberrent cells | 30 | 55 | 19 | 11 | 40 | 155 | |
| | % | 1.23 | 4.14 | 1.72 | 0.77 | 3.51 | | 2.097 |

Table IV.2E

Frequency of total aberrations in the root apices treated in different concentrations of Glycine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Total | Mean |
|---|---|---|---|---|---|---|---|
| 0.01 | 0.66 | 0.45 | 3.76 | 1.76 | - | 6.63 | 1.326 |
| 0.05 | 1.39 | 1.08 | - | 2.73 | 0.99 | 6.19 | 1.238 |
| 0.1 | 1.23 | 4.14 | 1.72 | 0.77 | 3.51 | 11.37 | 2.274 |
| Total | 3.28 | 5.67 | 5.48 | 5.26 | 4.50 | 24.19 | 4.8383 |

Table IV.2F

Frequency of individual aberrations in the root apices treated in different concentrations of Glycine

| | Aberration | Concentration of chemical 0.01% | 0.05% | 0.1% | Total |
|---|---|---|---|---|---|
| 1. | Somatic reduction | 0.065 | - | - | 0.065 |
| 2. | Breakages | 0.055 | - | 0.176 | 0.231 |
| 3. | Bridges | 0.334 | 0.131 | 0.176 | 0.641 |
| 4. | Chromosome dots | - | - | 0.122 | 0.122 |
| 5. | Chromosome clumping | - | - | 0.243 | 0.243 |
| 6. | Forwards | 0.160 | 0.083 | 0.149 | 0.392 |
| 7. | Spindle shifting | 0.065 | 0.617 | 0.622 | 1.304 |
| 8. | Phragmoplast shifting | - | 0.131 | - | 0.131 |
| 9. | Phragmoplast inhibition | 0.223 | 0.202 | 0.541 | 0.966 |
| 10. | Persistent nucleolus | 0.046 | - | 0.068 | 0.114 |
| 11. | Micronucleus | 0.232 | - | - | 0.232 |
| | Total | 1.18 | 1.164 | 2.097 | 4.441 |

Table IV.2G

Mitotic indices in the root apices treated in different concentrations of Glycine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Mean |
|---|---|---|---|---|---|---|
| 0.01 | 10.9 | 6.4 | 11.40 | 2.5 | 0.3 | 6.3 |
| 0.05 | 12.5 | 9.0 | 12.12 | 6.6 | 4.5 | 9.0 |
| 0.1 | 13.4 | 10.5 | 12.50 | 9.7 | 11.1 | 11.5 |
| Mean | 12.3 | 8.6 | 12.01 | 6.3 | 5.3 | 8.9 |

## 3. Methionine

Types and distribution of aberrations:
(Table IV.3A-3D and Photos IV.4,9-12;16)

In the root apices of Ephedra foliata, treated with 0.01%, 0.05% and 0.1% concentrations of Methionine for 1-24 hours 6 aberrations have been observed (Table IV.3A).

In 0.01%, only 1 aberration is observed - spindle shifting, occurring only after 3 hrs of treatment. The frequency is 0.151 (Table IV.3B).

In 0.05%, 4 aberrations are observed. Chromosome diminution and spindle inhibition occur after 6 hrs while spindle disruption and persistent nucleolus occur after 1 hr with respective frequencies of 0.178, 0.1, 0.078 and 0.061. The total percentage of aberrations in this concentration works out to 0.417 (Table IV.3C).

In 0.1%, 2 aberrations are present.- Forwards occur after 1 hr and 24 hrs while spindle shifting occurs only after 1 hr with frequencies of 0.175 and 0.1 respectively. The total percentage of aberrations in this concentration works out to 0.275 (Table IV.3D).

Frequency:

(Table IV.3E,3F)

Considering all the aberrations together, the mean aberration frequencies in 0.01%, 0.05%, and 0.1%, are 0.092, 0.376 and 0.21 respectively. In 0.01%, aberrations occur only in 3 hr treatment while in 0.05%, 0.1%, they occur after 1 and 6 and 1 and 24 hours. Taking durations only into account, the total aberration frequencies in 1,3,6 and 24 hrs are 1.30, 0.46, 1.09 and 0.54 respectively giving a total of 3.39. No aberrations are observed in 12 hr treatment (Table IV.3E).

Now the individual aberrations are considered after pooling together their readings in different durations. Chromosome diminution, spindle disruption, spindle inhibition and persistent nucleoli occur only in 0.05% while forwards occur in 0.1% with respective frequencies of 0.17%, 0.07%, 0.1,0.061 and 0.175. Spindle shifting occurs both in 0.01% and 0.1% giving a frequency of 0.251. The total percentage of aberrations produced by methionine sums up to 0.843 (Table IV.3F).

Mitotic indices

(Table IV.3G)

In 0.01%, the maximum mitotic index is after 12 hrs (13.58) and minimum (3.06) after 24 hours. In 0.05% the maximum index is after 1 hr (1372) and minimum after 12 hrs (5.55). In 0.1%, the maximum (8.02) and minimum (5.07) are respectively

after 24 hrs and 6 hrs. The means of mitotic indices in 0.01%, 0.05% and 0.1% are 8.89, 9.58 and 6.63.

On the whole, the mitotic indices in the material range between 3.06 and 13.72. Among the concentrations 0.05% gives higher readings of indices and 0.1% lower. Among the durations, maximum index has been recorded in 1 hr (11.22) and minimum in 6 hr (5.59) treatment. The mean mitotic index of the material works out to 8.36.

Table IV.3A

Presence of various aberrations in the root apices treated in different concentrations of Methionine for different durations

| Duration of treatment in hours | Concentration of the chemical | | |
|---|---|---|---|
| | 0.01% | 0.05% | 0.1% |
| 1 | - | Sd,Pn | Fr,Ss |
| 3 | Ss | - | - |
| 6 | - | Cd, Si | - |
| 12 | - | - | - |
| 24 | - | - | Fr |

Total number of aberrations : 6

Table IV.3B

Distribution of various aberrations in the root apices treated in 0.01% Methionine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 726 | 2584 | 432 | 648 | 2872 | 7262 | |
| Cells showing: | | | | | | | |
| 1. Spindle shifting | - | 11 | - | - | - | 11 | 0.151 |
| Total aberrent cells | - | 11 | - | - | - | 11 | |
| % | - | 0.46 | - | - | - | | 0.151 |

Table IV.3C

Distribution of various aberrations in the root apices treated in 0.05% Methionine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1632 | 1448 | 2200 | 2016 | 1672 | 8973 | |
| Cells showing: | | | | | | | |
| 1. Chromosome diminution | - | - | 15 | - | - | 15 | 0.178 |
| 2. Spindle disruption | 7 | - | - | - | - | 7 | 0.078 |
| 3. Spindle inhibition | - | - | 9 | - | - | 9 | 0.100 |
| 4. Persistent nucleolus | 6 | - | - | - | - | 6 | 0.061 |
| Total aberrent cells | 13 | - | 24 | - | - | 37 | |
| % | 0.79 | - | 1.09 | - | - | | 0.417 |

Table IV.3D

Distribution of various aberrations in the root apices treated in 0.1% Methionine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3112 | 1155 | 966 | 1862 | 1496 | 8591 | |
| Cells showing: | | | | | | | |
| 1. Forwards | 7 | - | - | - | 8 | 15 | 0.175 |
| 2. Spindle shifting | 9 | - | - | - | - | 9 | 0.1 |
| Total aberrent cells | 16 | - | - | - | 8 | 24 | |
| % | 0.51 | - | - | - | 0.54 | | 0.275 |

Table IV.3E

Frequency of total aberrations in the root apices treated in different concentrations of Methionine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Total | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| 0.01 | - | 0.46 | - | - | - | 0.46 | 0.092 |
| 0.05 | 0.79 | - | 1.09 | - | - | 1.88 | 0.376 |
| 0.1 | 0.51 | - | - | - | 0.54 | 1.05 | 0.210 |
| Total | 1.30 | 0.46 | 1.09 | - | 0.54 | 3.39 | 0.678 |

Table IV.3F

Frequency of individual aberrations in the root apices treated in different concentrations of Methionine

| Aberration | Concentration of chemical | | | Total |
|---|---|---|---|---|
| | 0.01% | 0.05% | 0.1% | |
| 1. Chromosome diminution | - | 0.178 | - | 0.178 |
| 2. Forwards | - | - | 0.175 | 0.175 |
| 3. Spindle disruption | - | 0.078 | - | 0.078 |
| 4. Spindle shifting | 0.151 | - | 0.1 | 0.251 |
| 5. Spindle inhibition | - | 0.1 | - | 0.1 |
| 6. Persistent nucleolus | - | 0.061 | - | 0.061 |
| Total | 0.151 | 0.417 | 0.275 | 0.843 |

Table IV.3G

Mitotic indices in the root apices treated in different concentrations of Methionine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Mean |
|---|---|---|---|---|---|---|
| 0.01 | 11.96 | 12.07 | 3.7 | 13.58 | 3.06 | 8.87 |
| 0.05 | 13.72 | 12.15 | 8.0 | 5.55 | 8.46 | 9.58 |
| 0.1 | 7.97 | 6.06 | 5.07 | 6.02 | 8.02 | 6.63 |
| Mean | 11.22 | 10.09 | 5.59 | 8.38 | 6.51 | 8.36 |

treatment. Their respective frequencies are 0.504, 1.36 and 0.815. The total percentage of aberrations in this concentration adds up to 2.679 (Table IV.4D).

Frequency:

(Table IV. 4E,4F)

Considering all the aberrations together, aberration frequencies in 0.01%, 0.05% and 0.1% are 0.112, 1.238 and 3.510. In 0.01% aberrations do not occur in 6, 12 and 24 hrs treatments while in the other two concentrations, they are noticed in all durations. Taking only the durations into account, total aberration frequencies in 1,3,6,12 and 24 hr treatments are 0.84, 1.99, 2,58, 7.59 and 11.29 respectively making up to 24.29 (Table IV.4E).

Now the individual aberrations are considered after pooling together their readings in different durations. Persistent nucleoli (0.01%), Micronuclei (0.05%) and Nuclear atrophy (0.01%), are present only in one concentration, with respective frequencies of 0.044, 0.089 and 1.36. Spindle disruption is seen in 0.01% and 0.05% while phragmoplast inhibition and nuclear ejection occur in 0.05% and 0.1% with total mean frequencies of 0.137,1.378 and 0.980. The total percentage of aberrations caused by threonine sums up to 3.988 (Table IV.4F).

Mitotic indices:

(Table IV.4G)

In 0.01%, the maximum mitotic index is in 1 hr.

treatment (8.91) and minimum after 24 hrs (3.09). In 0.05% and 0.1%, the maximum are recorded after 6 hours (10.33 and 9.58 respectively). In the former mitoses are absent after 24 hr treatment. The means of mitotic indices in 0.01%, 0.05%, and 0.1% concentrations are 5.54, 6.59 and 4.96 respectively.

On the whole, the mitotic indices range between 3.09 and 10.33. Among the concentrations, 0.05% given higher readings and 0.1%, lower. Among the durations, maximum is in 3 hr (8.36) and minimum in 24 hr (1.01) treatment. The mean mitotic index of the material works out to 5.9.

Table IV. 4A

Presence of various aberrations in the root apices treated in different concentrations of Threonine for different durations

| Duration of treatment in hours | Concentration of the chemical | | |
|---|---|---|---|
| | 0.01% | 0.05% | 0.1% |
| 1 | Sd | Mn | Pi |
| 3 | Pn | Sd | Pi |
| 6 | - | Pi | Pi |
| 12 | - | Pi | Na,Ne |
| 24 | - | Ne | Na,Ne |

Total number of aberrations : 6

Table IV.4B

Distribution of various aberrations in the root apices treated in 0.01% Threonine, for different duration

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 2424 | 1860 | 1946 | 2544 | 2136 | 11410 | |
| Cells shoing: | | | | | | | |
| 1. Spindle disruption | 7 | - | - | - | - | 7 | 0.061 |
| 2. Persistent nucleolus | - | 5 | - | - | - | 5 | 0.044 |
| Total aberrent cells | 7 | 5 | - | - | - | 12 | |
| % | 0.29 | 0.27 | - | - | - | | 0.105 |

Table IV.4C

Distribution of various aberrations in the root apices treated in 0.05% Threonine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 2472 | 1212 | 1438 | 1744 | 1021 | 7887 | |
| Cells showing: | | | | | | | |
| 1. Spindle disruption | - | 6 | - | - | - | 6 | 0.076 |
| 2. Phragmoplast inhibition | - | - | 21 | 48 | - | 69 | 0.874 |
| 3. Micronucleus | 7 | - | - | - | - | 7 | 0.089 |
| 4. Nuclear ejection | - | - | - | - | 13 | 13 | 0.165 |
| Total aberrent cells | 7 | 6 | 21 | 48 | 13 | 95 | |
| % | 0.28 | 0.49 | 1.46 | 2.75 | 1.21 | | 1.204 |

Table IV.4D

Distribution of various aberrations in the root apices treated in 0.1% Threonine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3276 | 1380 | 1878 | 1672 | 1123 | 9329 | |
| Cells showing: | | | | | | | |
| 1. Phragmoplast inhibition | 9 | 17 | 21 | - | - | 47 | 0.504 |
| 2. Nuclear atrophy | - | - | - | 23 | 53 | 127 | 1.360 |
| 3. Nuclear ejection | - | - | - | 58 | 69 | 76 | 0.815 |
| Total aberrent cells | 9 | 17 | 21 | 81 | 122 | 250 | |
| % | 0.27 | 1.23 | 1.12 | 4.84 | 10.08 | | 2.679 |

Table IV.4E

Frequency of total aberrations in the root apices treated in different concentrations of Threonine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Total | Mean |
|---|---|---|---|---|---|---|---|
| 0.01 | 0.29 | 0.27 | - | - | - | 0.56 | 0.112 |
| 0.05 | 0.28 | 0.49 | 1.46 | 2.75 | 1.21 | 6.19 | 1.238 |
| 0.1 | 0.27 | 1.23 | 1.12 | 4.84 | 10.08 | 17.54 | 3.510 |
| Total | 0.84 | 1.99 | 2.58 | 7.59 | 11.29 | 24.29 | 4.860 |

Table IV.4F

Frequency of individual aberrations in the root apices treated in different concentrations of Threonine

| Aberration | Concentration of chemical 0.01% | 0.05% | 0.1% | Total |
|---|---|---|---|---|
| 1. Spindle disruption | 0.061 | 0.076 | - | 0.137 |
| 2. Phragmoplast inhibition | - | 0.874 | 0.504 | 1.378 |
| 3. Persistent nucleolus | 0.044 | - | - | 0.044 |
| 4. Micronucleus | - | 0.089 | - | 0.089 |
| 5. Nuclear atrophy | - | - | 1.36 | 1.360 |
| 6. Nuclear ejection | - | 0.165 | 0.815 | 0.980 |
| Total | 0.105 | 1.204 | 2.679 | 3.988 |

Table IV.4G

Mitotic indices in the root apices treated in different concentrations of Threonine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Mean |
|---|---|---|---|---|---|---|
| 0.01 | 8.91 | 7.42 | 4.99 | 3.3 | 3.09 | 5.54 |
| 0.05 | 8.4 | 9.41 | 10.33 | 4.84 | - | 6.59 |
| 0.1 | 6.96 | 8.26 | 9.58 | - | - | 4.96 |
| Mean | 8.09 | 8.36 | 8.3 | 2.71 | 1.01 | 5.69 |

## 5. Valine

Types and distribution of aberrations:

(Table IV. 5A-5D and Photos IV.2,4,9,10,11,14,15)

In the root apices of Ephedra foliata treated with 0.01%, 0.05% and 0.1% concentrations of Valine, for 1-24 hours, 7 aberrations are observed (Table IV.5A).

In 0.01%, 4 aberrations are present. Of these, chromosome diminution and spindle disruption are caused both in 1 hr and 3 hr treatments with respective total mean frequencies of 0.204 and 0.17. Forwards and spindle shifting occur only after 1 hr with frequencies of 0.204 and 0.07 respectively. The total percentage of aberrations in this concentration is 0.704 (Table IV.5B).

In 0.05%, 3 aberrations are present. 1.Chromosome diminution, 2.Spindle disruption, and 3. Micronucleus. They occur after 6, 24 and 3 hrs of treatment respectively with corresponding frequencies of 0.099, 0.124 and 0.086. The total percentage of aberrations in this concentration adds up to 0.309 (Table IV.5C).

In 0.1%, 7 aberrations are present. Of these, chromosome diminution and spindle disruption occur only after 1 hr and

phragmoplast inhibition and micronucleus after 6 hrs with aberration frequencies of 0.041, 0.155, 0.041 and 0.057 respectively. Chromosome clumping occurs after 3 hrs with a frequency of 0.123. Forwards occur both in 1 hr as well as 3 hr treatments giving a frequency of 0.482. The total percentage of aberrations in this concentration adds up to 0.988 (Table IV.5D).

Frequency:

(Table IV. 5E,5F)

Considering all the aberrations together the mean aberration frequencies in 0.01%, 0.05% and 0.1% are 0.564, 0.432 and 0.576. In 0.01 no aberrations occur after 6,12 and 24 hrs. In 0.05% in 12 hr treatment and in 0.1%, after 12 and 24 hrs. Taking only the durations into account, the total aberration frequencies in 1,3,6 and 24 hr treatments are 3.23, 3.09, 1.11 and 0.43 totalling to 7.86. No aberrations occur after 12 hours (Table IV.5E).

Now the individual aberrations are considered after pooling together their readings in different durations. Chromosomes clumping and phragmoplast inhibition occur only in 0.1% with mean frequencies of 0.123 and 0.04 . Forwards and spindle shifting occur in 0.01% and 0.1% with respective frequencies of 0.742 and 0.159. Micronuclei occur in 0.05% and 0.1% while chromosome diminution and spindle disruption are present in all concentrations. Their frequencies are 0.143, 0.344 and 0.449 respectively. The total percentage of aberrations produced by

valine comes to 2.001 (Table IV.5F).

Mitotic indices:

(Table IV.5G)

In 0.01%, the maximum mitotic index is after 1 hr (6.44) and minimum after 6 hrs (2.76) of treatment. In 0.05%, maximum (9.87) occur afters 24 hours and minimum (3.45) after 3 hrs. In 0.1%, the maximum (7.72) and minimum (4.59) occur in 1 and 3 hr treatments respectively. The means of mitotic indices in 0.01%, 0.05% and 0.1% are 5.12, 5.61 and 6.24.

On the whole, the range of mitotic indices in the material lies between 2.76 and 9.87. Among the concentrations, indices are more in 0.1% and less in 0.01. Among the durations, maximum (7.59) is observed after 24 hours and minimum (4.35) after 6 hours of treatment. The mean mitotic index of the material works out to 5.66.

Table IV.5A

Presence of various aberrations in the root apices treated in different concentrations of valine for different durations

| Duration of treatment in hours | Concentration of the chemical | | |
|---|---|---|---|
| | 0.01% | 0.05% | 0.1% |
| 1 | Cd,Fr,Sd,Ss | - | Cd,Fr,Sd,Ss |
| 3 | Cd,Sd | Mn | Cc,Fr |
| 6 | - | Cd | Pi,Mn |
| 12 | - | - | - |
| 24 | - | Sd | - |

Total number of aberrations : 7

Table IV.5B

Distribution of various aberrations in the root apices treated in 0.01% Valine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3624 | 3344 | 4362 | 1398 | 1470 | 14198 | |
| Cells showing: | | | | | | | |
| 1. Chromosome diminution | 18 | 11 | - | - | - | 29 | 0.204 |
| 2. Forwards | 37 | - | - | - | - | 37 | 0.260 |
| 3. Spindle disruption | 11 | 13 | - | - | - | 24 | 0.170 |
| 4. Spindle shifting | 10 | - | - | - | - | 10 | 0.070 |
| Total aberrent cells | 76 | 24 | - | - | - | 100 | |
| % | 2.1 | 0.72 | - | - | - | | 0.704 |

Table IV.5C

Distribution of various aberrations in the root apices treated in 0.05% Valine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 972 | 670 | 1146 | 2958 | 2310 | 8056 | |
| Cells showing: | | | | | | | |
| 1. Chromosome diminution | - | - | 8 | - | - | 8 | 0.099 |
| 2. Spindle disruption | - | - | - | - | 10 | 10 | 0.124 |
| 3. Micronucleus | - | 7 | - | - | - | 7 | 0.086 |
| Total aberrent cells | - | 7 | 8 | - | 10 | 25 | |
| % | - | 1.04 | 0.69 | - | 0.43 | | 0.309 |

Table IV.5D

Distribution of various aberrations in the root apices treated in 0.1% Valine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 4350 | 4440 | 2844 | 3060 | 1896 | 12240 | |
| 1. Cells showing: Chromosome diminution | 5 | - | - | - | - | 5 | 0.041 |
| 2. Chromosome clumping | - | 15 | - | - | - | 15 | 0.123 |
| 3. Forwards | 15 | 44 | - | - | - | 59 | 0.482 |
| 4. Spindle disruption | 19 | - | - | - | - | 19 | 0.155 |
| 5. Spindle shifting | 11 | - | - | - | - | 11 | 0.089 |
| 6. Phragmoplast inhibition | - | - | 5 | - | - | 5 | 0.041 |
| 7. Micronucleus | - | - | 7 | - | - | 7 | 0.057 |
| Total aberrent cells | 50 | 59 | 12 | - | - | 121 | |
| % | 1.13 | 1.33 | 0.42 | - | - | | 0.988 |

## Table IV.5E

Frequency of total aberrations in the root apices treated in different concentrations of Valine for different durations

| Concentrations in % | Duration of treatment in hours | | | | | Total | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| 0.01 | 2.1 | 0.72 | - | - | - | 2.82 | 0.564 |
| 0.05 | - | 1.04 | 0.69 | - | 0.43 | 2.16 | 0.432 |
| 0.1 | 1.13 | 1.33 | 0.42 | - | - | 2.88 | 0.576 |
| Total | 3.23 | 3.09 | 1.11 | - | 0.43 | 7.86 | 1.572 |

Table IV.5F

Frequency of individual aberrations in the root apices treated in different concentrations of Valine

| | Aberration | Concentration of chemical 0.01% | 0.05% | 0.1% | Total |
|---|---|---|---|---|---|
| 1. | Chromosome diminution | 0.204 | 0.099 | 0.041 | 0.344 |
| 2. | Chromosome clumping | - | - | 0.123 | 0.123 |
| 3. | Forwards | 0.26 | - | 0.482 | 0.742 |
| 4. | Spindle disruption | 0.17 | 0.124 | 0.155 | 0.449 |
| 5. | Spindle shifting | 0.07 | - | 0.089 | 0.159 |
| 6. | Phragmoplast inhibition | - | - | 0.041 | 0.041 |
| 7. | Micronucleus | - | 0.086 | 0.057 | 0.143 |
| | Total | 0.704 | 0.309 | 0.988 | 2.001 |

Table IV.5G

Mitotic indices in the root apices treated in different concentrations of Valine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.01 | 6.44 | 5.53 | 2.76 | 5.58 | 5.31 | 5.12 |
| 0.05 | 3.7 | 3.45 | 5.23 | 5.79 | 9.87 | 5.61 |
| 0.1 | 7.72 | 4.59 | 5.06 | 6.25 | 7.59 | 6.24 |
| Mean | 5.95 | 4.52 | 4.35 | 5.87 | 7.59 | 5.66 |

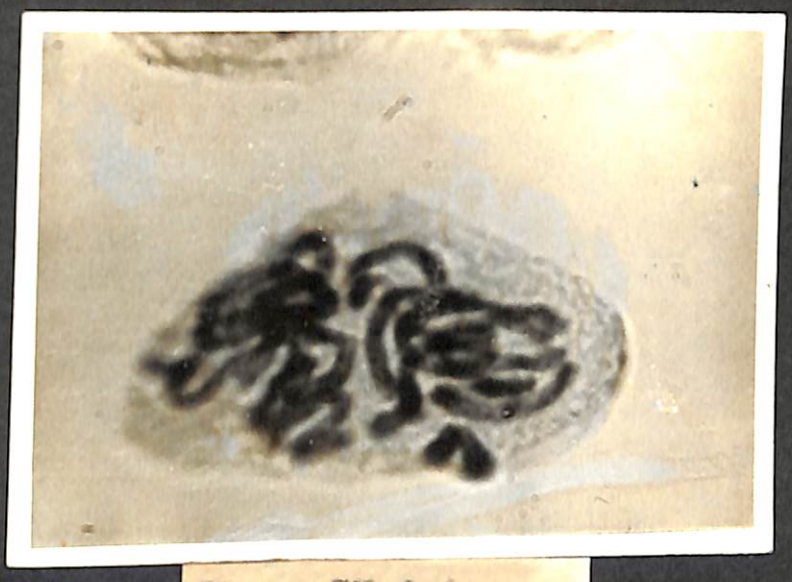


Photo IV.1 A:
Somatic reduction
in Prophase.
X 1280

Photo IV.1 B:
Somatic reduction
in metaphase
X 800

Photo IV.1 C:
Somatic reduction:
distributive metaphase.
X 1280

Photo IV.2 A:
Chromosome clumping
X 1024

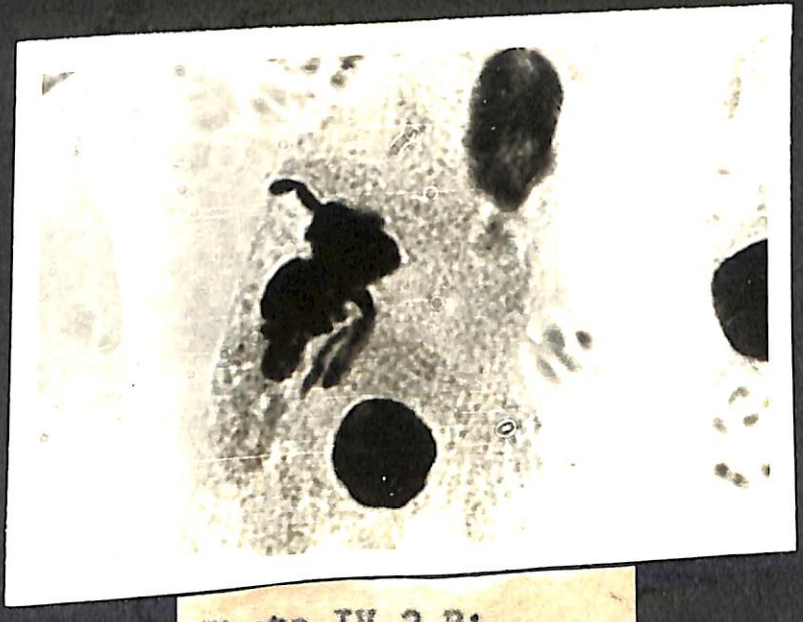


Photo IV.2 B:
Chromosome clumping
X 800

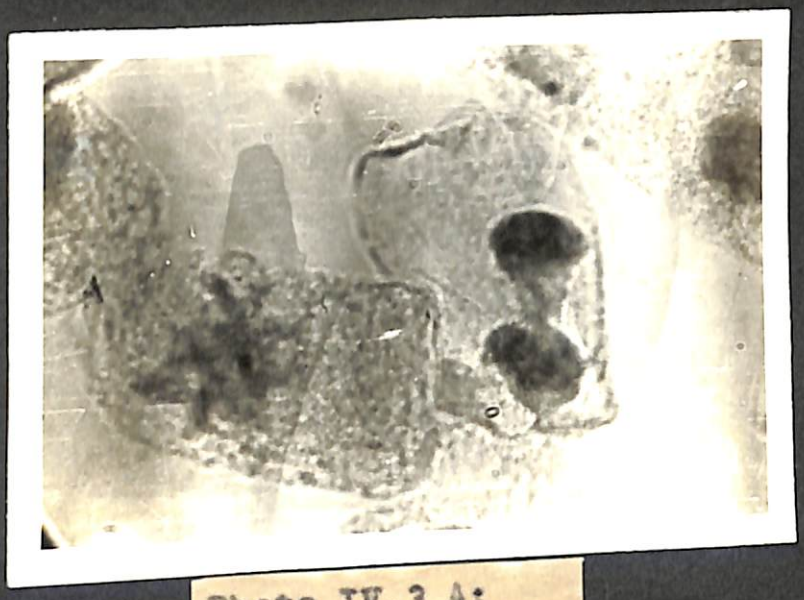


Photo IV.3 A:
Molten metaphase
X 800

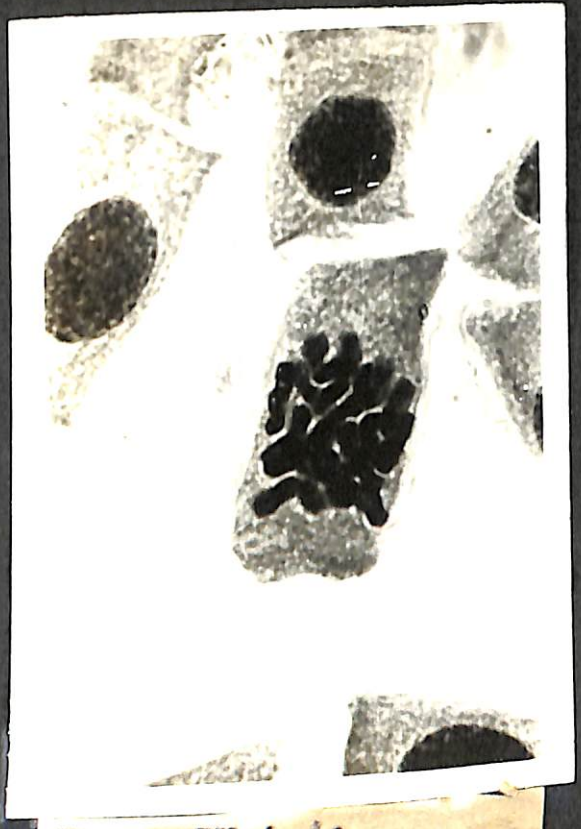


Photo IV.4 A:
Chromosome diminution
X 800

Photo IV.4 B:
Chromosome diminution
X 800

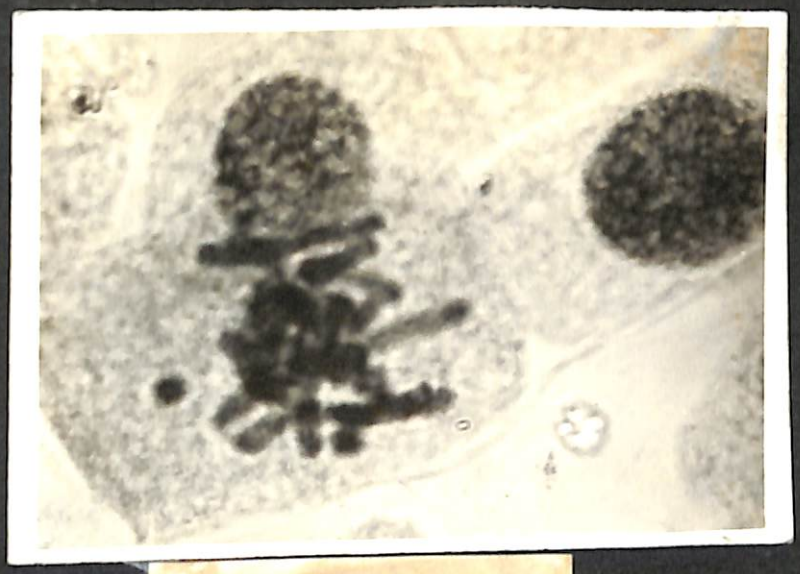


Photo IV.5 A:
Chromosome dot in metaphase.
X 1024

Photo IV.5 B
Chromosome dots(4)
X 800

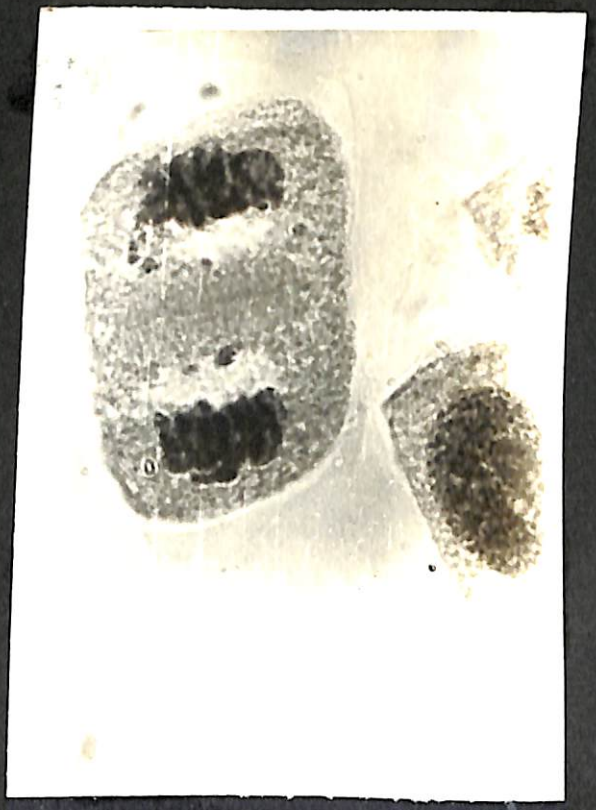


Photo IV. 5 C:
Chromosome dots in telophase
X 1024

Photo IV.7 A:
Prophase breaks
X 800

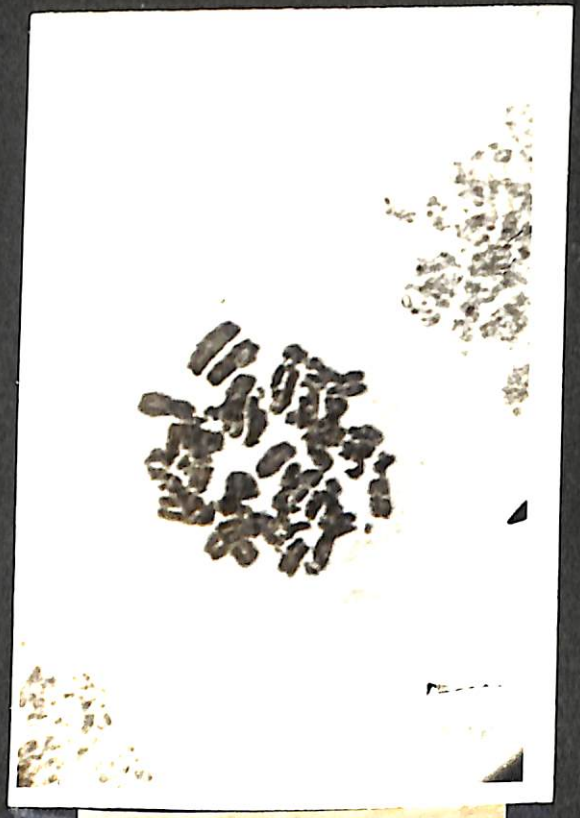


Photo IV.7 B:
Metaphase breaks
(diplochromosomal)
X 800

Photo IV.7 C:
Metaphase breaks
X 800

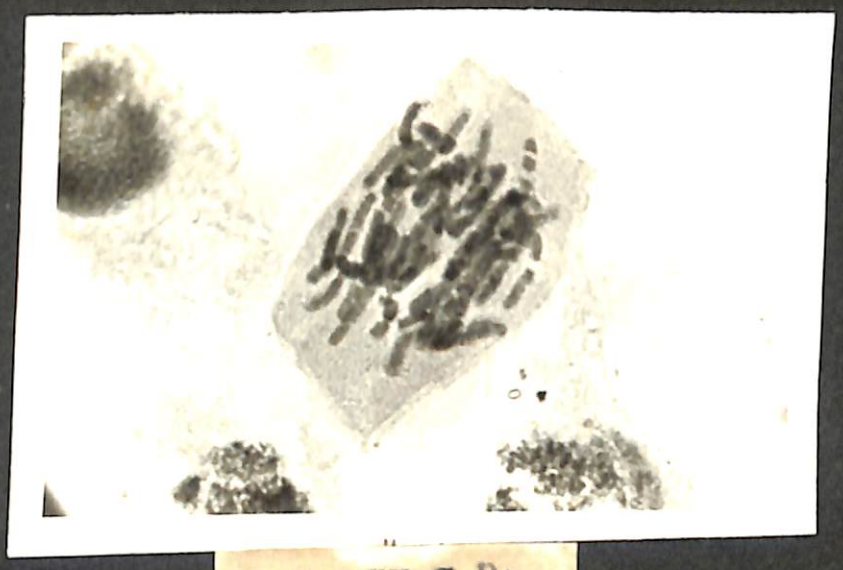


Photo IV.7 D:
Anaphase breaks
X 800

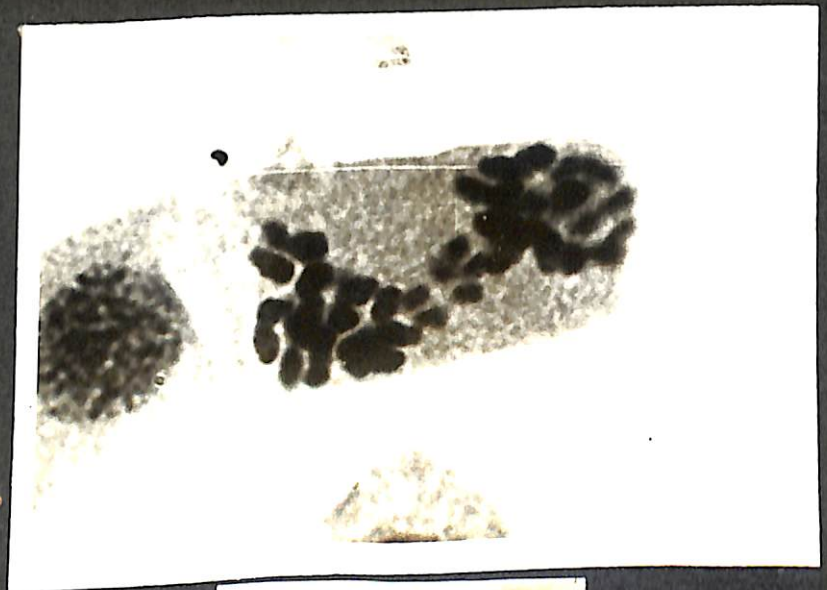


Photo IV.7 E:
Anaphase breaks
X 800

Photo IV.8 A:
Acrocentric laggard
X 1024

Photo IV.8 B:
Submeta-centric
laggard.
X 800

Photo IV.9 A:
Forwards (Acrocentric,
submetacentric
Diplochromosomal)
X 1024

Photo IV.9 B:
Forwards (Acrocentric
submeta-centric).
X 1280

## 6. Lochnerinine

Types and distribution of aberrations:
(Tables IV.6A-6D and Photos IV.6,10,15)

In the root apices of Ephedra foliata treated in 0.001%, 0.002% and 0.004% solutions of Lochnernine, for 1-24 hours duration, 3 aberrations are produced (Table IV.6A).

In 0.001%, only one aberration is present - micronuclei, occurring after 12 hours as well as 24 hours of treatment. Their mean frequencies in this concentration is 0.37(Table IV.6B).

In 0.002%, also only one aberration is present - bridges, observed after 3 hr treatment. Their mean frequency in this concentration is 0.17 (Table IV.6C).

In 0.004%, two aberrations are present. The bridges are observed after 6 hrs treatment and spindle shifting, after 3 hours. Their mean frequencies are 0.15 and 0.34 respectively. Thus the total percentage of aberrations in this concentration come to 0.49 (Table IV.6D).

Frequency:
(Tables IV.6E and 6F)

Considering all the aberrations together the respective mean aberration frequencies in 0.001%, 0.002% and 0.004%

concentrations are 0.214, 0.156 and 0.558. In 0.001%, aberrations occur in 12 hr (0.7) and 24 hr (0.37) durations. In 0.002%, they occur only in 3 hr (0.78) duration. In 0.004%, they occur in 3 hr (1.8) and 6 hr (0.99) durations. Taking the durations into account, the aberration frequencies in 3,6,12 and 24 hr treatments sum up to 2.58, 0.99, 0.7 and 0.37 respectively giving a total of 4.64. No aberrations are observed 1 hr treatment (Table IV.6E).

Now the individual aberrations are considered after pooling their readings in different durations. Bridges occur both in 0.002% and 0.004% concentrations giving a total frequency of 0.32. Spindle shifting occurs only in 0.004% with a frequency of 0.34. Micronuclei are present only in 0.001% with a frequency of 0.37. Thus the total percentage of aberrations caused by Lochner's nine works out to 1.03 (Table IV.6F).

Mitotic indices:

(Table IV.6G)

In 0.001%, the maximum mitotic index is after 6 hrs (3.01) and minimum after 1 hr (2.35) treatment. In 0.002%, the maximum is after 1 hr (5.76) and the minimum after 12 hrs(2.13). In 0.004% the maximum is after 12 hrs (2.88) and minimum after 24 hrs (0.53) of treatment. The means of mitotic indices in 0.001%, 0.002% and 0.004% concentrations are 2.75, 3.48 and 2.27 respectively.

On the whole, the range of mitotic indices in the material lies between 0.53 and 5.76. Among the concentrations they are more in 0.002% and less in 0.004%, and among durations, after 1 hr and 24 hrs respectively. The mean mitotic index of the material works out to 2.83.

*Table IV.6A*

Presence of various aberrations in the root apices treated with different concentrations of Lochnerinine for different durations

| Duration of treatment in hours | concentration of the chemical | | |
|---|---|---|---|
| | 0.001% | 0.002% | 0.004% |
| 1 | - | - | - |
| 3 | - | Br | Ss |
| 6 | - | - | Br |
| 12 | Mn | - | - |
| 24 | Mn | - | - |

Total number of aberrations : 3

Table IV.6B

Distribution of various aberrations in the root apices treated with 0.001% Lochnerinine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 558 | 1924 | 2576 | 1152 | 2421 | 7631 | |
| Cells showing: | | | | | | | |
| 1. Micronucleus | - | - | - | 11 | 17 | 28 | 0.37 |
| Total aberrent cells | - | - | - | 11 | 17 | 28 | |
| % | - | - | - | 0.7 | 0.37 | | 0.37 |

Table IV.6C

Distribution of various aberrations in the root apices treated with 0.002% Lochneninine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| Cells observed | 1323 | 1960 | 1764 | 1557 | 2178 | 8780 | |
| Cells showing: | | | | | | | |
| 1. Bridges | - | 15 | - | - | - | 15 | 0.17 |
| Total aberrent cells | - | 15 | - | - | - | 15 | |
| % | - | 0.78 | - | - | - | | 0.17 |

Table IV.6D

Distribution of various aberrations in the root apices treated with 0.004% Lochnerinine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1386 | 1540 | 1209 | 2853 | 1201 | 8189 | |
| Cells showing: | | | | | | | |
| 1. Bridges | - | - | 12 | - | - | 12 | 0.15 |
| 2. Spindle shifting | - | 28 | - | - | - | 28 | 0.34 |
| Total aberrent cells | - | 28 | 12 | - | - | 40 | |
| % | - | 1.8 | 0.99 | - | - | | 0.49 |

Table IV.6E

Frequency of total aberrations in the root apices treated with different concentrations of Lochnerinine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Total in % | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| 0.001 | - | - | - | 0.7 | 0.37 | 1.07 | 0.24 |
| 0.002 | - | 0.78 | - | - | - | 0.78 | 0.156 |
| 0.004 | - | 1.8 | 0.99 | - | - | 2.79 | 0.558 |
| Total in % | - | 2.58 | 0.99 | 0.7 | 0.37 | 4.64 | 0.928 |

Table IV.6F

Frequency of individual aberrations in the root apices treated with different concentrations of Lochnerinine

| | Aberration | Concentration of the chemical | | | Total |
|---|---|---|---|---|---|
| | | 0.001% | 0.002% | 0.004% | |
| 1. | Bridges | - | 0.17 | 0.15 | 0.32 |
| 2. | Spindle shifting | - | - | 0.34 | 0.34 |
| 3. | Micronucleus | 0.37 | - | - | 0.37 |
| | Total | 0.37 | 0.17 | 0.49 | 1.03 |

Table IV.6G

Mitotic indices in the root apices treated with different concentrations of Lochnerinine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.001 | 2.35 | 2.92 | 3.01 | 2.97 | 2.5 | 2.75 |
| 0.002 | 5.76 | 3.49 | 2.26 | 2.13 | 3.76 | 3.48 |
| 0.004 | 2.58 | 2.63 | 2.74 | 2.88 | 0.53 | 2.27 |
| Mean | 3.56 | 3.01 | 2.67 | 2.66 | 2.26 | 2.83 |

## 7. Sitsirikine

Types and distribution of aberrations:

(Table IV.7A-7D and Photos IV.6,7,12,24)

In the root apices of Ephedra foliata treated in 0.001%,0.002% and 0.004% concentrations of sitsirikine, for 1-24 hours, 4 aberrations are noticed (Table IV.7A).

In 0.001% only one aberration is seen - bridges occurring after 6 hr treatment, with a mean frequency of 0.49 (Table IV.7B).

In 0.002% also, only one aberration is seen - phragmoplast inhibition occurring after 24 hr treatment, with a mean frequency of 0.22 (Table IV.7C).

In 0.004% two aberrations are seen. Of these, breakages are observed after 1 hr spindle shifting after 3 hr treatments with respective mean frequencies of 0.25 and 0.275. The total percentage of aberrations in this concentration sums up to 0.525 (Table IV.7D).

Frequency:

(Table IV.7E,7F)

Considering all the aberrations together, the respective

mean aberration frequencies in 0.001%, 0.002% and 0.004% concentrations, are 0.334,0.34 and 0.365. In 0.001%, aberrations occur only after 6 hrs (1.67) treatment and in 0.002% after 24 hrs (1.7). In 0.004%, they occur after 1 hr (1.05) and 3 hr (0.775) durations. Taking the durations only into account, aberrations are absent only in 12 hr treatment. The aberration frequencies in 1,3,6 and 24 hr treatments are 1.05, 0.775, 1.67 and 1.7 respectively giving a total of 5.195 (Table IV.7E).

Now the individual aberrations are considered after pooling together their readings in different durations. Breakages and spindle inhibition are present only in 0.004% with respective frequencies of 0.25 and 0.275. Bridges occur only in 0.001% and phragmoplast inhibition in 0.002%. Their respective frequencies are 0.49 and 0.22. Thus the total percentage of aberrations caused by sitsirkine works out to 1.235 (Table IV.7F).

Mitotic indices:

(Table IV.7G)

In 0.001% concentration, the maximum mitotic index is after 1 hr (6.58) duration. No mitoses occur in 24 hour duration. In 0.002% the maximum mitotic index is after 1 hr (14.73) and minimum after 24 hrs (1.73). In 0.004% the maximum is after 12 hr (8.47) duration, and no mitoses occur after 24 hrs. The means of mitotic indices in 0.001%, 0.002% and 0.004% concentrations are 3.14, 7.38 and 5.33 respectively.

On the whole, the range of mitotic indices in the

material lies between 1.73 and 14.73. Among the concentrations, the indices are least in 0.001% and more in 0.002% treatments. Among the durations the maximum is after 1 hr and minimum after 24 hours. The mean mitotic index of the material works to 5.28.

Table IV.7A

Presence of various aberrations in the root apices treated with different concentrations of Sitsirikine for different durations

| Duration of treatment in hours | Concentration of the chemical | | |
|---|---|---|---|
| | 0.001% | 0.002% | 0.004% |
| 1 | - | - | Bk |
| 3 | - | - | Si |
| 6 | Br | - | - |
| 12 | - | - | - |
| 24 | - | Pi | - |

Total number of aberrations : 4

Table IV.7C

Distribution of various aberrations in the root apices treated with 0.002% Sitsirikine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 790 | 935 | 1180 | 1130 | 590 | 4625 | |
| Cells showing: | | | | | | | |
| 1. Phragmoplast inhibition | - | - | - | - | 10 | 10 | 0.22 |
| Total aberrent cells | - | - | - | - | 10 | 10 | |
| % | - | - | - | - | 1.7 | | 0.22 |

Table IV.7D

Distribution various aberrations in the root apices treated with 0.004% Sitsirikine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 995 | 1420 | 305 | 565 | 720 | 4005 | |
| Cells showing: | | | | | | | |
| 1. Breakages | 10 | - | - | - | - | 10 | 0.25 |
| 2. Spindle inhibition | - | 11 | - | - | - | 11 | 0.275 |
| Total aberrent cells | 10 | 11 | - | - | - | 21 | |
| % | 1.05 | 0.775 | - | - | - | | 0.525 |

## Table IV.7E

Frequency of total aberrations in the root apices treated different concentrations of Sitsirikine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Total in % | Mean |
|---|---|---|---|---|---|---|---|
| 0.001 | - | - | 1.67 | - | - | 1.67 | 0.334 |
| 0.002 | - | - | - | - | 1.7 | 1.7 | 0.34 |
| 0.004 | 1.05 | 0.775 | - | - | - | 1.825 | 0.365 |
| Total in % | 1.05 | 0.775 | 1.67 | - | 1.7 | 5.195 | 1.039 |

Table IV.7F

Frequency of individual aberrations in the root apices treated with different concentrations of Sitsirikine

| | Aberration | Concentration of the chemical | | | Total |
|---|---|---|---|---|---|
| | | 0.001% | 0.002% | 0.004% | |
| 1. | Breakages | - | - | 0.25 | 0.25 |
| 2. | Bridges | 0.49 | - | - | 0.49 |
| 3. | Spindle inhibition | - | - | 0.275 | 0.275 |
| 4. | Phragmoplast inhibition | - | 0.22 | - | 0.22 |
| | Total | 0.49 | 0.22 | 0.525 | 1.235 |

Table IV.7G

Mitotic indices in the root apices treated with different concentrations of Sitsirikine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.001 | 6.58 | 3.81 | 3.2 | 2.12 | - | 3.14 |
| 0.002 | 14.73 | 6.16 | 7.54 | 6.74 | 1.73 | 7.38 |
| 0.004 | 7.30 | 5.59 | 5.28 | 8.47 | - | 5.33 |
| Mean | 9.54 | 5.19 | 5.34 | 5.78 | 0.58 | 5.28 |

## 8. Vinblastine

Types and distribution of aberrations:

(Table IV.8A-8D and Photos IV.1-3,6,7,9-15,17)

In the root apices of Ephedra foliata treated with 0.001%, 0.002% and 0.004% concentrations of Vinblastine, for 1-24 hours duration, 13 aberrations are produced (Table IV.8A).

In 0.001%, 3 aberrations are produced. Breakages occur after 1 hr and 24 hour durations with a total mean frequency of 0.17. Spindle inhibition and micronucleus are observed only in 1 hr duration with mean frequencies of 0.08 and 0.13 respectively. The aberration frequencies after 1 hr and 24 hrs respectively amount to 1.9 and 0.58. No aberrations are observed in 3,6 and 12 hr durations. The total percentage of aberrations in this concentration sums up to 0.38 (Table IV.8B).

In 0.002% 5 aberrations are noticed. Somatic reduction is found after 6 hrs duration with a mean frequency of 0.09. Bridges and spindle disruption are noticed after 12 hrs duration giving mean frequencies of 0.18 and 0.135 respectively. Micronuclei occur in 1 hr and 3 hr durations. Their mean frequency is 0.54. Nucleic acid starvation is observed after 1 hr with a frequency of 0.84. The aberration frequencies in 1,3,6 and 12 hr durations, amount to 6.57,1.3, 0.37 and 1.8 respectively. No

aberrations occur after 24 hours. The total percentage of aberrations in this concentration sums up to 1.785 (Table IV.8C).

In 0.004% concentration, 12 aberrations are caused. Somatic reduction occurs after 1 hr duration with a mean frequency of 0.11. Chromosome clumping and molten metaphases are seen after 6 and 24 hrs of treatment with respective frequencies of 0.12 and 0.055. Breakages occur after 3 and 6 hrs of treatment giving a frequency of 0.495. Bridges are observed after 1,6 and 12 hr durations with a frequency of 1.2. Forwards occur after 1 hr and 6 hrs durations giving a frequency of 0.36. Spindle shifting is observed in 3,6 and 12 hrs of treatment contributing a frequency of 2.02. Spindle is disrupted after ½ hr whereas its complete inhibition occurs after 3 and 6 hrs durations. Their respective frequencies are 0.22 and 0.76. Phragmoplast is shifted after 12 hours of treatment and is inhibited after 12 hours as well as 24 hours. Their frequencies work out to 0.21 and 0.36 respectively. The most singularly important observation is nucleicacid starvation, occuring only in 1 hour treatment. Its frequency is 1.35. The aberration frequencies after 1,3,6, 12 and 24 hrs of treatment are 9.63, 7.63, 7.9,8.5 and 1.88 respectively. The total percentage of aberrations in this concentration sums up to 7.46 (Table IV.8D).

concentrations, are 0.496, 2.008, and 7.118. In 0.001%, aberrations occur only in 1 hr (1.9) and 24 hr (0.58) durations. In 0.002%, they occur in all except 24 hr duration. The frequencies are 6.57 (1 hr), 1.3 (3hr), 0.37 (6 hr) and 1.8 (12 hr). In 0.004% aberrations are observed in all durations. The frequencies are 9.63 (1 hr) 7.63 (3 hrs), 7.9(6 hrs), 8.5 (12 hrs) and 1.88 (24 hrs). Taking the durations only into account the aberration frequencies in 1,3,6, 12 and 24 hr of durations are 18.10, 8.93, 8.27, 10.3 and 2.46 respectively mounting upto a total of 48.06 (Table IV.8E).

Now the individual aberrations are considered after pooling together their readings in different durations. Chromosome clumping, molten metophases, forwards, spindle shifting, phragmoplast shifting, and phragmoplast inhibition are observed only in 0.004% concentration. Their mean frequencies are found to be 0.12, 0.55, 0.36, 2.02, 0.21, and 0.36 respectively. Somatic reduction, bridges, spindle disruption, and nucleicacid starvation are represented both in 0.002% and 0.004% concentrations with total mean frequencies of 0.20, 1.38, 0.355, and 2.19 respectively. While breakages and spindle inhibition are noticed in 0.001% and 0.004%, micronuclei occur in 0.001% and 0.002%. Their respective mean frequencies are 0.665, 1.04 and 0.67. Thus, the total percentage of aberrations caused by Vinblastine is 9.625 (Table IV.8F).

(3.39) and minimum (1.16) after 12 hrs. In 0.002% the maximum index is shown after 3 hrs (3.45) and minimum (1.25) after 24 hours. In 0.004%, the mitotic index is maximum after 1 hour (5.83) and minimum (1.30) after 24 hours. The mean mitotic indices in 0.001%, 0.002% and 0.004% concentrations work out to be 2.18, 2.37 and 3.76 respectively.

On the whole, the range of mitotic indices in the material lies between 1.16 and 5.83. Among the concentrations, indices are least in 0.001% and maximum in 0.004% concentration. Among durations, maximum is after 3 hrs and minimum after 24 hrs. The mean mitotic index of thematerial works out to 2.75.

Table IV.8A

Presence of various aberrations in the root apices treated with different concentrations of Vinblastine for different durations

| Duration of treatment in hours | Concentration of the chemical 0.001% | 0.002% | 0.004% |
|---|---|---|---|
| 1 | Bk,Si,Mn | Mn,Ns | Br,Sr,Fr,Sd,Ns |
| 3 | - | Mn | Br,Bk,Ss,Si |
| 6 | - | Sr | Cc,Br,Bk,Fr,Ss,Si |
| 12 | - | Br,Sd | Br,Ss,Ps,Pi |
| 24 | Bk | - | Mm,Pi |

Total number of aberrations : 13

Table IV.8B

Distribution of various aberrations in the root apices treated with 0.001% Vinblastine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1256 | 2480 | 1576 | 1360 | 856 | 7528 | |
| Cells showing: | | | | | | | |
| 1. Breakages | 8 | - | - | - | 5 | 13 | 0.17 |
| 2. Spindle inhibition | 6 | - | - | - | - | 6 | 0.08 |
| 3. Micronuclei | 10 | - | - | - | - | 10 | 0.13 |
| Total aberrent cells | 24 | - | - | - | 5 | 29 | |
| % | 1.9 | - | - | - | 0.58 | | 0.38 |

Table IV.8C

Distribution of various aberrations in the root apices treated with 0.002% Vinblastine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1096 | 1536 | 1635 | 1152 | 1272 | 6691 | |
| Cells showing: | | | | | | | |
| 1. Somatic reduction | - | - | 6 | - | - | 6 | 0.09 |
| 2. Bridges | - | - | - | 12 | - | 12 | 0.18 |
| 3. Spindle disruption | - | - | - | 9 | - | 9 | 0.135 |
| 4. Micronuclei | 16 | 20 | - | - | - | 36 | 0.54 |
| 5. Nucleic acid starvation | 56 | - | - | - | - | 56 | 0.84 |
| Total aberrent cells | 72 | 20 | 6 | 21 | - | 119 | |
| % | 6.57 | 1.3 | 0.37 | 1.8 | - | | 1.785 |

Table IV.8D

Distribution of various aberrations in the root apices treated with 0.004% Vinblastine for different durations

| | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Total | % |
|---|---|---|---|---|---|---|---|
| Cells observed | 1744 | 1560 | 1760 | 1152 | 1064 | 7280 | |
| Cells showing: | | | | | | | |
| 1. Somatic reduction | 8 | - | - | - | - | 8 | 0.11 |
| 2. Chromosome clumping | - | - | 9 | - | - | 9 | 0.12 |
| 3. Molten metaphase | - | - | - | - | 4 | 4 | 0.055 |
| 4. Breakages | - | 29 | 7 | - | - | 36 | 0.495 |
| 5. Bridges | 31 | - | 35 | 24 | - | 90 | 1.2 |
| 6. Forwards | 15 | - | 11 | - | - | 26 | 0.36 |
| 7. Spindle shifting | - | 51 | 46 | 49 | - | 146 | 2.02 |
| 8. Spindle disruption | 16 | - | - | - | - | 16 | 0.22 |
| 9. Spindle inhibition | - | 39 | 31 | - | - | 70 | 0.96 |
| 10. Phragmoplast shifting | - | - | - | 15 | - | 15 | 0.21 |
| 11. Phragmoplast inhibition | - | - | - | 10 | 16 | 26 | 0.36 |
| 12. Nucleic acid starvation | 98 | - | - | - | - | 98 | 1.35 |
| Total aberrent cells | 168 | 119 | 139 | 98 | 20 | 544 | |
| % | 9.63 | 7.63 | 7.9 | 8.5 | 1.88 | | 7.46 |

## Table IV.8E

Frequency of total aberrations in the root apices treated with different concentrations of Vinblastine for different durations

| Concentration in % | Duration of treatment in hours 1 | 3 | 6 | 12 | 24 | Total in % | Mean |
|---|---|---|---|---|---|---|---|
| 0.001 | 1.9 | - | - | - | 0.58 | 2.48 | 0.496 |
| 0.002 | 6.57 | 1.3 | 0.37 | 1.8 | - | 10.04 | 2.008 |
| 0.004 | 9.63 | 7.63 | 7.9 | 8.5 | 1.88 | 35.54 | 7.118 |
| Total in % | 18.10 | 8.93 | 8.27 | 10.3 | 2.46 | 48.06 | 9.612 |

Table IV.8F

Frequency of individual aberrations in the root apices treated with different concentrations of Vinblastine

| Aberration | Concentration of the chemical 0.001% | 0.002% | 0.004% | Total |
|---|---|---|---|---|
| 1. Somatic reduction | - | 0.09 | 0.11 | 0.20 |
| 2. Chromosome clumping | - | - | 0.12 | 0.12 |
| 3. Molten metaphase | - | - | 0.055 | 0.055 |
| 4. Breakages | 0.17 | - | 0.495 | 0.665 |
| 5. Bridges | - | 0.18 | 1.20 | 1.38 |
| 6. Forwards | - | - | 0.36 | 0.36 |
| 7. Spindle shifting | - | - | 2.02 | 2.02 |
| 8. Spindle disruption | - | 0.135 | 0.22 | 0.355 |
| 9. Phragmoplast shifting | - | - | 0.21 | 0.21 |
| 10. Spindle inhibition | 0.08 | - | 0.96 | 1.04 |
| 11. Phragmoplast inhibition | - | - | 0.36 | 0.36 |
| 12. Micronucleus | 0.13 | 0.54 | - | 0.67 |
| 13. Nucleic acid starvation | - | 0.84 | 1.35 | 2.19 |
| Total | 0.38 | 1.785 | 7.460 | 9.625 |

Table IV.8G

Mitotic indices in the root apices treated with different concentrations of Vinblastine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.001 | 2.03 | 3.39 | 2.88 | 1.16 | 1.42 | 2.18 |
| 0.002 | 1.41 | 3.45 | 2.50 | 3.23 | 1.25 | 2.37 |
| 0.004 | 5.83 | 5.04 | 3.71 | 2.65 | 1.30 | 3.76 |
| Mean | 3.09 | 3.96 | 3.03 | 2.35 | 1.32 | 2.75 |

## 9. Vindoline

Types and distribution of aberrations:

(Table IV.9 A-9D and Photos IV.6,7,9,10-12;15,17)

In the root apices of Ephedra foliata treated with 0.001%, 0.002% and 0.004% concentrations of Vindoline, for 1-24 hrs 8 aberrations are produced (Table IV.9A).

In 0.001%, 3 aberrations are present. Bridges occur in 3 hr and 24 hr durations giving a mean frequency of 0.1. Spindle inhibition is noticed only after 6 hr. while Nucleic acid starvation is found after 24 hrs. Their respective mean frequencies are 0.15 and 0.2. The aberration frequencies after 3, 6 and 24 hours are 0.305, 0.96 and 1.06 respectively. No aberrations occur in 1 hour and 12 hr durations. The total percentage of aberrations in this concentration works out to 0.45 (Table IV.9B).

In 0.002% 2 aberrations are present. Bridges occur after 3 hr treatment and spindle disruption after 6 hrs, with mean frequencies of 0.1 and 0.13 respectively. The aberration frequencies after 3 and 6 hours are 0.34 and 0.61 respectively. No aberrations are observed in 1,12 and 24 hr treatments. The total percentage of aberrations in this concentration works out to 0.23 (Table IV.9C).

In 0.004% there are 7 aberrations. Breakages and bridges occur after 24 and 12 hours of treatments with respective mean frequencies of 0.11 and 0.14. Forwards occur both after 1 as well as 24 hours with a total mean frequency of 0.42. Spindle shifting and micronuclei occur after 3 hours and spindle inhibition after 6 hours. Their respective mean frequencies are 0.25, 0.25 and 0.21. Nucleic acid starvation occurs after 1 hour with a mean frequency of 0.17. The aberration frequencies after 1,3,6,12 and 24 hours of treatment are 1.87, 2.53, 0.82, 0.78 and 2.29 respectively. The total percentage of aberrations in this concentration works out to 1.55 (Table IV.9D).

Frequency:

(Table IV.9E,9F)

Considering all the aberrations together, the respective mean aberration frequencies in 0.001%, 0.002% and 0.004% concentrations are 0.465,0.19 and 1.65% respectively. In 0.001%, aberrations occur after 3 hours (0.305), 6 hours (0.96) and 24 hrs (1.06). In 0.002%, they occur only after 3 hrs (0.34) and 6 hrs (0.61), whereas in 0.004% they are observed in all durations. Their frequencies are 1.87 (1 hr), 2.53 (3hrs), 0.82 (6 hrs), 0.78 (12 hrs) and 2.29 (24 hrs). Taking the durations only into account, the aberration frequencies in 1,3,6,12 and 24 hr treatments are 1.87, 3.175, 2.39, 0.78 and 3.35 respectively, forming a total of 11.565 (Table IV.9E).

Now the individual aberrations are considered after pooling together their readings in different durations. Breakages,

forwards, spindle shifting and micronuclei are present only in 0.004% concentration. Their frequencies are 0.11, 0.42, 0.25 and 0.25 respectively. Spindle disruption is present only in 0.002% with a frequency of 0.13. Spindle inhibition and nucleic acid starvation occur both in 0.001% and 0.004%, with respective frequencies of 0.36 and 0.37. Bridges are the only aberrations present in all the concentrations. The frequency is 0.34. The total percentage of aberrations caused by vindoline sums up to 2.23 (Table IV.9F).

Mitotic indices:

(Table IV.10G)

In 0.001%, the maximum mitotic index is after 12 hour treatment (6.74) and minimum after 1 hour (3.45). In 0.002% also, the maximum index is after 12 hours (5.31) but minimum after 6 hrs duration (3.07). In 0.004%, the maximum is after 3 hrs (4.88) and minimum after 6 hours (2.22). The mean mitotic indices in 0.001%, 0.002% and 0.004% concentration are calculated to be 4.44, 4.13 and 3.38 respectively.

On the whole, the range of mitotic indices in the material lies between 2.22 and 6.74. Among the concentrations, they are more in 0.001% and least in 0.004%. Among the durations, maximum is in 12 hours treatment and minimum in 6 hours. The mean mitotic index in this chemical works out to 3.98.

Table IV.9A

Presence of various aberrations in the root apices treated with different concentrations of Vindoline for different durations

| Duration of treatment in hours | Concentration of the chemical | | |
|---|---|---|---|
| | 0.001% | 0.002% | 0.004% |
| 1 | - | - | Ns |
| 3 | Br | Br | Ss,Mn |
| 6 | Si | Sd | Si |
| 12 | - | - | Br |
| 24 | Ns,Br | - | Bk,Fr |

Total number of aberrations : 8

Table IV.9B

Distribution of various aberrations in the root apices treated with 0.001% Vindoline, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3172 | 3940 | 2396 | 2676 | 3220 | 15404 | |
| Cells showing: | | | | | | | |
| 1. Bridges | - | 12 | - | - | 3 | 15 | 0.1 |
| 2. Spindle inhibition | - | - | 23 | - | - | 23 | 0.15 |
| 3. Nucleic acid starvation | - | - | - | - | 31 | 31 | 0.2 |
| Total aberrent cells | - | 12 | 23 | - | 34 | 69 | |
| % | - | 0.305 | 0.96 | - | 1.06 | | 0.45 |

## Table IV.9C

Distribution of various aberrations in the root apices treated with 0.002% Vindoline for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1824 | 3876 | 2940 | 1848 | 2700 | 13188 | |
| Cells showing: | | | | | | | |
| 1. Bridges | - | 13 | - | - | - | 13 | 0.1 |
| 2. Spindle disruption | - | - | 18 | - | - | 18 | 0.13 |
| Total aberrent cells | - | 13 | 18 | - | - | 31 | |
| % | - | 0.34 | 0.61 | - | - | | 0.23 |

Table IV.9D

Distribution of various aberrations in the root apices treated with 0.004% Vindoline for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3900 | 3516 | 4392 | 3072 | 1968 | 16848 | |
| Cells showing: | | | | | | | |
| 1. Breakages | - | - | - | - | 18 | 18 | 0.11 |
| 2. Bridges | - | - | - | 24 | - | 24 | 0.14 |
| 3. Forwards | 45 | - | - | - | 27 | 72 | 0.42 |
| 4. Spindle shifting | - | 42 | - | - | - | 42 | 0.25 |
| 5. Spindle inhibition | - | - | 36 | - | - | 36 | 0.21 |
| 6. Micronucleus | - | 48 | - | - | - | 48 | 0.25 |
| 7. Nucleic acid starvation | 28 | - | - | - | - | 28 | 0.17 |
| Total aberrent cells | 73 | 90 | 36 | 24 | 45 | 268 | |
| % | 1.87 | 2.53 | 0.82 | 0.78 | 2.29 | | 1.55 |

*Table IV.9F*

Frequency of individual aberrations in the root apices treated with different concentrations of Vindoline

| Aberration | Concentration of the chemical 0.001% | 0.002% | 0.004% | Total |
|---|---|---|---|---|
| 1. Breakages | - | - | 0.11 | 0.11 |
| 2. Bridges | 0.1 | 0.1 | 0.14 | 0.34 |
| 3. Forwards | - | - | 0.42 | 0.42 |
| 4. Spindle shifting | - | - | 0.25 | 0.25 |
| 5. Spindle disruption | - | 0.13 | - | 0.13 |
| 6. Spindle inhibition | 0.15 | - | 0.21 | 0.36 |
| 7. Micronucleus | - | - | 0.25 | 0.25 |
| 8. Nucleic acid starvation | 0.20 | - | 0.17 | 0.37 |
| Total | 0.45 | 0.23 | 1.55 | 2.23 |

Table IV.9G

Mitotic indices in the root apices treated with different concentrations of Vindoline for different durations

| Concentrations in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.001 | 3.45 | 3.58 | 3.85 | 6.74 | 4.58 | 4.44 |
| 0.002 | 5.28 | 3.53 | 3.07 | 5.31 | 3.46 | 4.13 |
| 0.004 | 3.92 | 4.88 | 2.22 | 3.05 | 2.82 | 3.38 |
| Mean | 4.22 | 4.00 | 3.05 | 5.03 | 3.62 | 3.98 |

## 10. Yohimbine

Types and distribution of aberrations:
(Tables IV.10A-10D and Photos IV.1,2,4,6,7,9-11,14)

In the root apices of Ephedra foliata treated with 0.001%, 0.002% and 0.004% concentrations of yohimbine for 1-24 hours 9 aberrations are observed (Table IV.10A).

In 0.001%, 2 types of aberrations are present. Chromosome diminution is affected in 3 hr and 6 hr duration resulting in a mean frequency of 0.29, while spindle shifting occurs after 3 hrs, with a mean frequency of 0.22. The aberration frequencies after 3 hrs and 6 hrs are 1.45 and 1.35 respectively. No aberrations are caused in 1,12 and 24 hr durations. The total percentage of aberrations in this concentration is 0.51 (Table IV.10B).

In 0.002%, 5 aberrations are noticed. Somatic reduction occurs only in 1 hr duration with a mean frequency of 0.09. Chromosome clumping occurs after 6 hours treatment with a mean frequency of 0.66. Breakages, bridges and spindle shifting occur only in 1 hour duration with mean frequencies of 0.12, 0.152 and 0.36, respectively. The respective aberration frequencies after 1, 3 and 6 hours duration work out to 2.30, 1.11 and 0.49. No aberrations occur in 12 and 24 hours duration. The total percentage of aberrations in this concentration is 0.782 (Table IV.10C).

In 0.004%, 6 aberrations are caused. Breakages and bridges occur after 12 and 24 hours respectively, with mean frequencies of 0.06 and 0.2. Spindle shifting is affected after 1 hr with a mean frequency of 0.2. Forwards, spindle disruption and phrogmoplast inhibition occur only after 3 hrs of treatment giving frequencies of 0.18, 0.28 and 0.36 respectively. The respective aberration frequencies after 1,3,12 and 24 hrs durations are 0.64,3.13, 1.82 and 1.80. The total percentage of aberrations in this concentration is 1.28(Table IV.10D).

Frequency:

(Tables IV.10E,10F)

Considering all the aberrations together, the respective mean aberration frequencies in 0.001%,0.002% and 0.004% concentrations are 0.56, 0.78 and 1.478. In 0.001%, aberrations occur only in 3 hrs (1.45) and 6 hrs (1.35) duration. In 0.002%, they occur after 1 hr (2.30), 3 hrs (1.11) as well as 6 hrs (0.49). But in 0.004%, aberrations are recorded in all treatments except that of 6 hr duration. Their frequencies are 0.64 (1 hr), 3.13 (3 hrs),1.82 (12 hrs) and 1.80 (24 hrs). Taking the durations only into account, the aberration frequencies in 1,3,6,12 and 24 hrs duration are 2.94,5.69,1.84,1.82 and 1.80 respectively forming a total of 14.09 (Table IV.10E).

Now the individual aberrations are considered after pooling together their readings in different durations. Chromosome diminution is present only in 0.001% with a frequency of

0.29. Somatic reduction, chromosome clumping are recorded only in 0.002% concentration both respective frequencies of 0.09 and 0.06. Forwards, spindle disruption, and phragmoplast inhibition are reported only in 0.004% with frequencies of 0.18,0.28 and 0.36. Breakages and bridges occur both in 0.002% as well as 0.004% concentration with respective frequencies of 0.18 and 0.352. Spindle shifting alone occurs in all the three concentrations. The frequency 0.78. The total percentage of aberrations produced by yohimbine is calculated to be 2.572 (Table IV.10F).

Mitotic indices:

(Table IV.10G)

In 0.001%, the maximum mitotic index is after 3 hrs (4.8) and minimum after 6 hrs (1.08) treatment. In 0.002%, the maximum is after 6 hrs (5.76) and minimum after 24 hrs (2.82). In 0.004%, maximum is in 1 hr (5.05) duration and minimum index after 24 hours (2.1). The means of mitotic indices in 0.001%, 0.002% and 0.004% concentrations are found tobe 5.56, 6.81 and 6.12 respectively.

On the whole, the range of mitotic indices in the material lies between 1.08 and 5.76. Among the concentrations the indices are more in 0.002% and least in 0.001%. Among durations maximum is in 1 hr treatment and minimum after 24 hours. The mean mitotic index of the material works out to 3.70.

***

Table IV.10A

Presence of various aberrations in the root apices treated with different concentrations of Yohimbine for different durations

| Duration of treatment in hours | Concentration of the chemical 0.001% | 0.002% | 0.004% |
|---|---|---|---|
| 1 | - | Sr,Br,Bk | Ss |
| 3 | Cd,Ss | Ss | Fr,Sd,Pi |
| 6 | Cd | Cc | - |
| 12 | - | - | Bk |
| 24 | - | - | Br |

Total number of aberrations : 9

Table IV.10B

Distribution of various aberrations in the root apices treated with 0.001% Yohimbine, for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 2639 | 2555 | 1554 | 2646 | 2093 | 11487 | |
| Cells showing: | | | | | | | |
| 1. Chromosome diminution | - | 12 | 21 | - | - | 33 | 0.29 |
| 2. Spindle shifting | - | 25 | - | - | - | 25 | 0.22 |
| Total aberrent cells | - | 37 | 21 | - | - | 58 | |
| % | - | 1.45 | 1.35 | - | - | | 0.51 |

Table IV.10C

Distribution of various aberrations in the root apices treated with 0.002% Yohimbine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 1561 | 3234 | 1211 | 2009 | 1820 | 9835 | |
| Cells showing: | | | | | | | |
| 1. Somatic reduction | 9 | - | - | - | - | 9 | 0.09 |
| 2. Chromosome clumping | - | - | 6 | - | - | 6 | 0.06 |
| 3. Breakages | 12 | - | - | - | - | 12 | 0.12 |
| 4. Bridges | 15 | - | - | - | - | 15 | 0.152 |
| 5. Spindle shifting | - | 36 | - | - | - | 36 | 0.36 |
| Total aberrent cells | 36 | 36 | 6 | - | - | 78 | |
| % | 2.30 | 1.11 | 0.49 | - | - | | 0.782 |

Table IV.10D

Distribution of various aberrations in the root apices treated with 0.004% Yohimbine for different durations

| | Duration of treatment in hours | | | | | Total | % |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| Cells observed | 3290 | 2625 | 2940 | 329 | 1162 | 10346 | |
| Cells showing: | | | | | | | |
| 1. Breakages | - | - | - | 6 | - | 6 | 0.06 |
| 2. Bridges | - | - | - | - | 21 | 21 | 0.2 |
| 3. Forwards | - | 18 | - | - | - | 18 | 0.18 |
| 4. Spindle shifting | 21 | - | - | - | - | 21 | 0.2 |
| 5. Spindle disruption | - | 28 | - | - | - | 28 | 0.28 |
| 6. Phragmoplast inhibition | - | 36 | - | - | - | 36 | 0.36 |
| Total aberrent cells | 21 | 82 | - | 6 | 21 | 130 | |
| % | 0.64 | 3.13 | - | 1.82 | 1.80 | | 1.28 |

Table IV.10E

Frequency of total aberrations in the root apices treated with different concentrations of Yohimbine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Total in % | Mean |
|---|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | | |
| 0.001 | - | 1.45 | 1.35 | - | - | 2.80 | 0.56 |
| 0.002 | 2.30 | 1.11 | 0.49 | - | - | 3.90 | 0.78 |
| 0.004 | 0.64 | 3.13 | - | 1.82 | 1.80 | 7.39 | 1.478 |
| Total in % | 2.94 | 5.69 | 1.84 | 1.82 | 1.80 | 14.09 | 2.818 |

Table IV.10F

Frequency of individual aberrations in the root apices treated with different concentrations of Yohimbine

| | Aberration | Concentration of the chemical 0.001% | 0.002% | 0.004% | Total |
|---|---|---|---|---|---|
| 1. | Somatic reduction | - | 0.09 | - | 0.09 |
| 2. | Chromosome clumping | - | 0.06 | - | 0.06 |
| 3. | Chromosome diminution | 0.29 | - | - | 0.29 |
| 4. | Breakages | - | 0.12 | 0.06 | 0.18 |
| 5. | Bridges | - | 0.152 | 0.2 | 0.352 |
| 6. | Forwards | - | - | 0.18 | 0.18 |
| 7. | Spindle shifting | 0.22 | 0.36 | 0.2 | 0.78 |
| 8. | Spindle disruption | - | - | 0.28 | 0.28 |
| 9. | Phragmoplast inhibition | - | - | 0.36 | 0.36 |
| | Total | 0.51 | 0.782 | 1.28 | 2.572 |

Table IV.10G

Mitotic indices in the root apices treated with different concentrations of Yohimbine for different durations

| Concentration in % | Duration of treatment in hours | | | | | Mean |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 12 | 24 | |
| 0.001 | 3.76 | 4.8 | 1.08 | 4.12 | 2.94 | 5.56 |
| 0.002 | 4.08 | 3.7 | 5.76 | 4.08 | 2.82 | 6.81 |
| 0.004 | 5.05 | 4.13 | 4.96 | 2.13 | 2.1 | 6.12 |
| Mean | 2.58 | 2.53 | 2.36 | 2.07 | 1.57 | 3.70 |

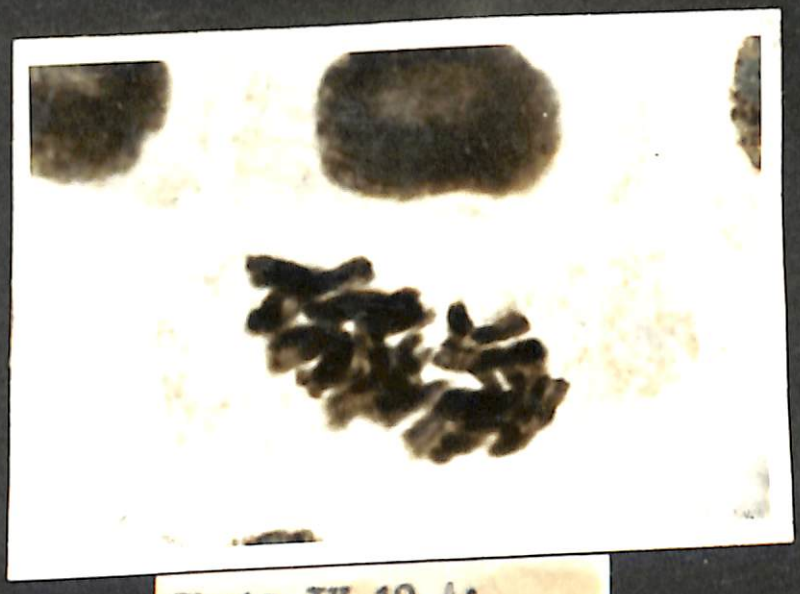


Photo IV.10 A:
Spindle shifting in metaphase.
X 1280

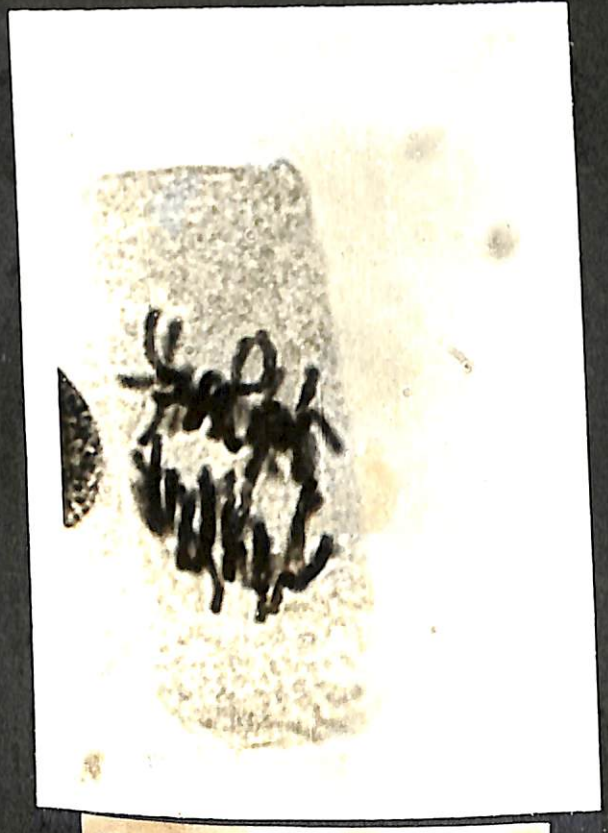


Photo IV.10 B:
Spindle shifting in anaphase(oblique).
X 1024

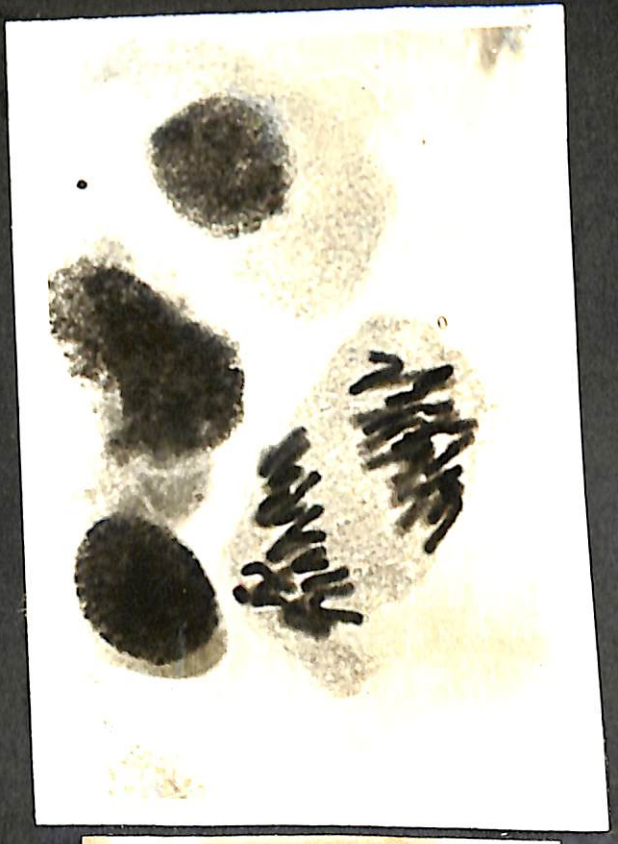


Photo IV.10 C:
Spindle shifting in anaphase(diagonal).
X 800

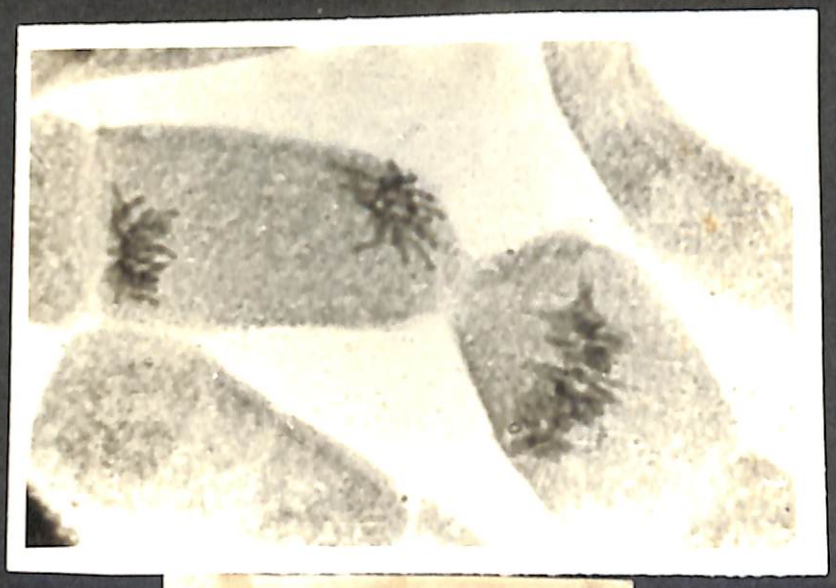


Photo IV.10 D:
Spindle shifting in meta- and telophase.
X 800

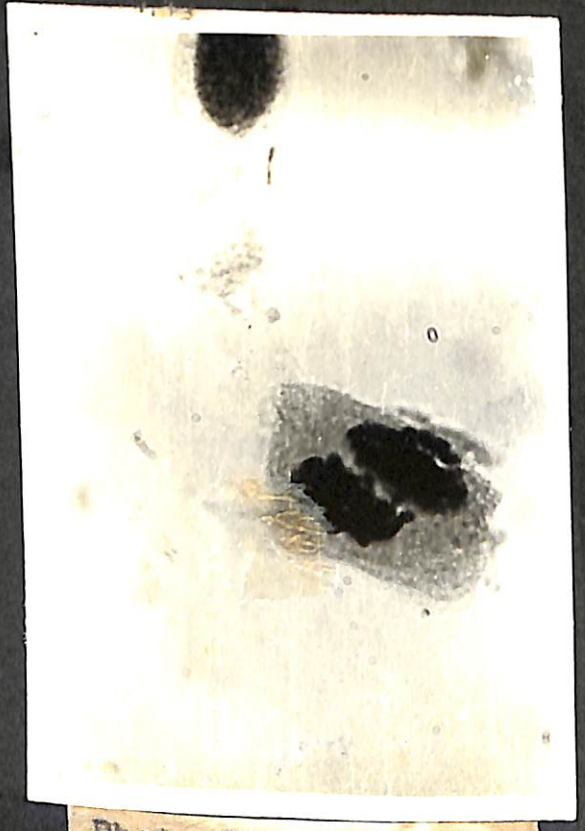


Photo IV.10 E:
Spindle shifting in Telophase (oblique).
X 800

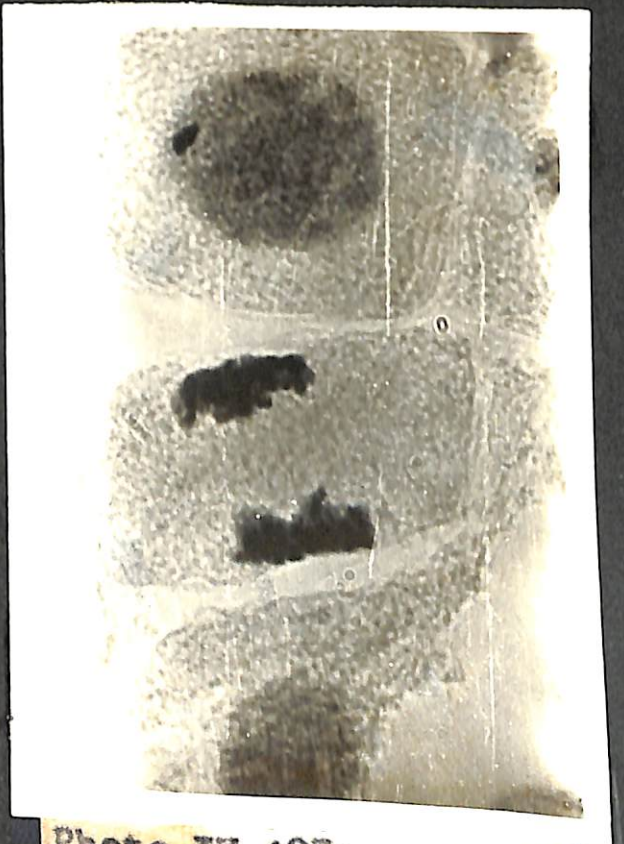


Photo IV.10F:
Spindle shifting in Telophase(transverse).
X 1024

Photo IV.11 A:
Spindle divurgence at one pole
X 1280

Photo IV.11 B:
Spindle divergence at both poles.
X 1280

Photo IV.11 C:
Spindle disturbance at equatorial region.
X 1024

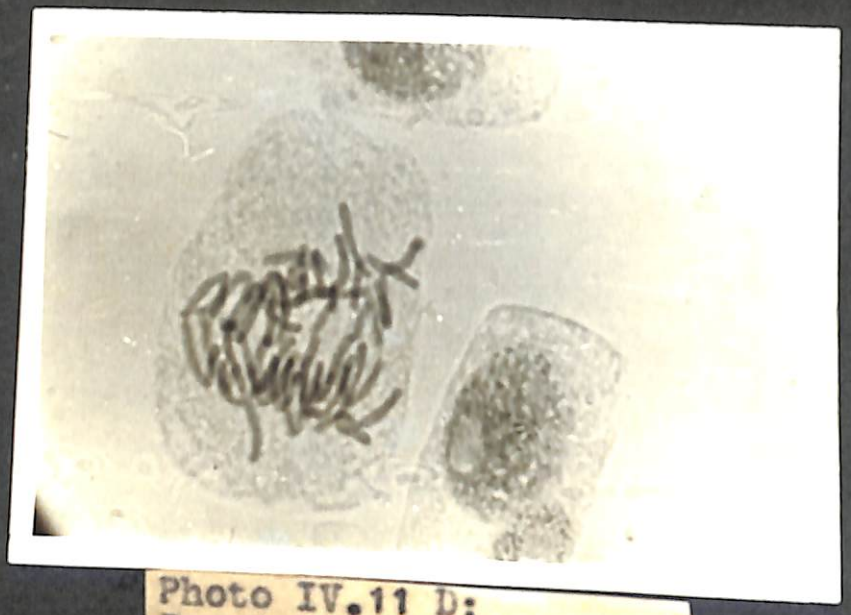
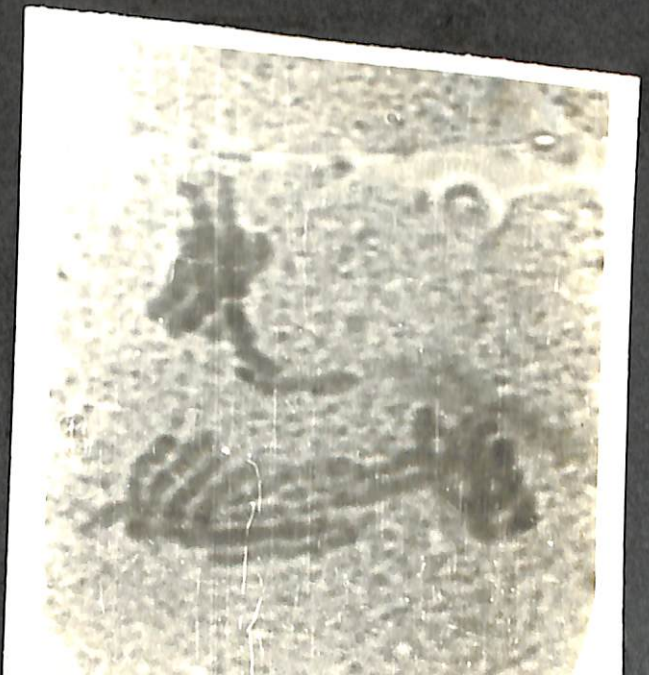
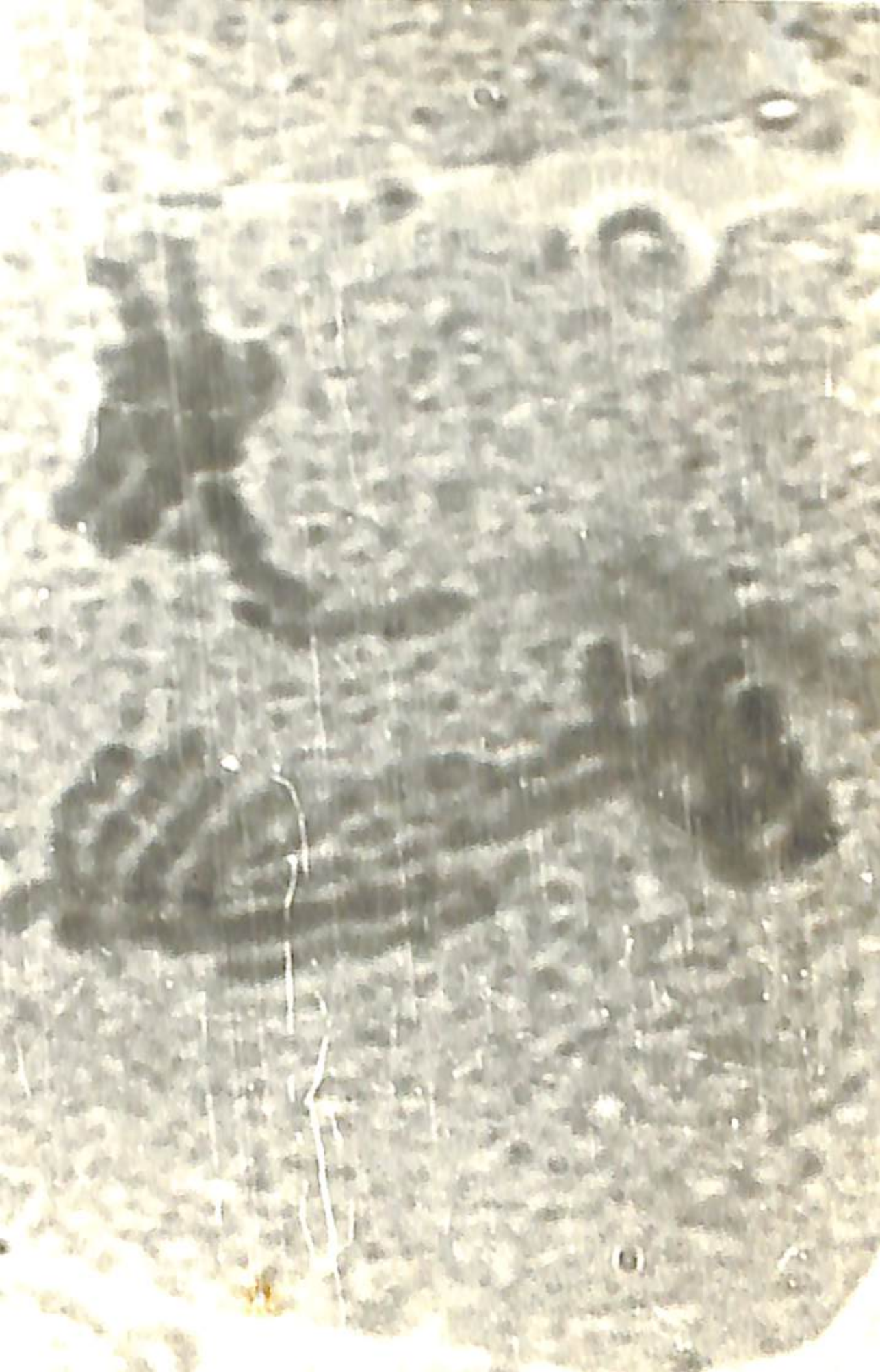


Photo IV.11 D:
Partial spindle furcation at one pole.
X 800





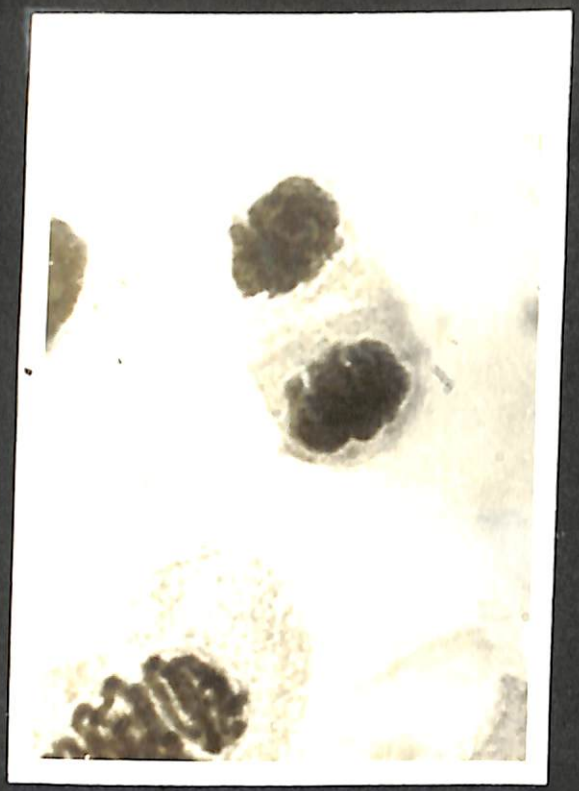


Photo IV. 13A.
Phragmoplast Shifting.
X 800.

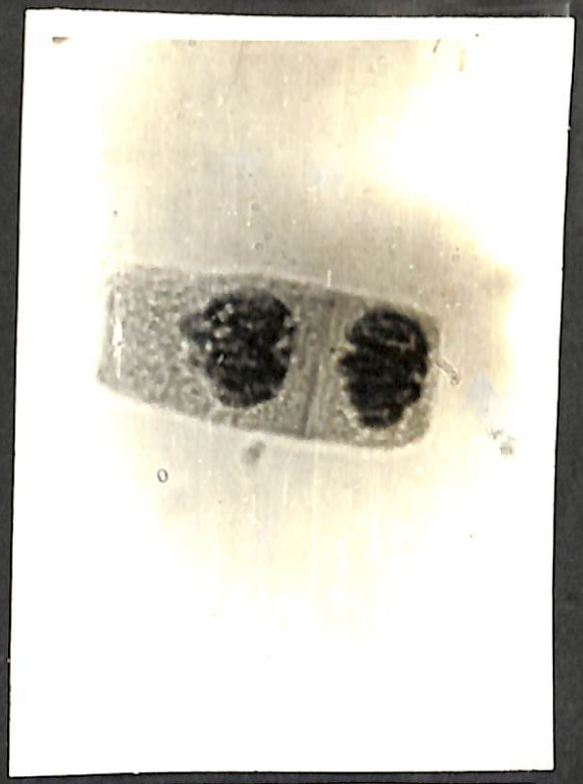
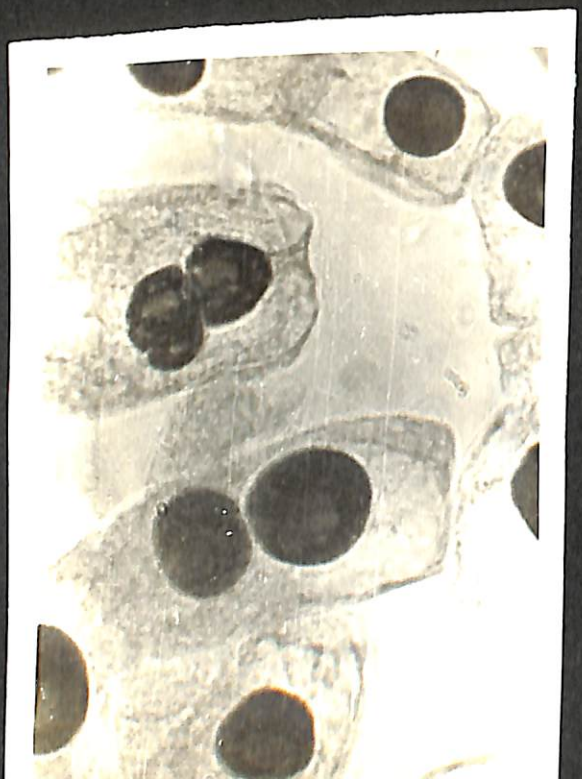
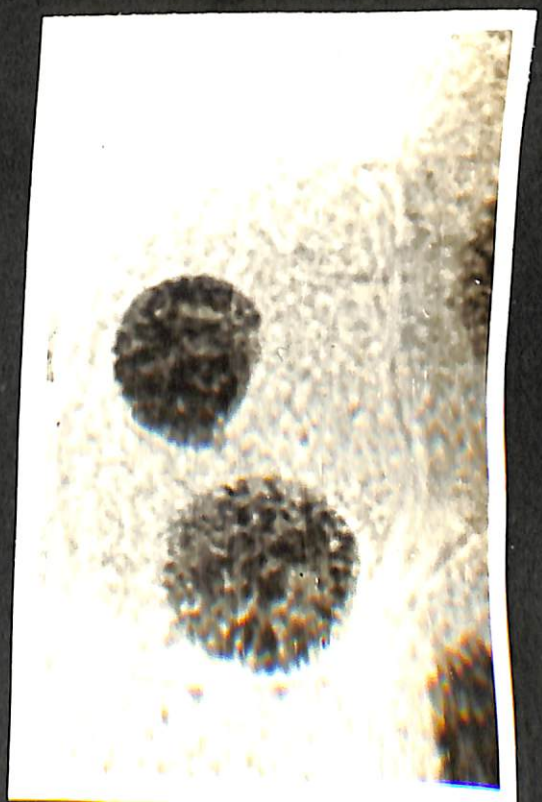


Photo IV.13 B:
Phragmoplast shifting
X 800





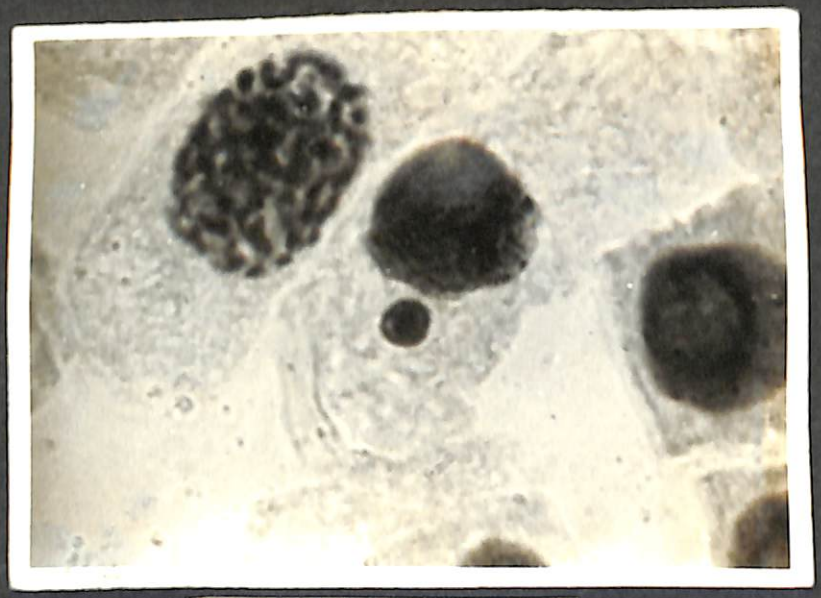
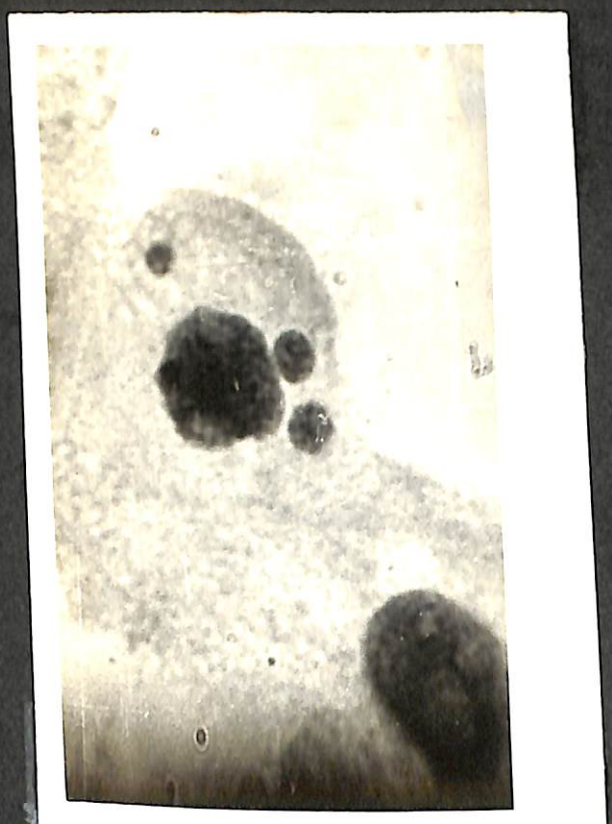
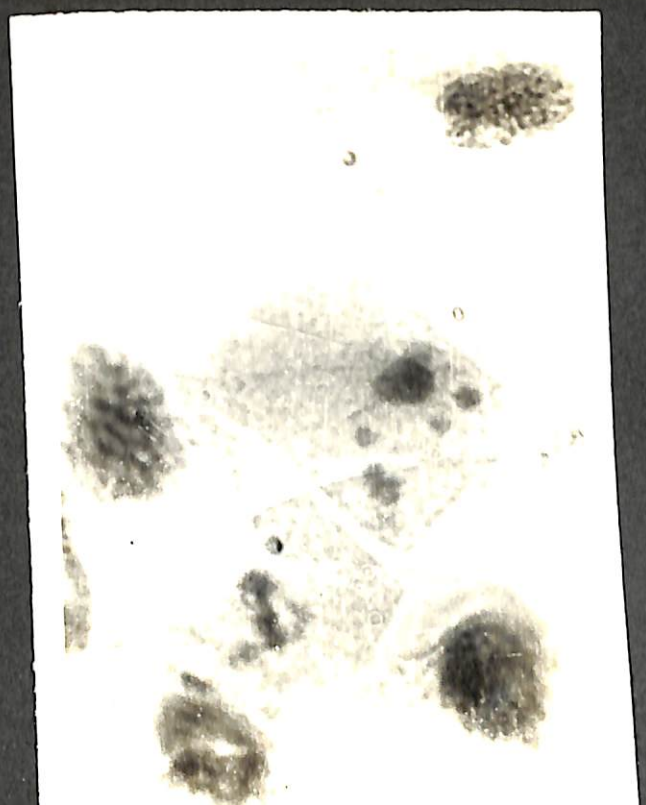


Photo IV.15 A:
One micronucleus
X 1024





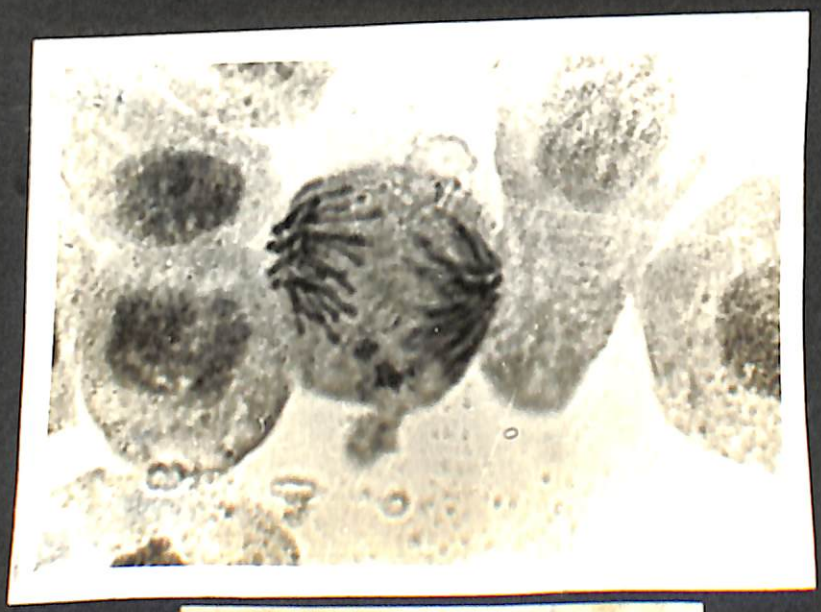


Photo IV.15D:
Micronuclear formation
due to laggards
X 1024

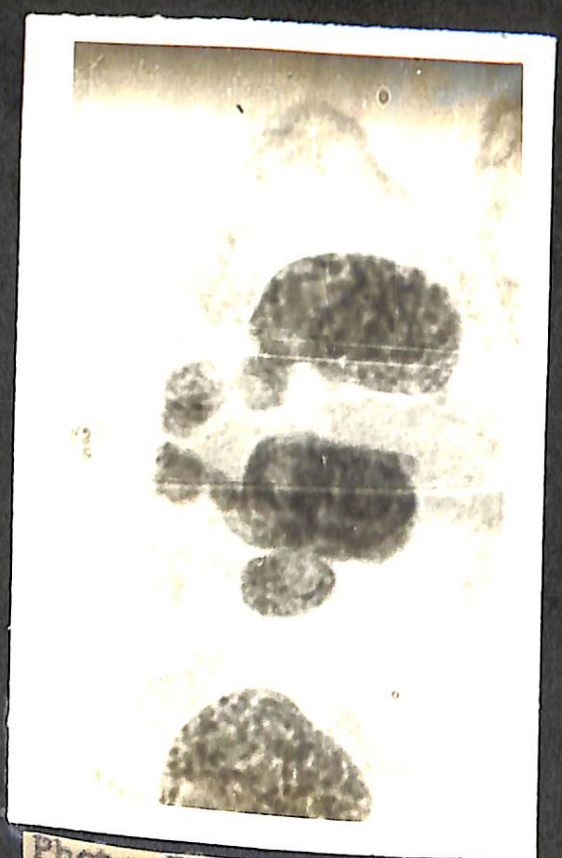


Photo IV.15 F:
Micronuclear formation
due to budding.
X 1280

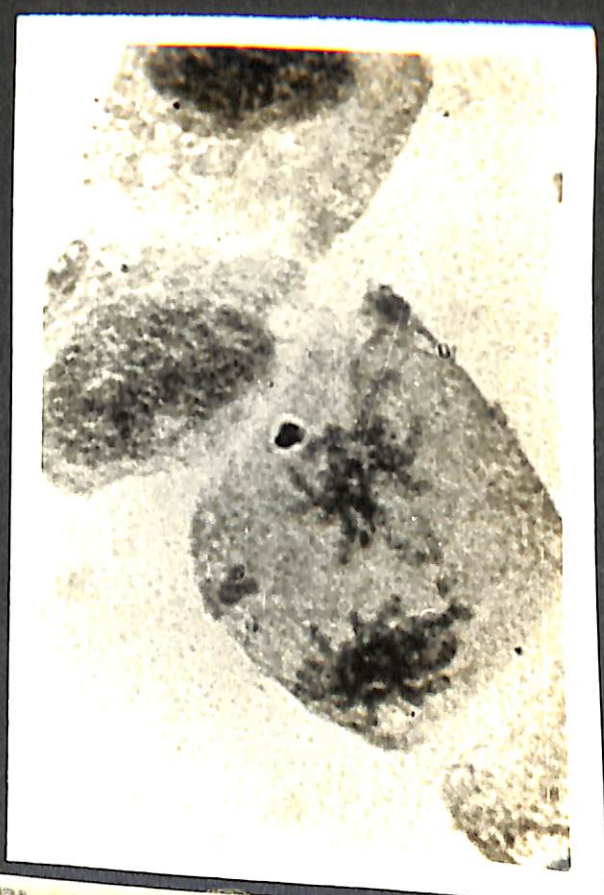


Photo IV.15 E:
Micronuclear formation
due to laggards & forwards.
X 1024

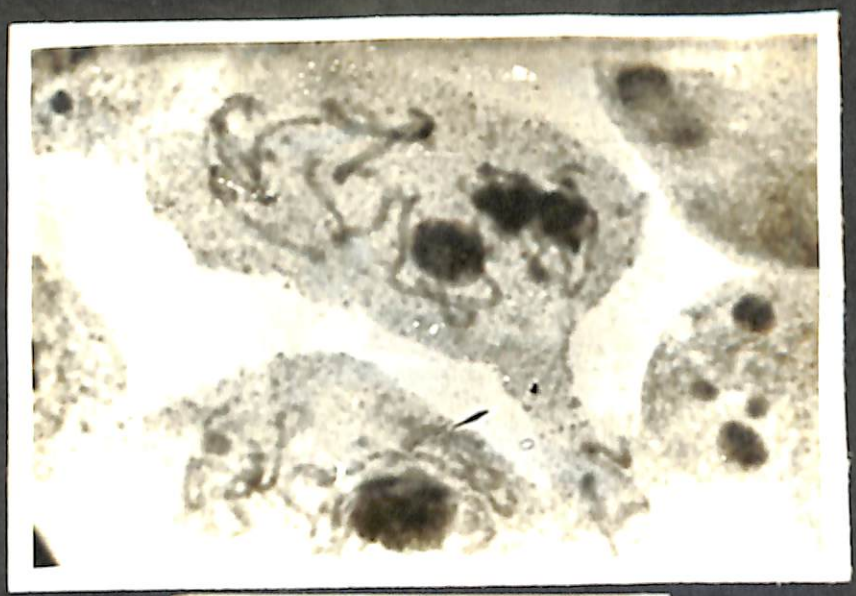
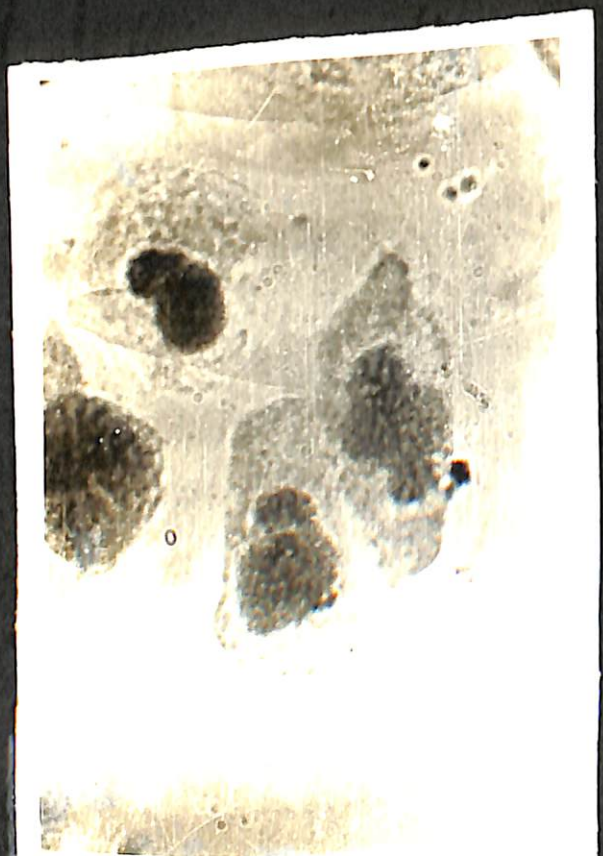
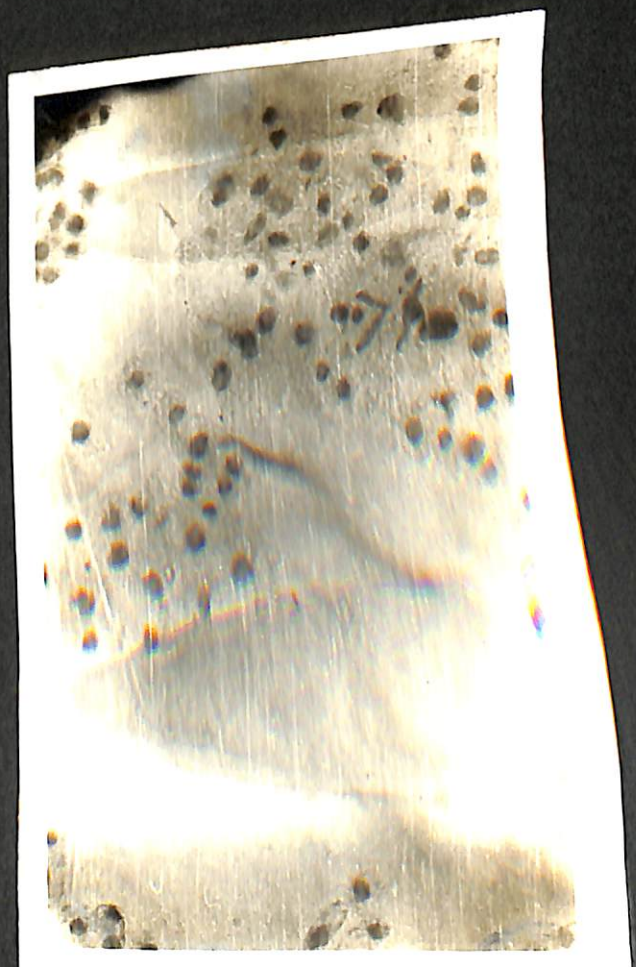


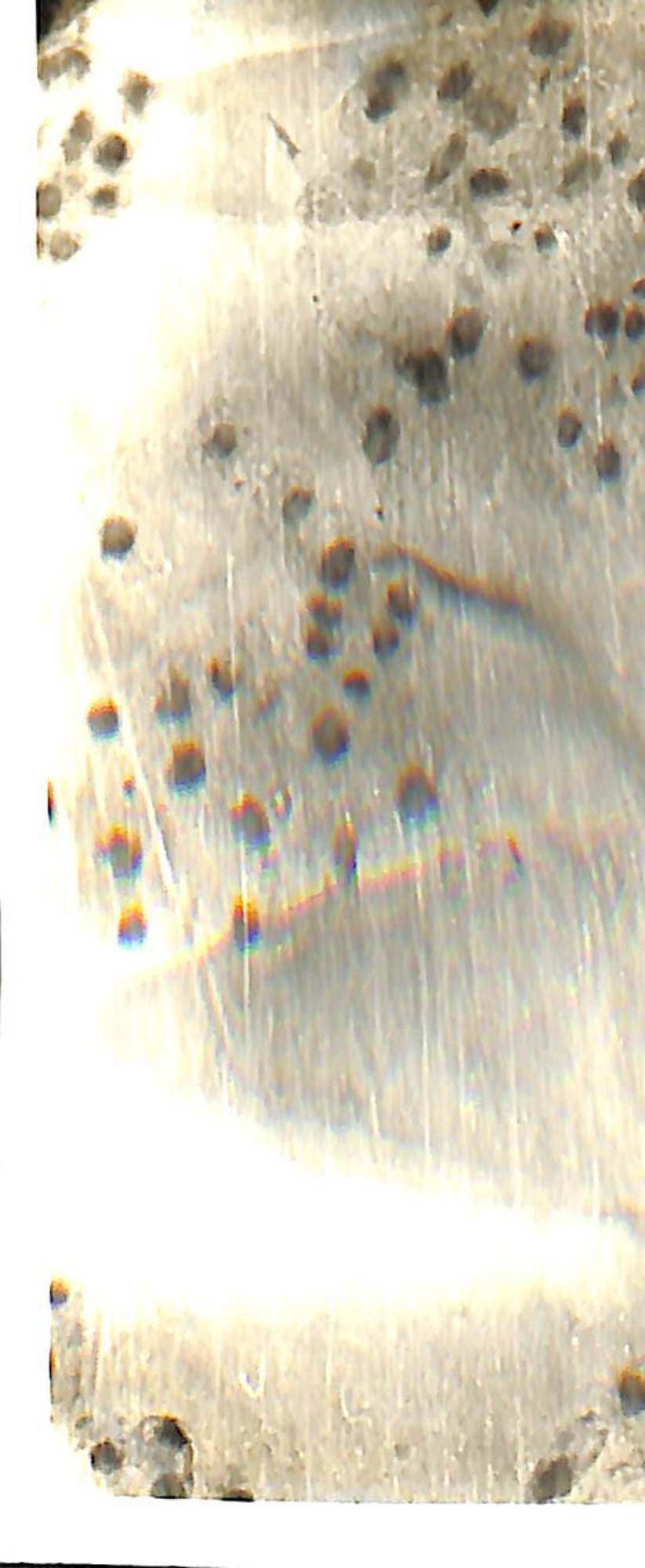
Photo IV.16 A:
Persistant nucleolus
in late prophase.
X 800

Photo IV.16 B:
Persistent nucleolus
in metaphase.
X 800

Photo IV. 17A:
Nucleic acid Starvation
X 526



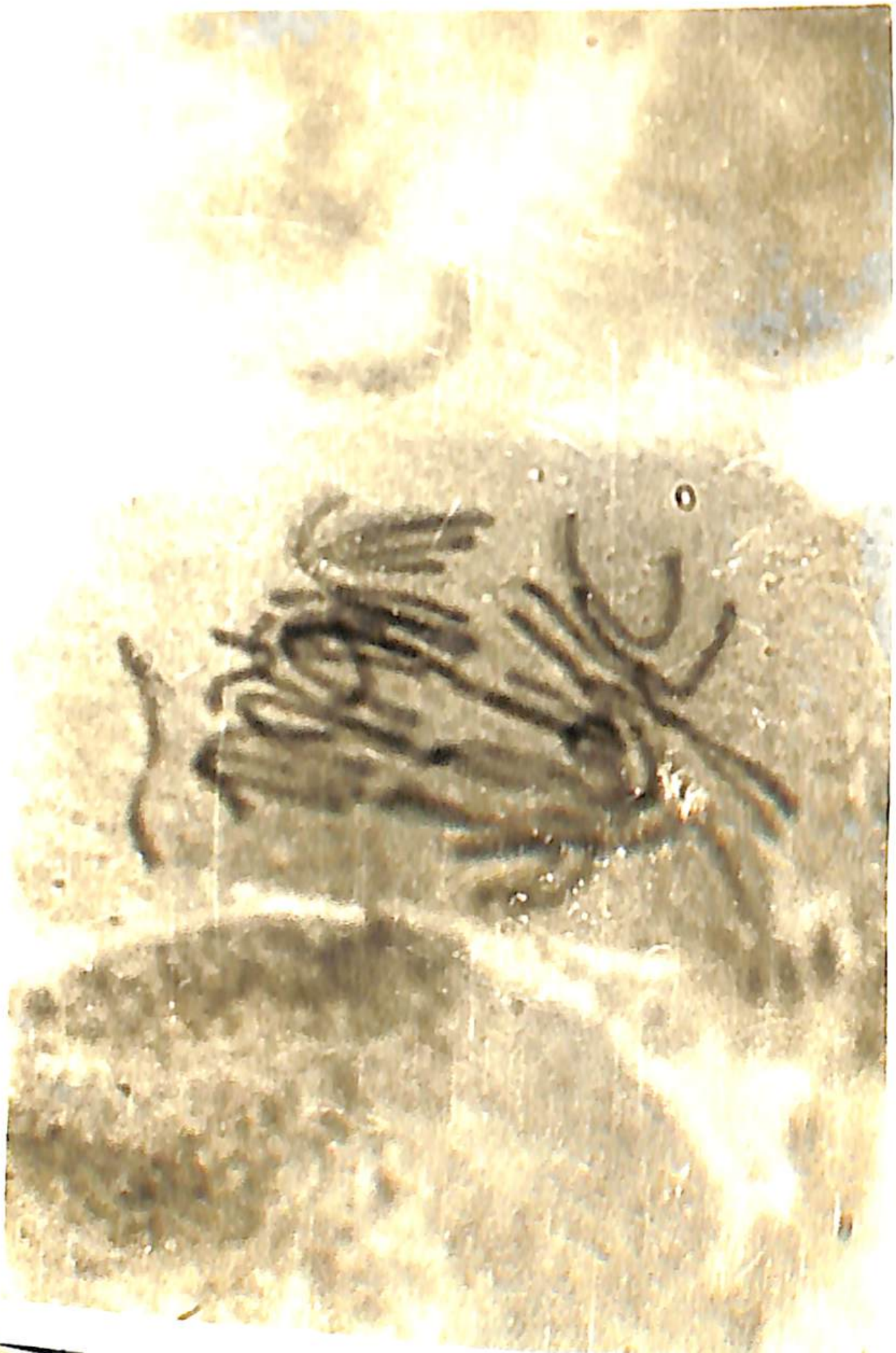




hoto 20:
icronuclear formation
a forward, laggard,
d phragmoplast shifting
1024

oto 20:
cronuclear formation
a forward, laggard,
phragmoplast shifting
1024

Photo 21:
Metaphase forward and
tetraploid metaphase in
adjacent cells.
X 576



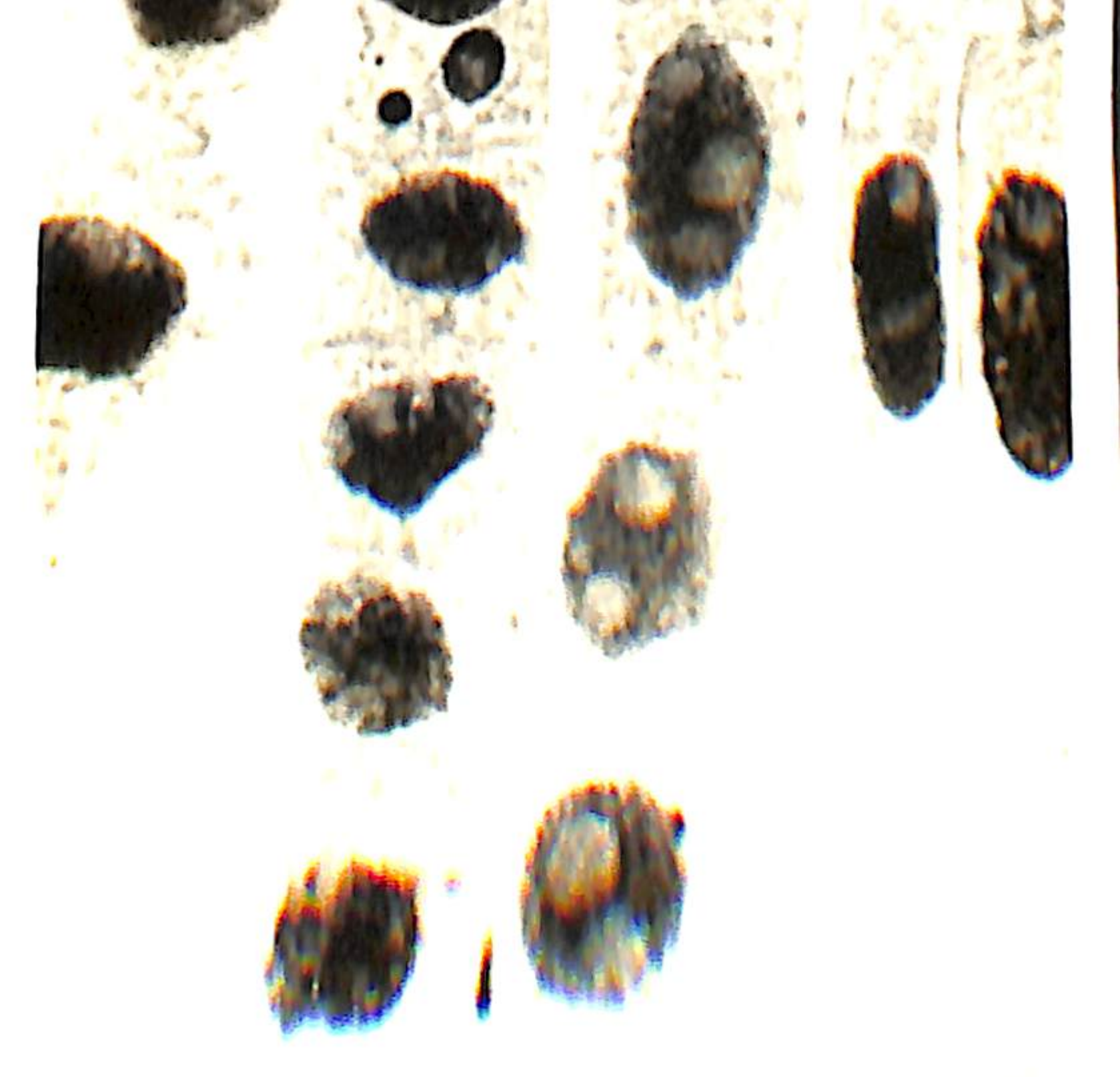


Table IV.11

Relative frequency of various aberrations in the root apices treated with aminoacids

| Type of aberration | | Aberration | Argi-nine | Gly-cine | Methio-nine | Threo-nine | Valine | Total |
|---|---|---|---|---|---|---|---|---|
| I. KARYOKINETIC | | | | | | | | |
| a. Chromosomal | 1. | Somatic reduction | 0.018 | 0.065 | - | - | - | 0.083 |
| | 2. | Chromosome clumping | 1.221 | 0.243 | - | - | 0.123 | 1.587 |
| | 3. | Molten metaphase | 0.180 | - | - | - | - | 0.180 |
| | 4. | Chromosome diminution | 0.065 | - | 0.178 | - | 0.344 | 0.587 |
| | 5. | Chromosome dots | - | 0.122 | - | - | - | 0.122 |
| | 6. | Breakages | 0.043 | 0.231 | - | - | - | 0.274 |
| | 7. | Bridges | 0.560 | 0.641 | - | - | - | 1.201 |
| | 8. | Forwards | 0.464 | 0.392 | 0.175 | - | 0.742 | 1.773 |
| | 9. | Laggards | 0.024 | - | - | - | - | 0.024 |
| b. Spindle | 10. | Spindle shifting | 0.529 | 1.304 | 0.251 | - | 0.159 | 2.243 |
| | 11. | Spindle disruption | 0.284 | - | 0.078 | 0.137 | 0.449 | 0.948 |
| | 12. | Spindle inhibition | - | - | 0.100 | - | - | 0.100 |
| II. CYTOKINETIC | 13. | Phragmoplast shifting | 0.297 | 0.131 | - | | | 0.428 |
| | 14. | Phragmoplast inhibition | - | 0.966 | - | 1.378 | 0.041 | 2.385 |
| III. NONKINETIC | 15. | Persistent nucleolus | 0.073 | 0.114 | 0.061 | 0.044 | - | 0.292 |
| | 16. | Micronucleus | 0.174 | 0.232 | - | 0.089 | 0.143 | 0.638 |
| | 17. | Nuclear atrophy | 0.356 | - | - | 1.360 | - | 1.716 |
| | 18. | Nuclear ejection | 0.503 | - | - | 0.98 | - | 1.483 |
| TOTAL | | | 4.791 | 4.441 | 0.843 | 3.988 | 2.001 | |

Table IV.12

Relative frequency of different types of aberrations in the root apices treated with various aminoacids

| CHEMICAL | KARYOKINETIC | | CYTOKINETIC | NONKINETIC | TOTAL |
|---|---|---|---|---|---|
| | Chromosomal | Spindle | | | |
| 1. Arginine | 2.575 | 0.813 | 0.297 | 1.106 | 4.791 |
| 2. Glycine | 1.694 | 1.304 | 1.097 | 0.346 | 4.441 |
| 3. Methionine | 0.353 | 0.429 | - | 0.061 | 0.843 |
| 4. Threonine | - | 0.137 | 1.378 | 2.473 | 3.988 |
| 5. Valine | 1.209 | 0.608 | 0.041 | 0.143 | 2.001 |
| Total | 5.781 | 3.291 | 2.813 | 4.129 | 16.064 |
| | 9.072 | | | | |

## Table IV.13

Total percentage of aberrations in the root apices treated with different concentrations of Aminoacids

| Concentration | Arginine | Glycine | Methionine | Threonine | Valine | Total |
|---|---|---|---|---|---|---|
| 0.01% | 1.717 | 1.186 | 0.151 | 0.105 | 0.704 | |
| 0.05% | 0.761 | 1.164 | 0.417 | 1.204 | 0.309 | |
| 0.1% | 2.313 | 2.097 | 0.275 | 2.679 | 0.988 | |
| Total | 4.791 | 4.441 | 0.843 | 3.988 | 2.001 | 16.064 |

Mean : 3.213

Table IV.14

Mean mitotic indices in the root apices treated with different concentrations of Amino acids

| Concentration | Arginine | Glycine | Methionine | Threonine | Valine | Mean |
|---|---|---|---|---|---|---|
| 0.01% | 5.84 | 6.30 | 8.87 | 5.54 | 5.12 | |
| 0.05% | 9.24 | 9.00 | 9.58 | 6.59 | 5.61 | |
| 0.1% | 7.36 | 11.50 | 6.63 | 4.96 | 6.24 | |
| Mean | 7.48 | 8.90 | 8.36 | 5.69 | 5.66 | 7.22 |

Table IV.15

Relative frequency of various aberrations in the root apices treated in alkaloids

| Type of aberration | Aberration | Lochnerinine | Sitsirikine | Vinblastine | Vindoline | Yohimbine | Total |
|---|---|---|---|---|---|---|---|
| I. KARYOKINETIC | | | | | | | |
| a. Chromosomal | 1. Somatic reduction | - | - | 0.20 | - | 0.09 | 0.29 |
| | 2. Chromosome clumping | - | - | 0.12 | - | 0.06 | 0.18 |
| | 3. Molten metaphase | - | - | 0.055 | - | | 0.055 |
| | 4. Chromosome diminution | - | - | - | - | 0.29 | 0.29 |
| | 5. Breakages | - | 0.25 | 0.665 | 0.11 | 0.18 | 1.205 |
| | 6. Bridges | 0.32 | 0.49 | 1.38 | 0.34 | 0.352 | 2.882 |
| | 7. Forwards | - | - | 0.36 | 0.42 | 0.18 | 0.96 |
| b. Spindle | 8. Spindle shifting | 0.34 | - | 2.02 | 0.25 | 0.78 | 3.39 |
| | 9. Spindle disruption | - | - | 0.355 | 0.13 | 0.28 | 0.765 |
| | 10. Spindle inhibition | - | 0.275 | 1.04 | 0.36 | - | 1.675 |
| II. CYTOKINETIC | 11. Phragmoplast shifting | - | - | 0.21 | - | - | 0.21 |
| | 12. Phragmoplast inhibition | - | 0.22 | 0.36 | - | 0.36 | 0.94 |
| III. NONKINETIC | 13. Micronucleus | 0.37 | - | 0.67 | 0.25 | - | 1.29 |
| | 14. Nucleic acid starvation | - | - | 2.19 | 0.37 | - | 2.56 |
| TOTAL | | 1.03 | 1.235 | 9.625 | 2.23 | 2.572 | 16.692 |

## Table IV.16

Relative frequency of different types of aberrations in the root apices treated with various alkaloids

| CHEMICAL | KARYOKINETIC | | CYTOKINETIC | NONKINETIC | TOTAL |
|---|---|---|---|---|---|
| | Chromosomal | Spindle | | | |
| 1. Lochnerinine | 0.32 | 0.34 | - | 0.37 | 1.03 |
| 2. Sitsirikine | 0.74 | 0.275 | 0.22 | - | 1.235 |
| 3. Vinblastine | 2.78 | 3.415 | 0.57 | 2.86 | 9.625 |
| 4. Vindoline | 0.87 | 0.74 | - | 0.62 | 2.23 |
| 5. Yohimbine | 1.152 | 1.06 | 0.36 | - | 2.572 |
| Total | 5.862 | 5.830 | 1.15 | 3.85 | 16.692 |
| | 11.692 | | | | |

Table IV.17

Total percentage of aberrations in the root apices treated with different concentrations of Alkaloids

| Concentration | Lochnerinine | Sitsirikine | Vunblastine | Vindoline | Yohimbine | Total |
|---|---|---|---|---|---|---|
| 0.001% | 0.37 | 0.49 | 0.38 | 0.45 | 0.51 | |
| 0.002% | 0.17 | 0.22 | 1.785 | 0.23 | 0.79 | |
| 0.004% | 0.49 | 0.525 | 7.46 | 1.55 | 1.28 | |
| Total | 1.03 | 1.235 | 9.625 | 2.23 | 2.58 | 16.7 |

Mean : 3.334

Table IV.18

Mean mitotic indices in the root apices treated with different concentrations of alkaloids

| Concentration | Lochnerinine | Sitsirikine | Vinblastine | Vindoline | Yohimbine | Mean |
|---|---|---|---|---|---|---|
| 0.001% | 2.75 | 3.14 | 2.18 | 4.44 | 5.56 | |
| 0.002% | 3.48 | 7.38 | 2.37 | 4.13 | 6.81 | |
| 0.004% | 2.27 | 5.33 | 3.71 | 3.38 | 6.12 | |
| Mean | 2.83 | 5.28 | 2.75 | 3.98 | 3.7 | 3.71 |

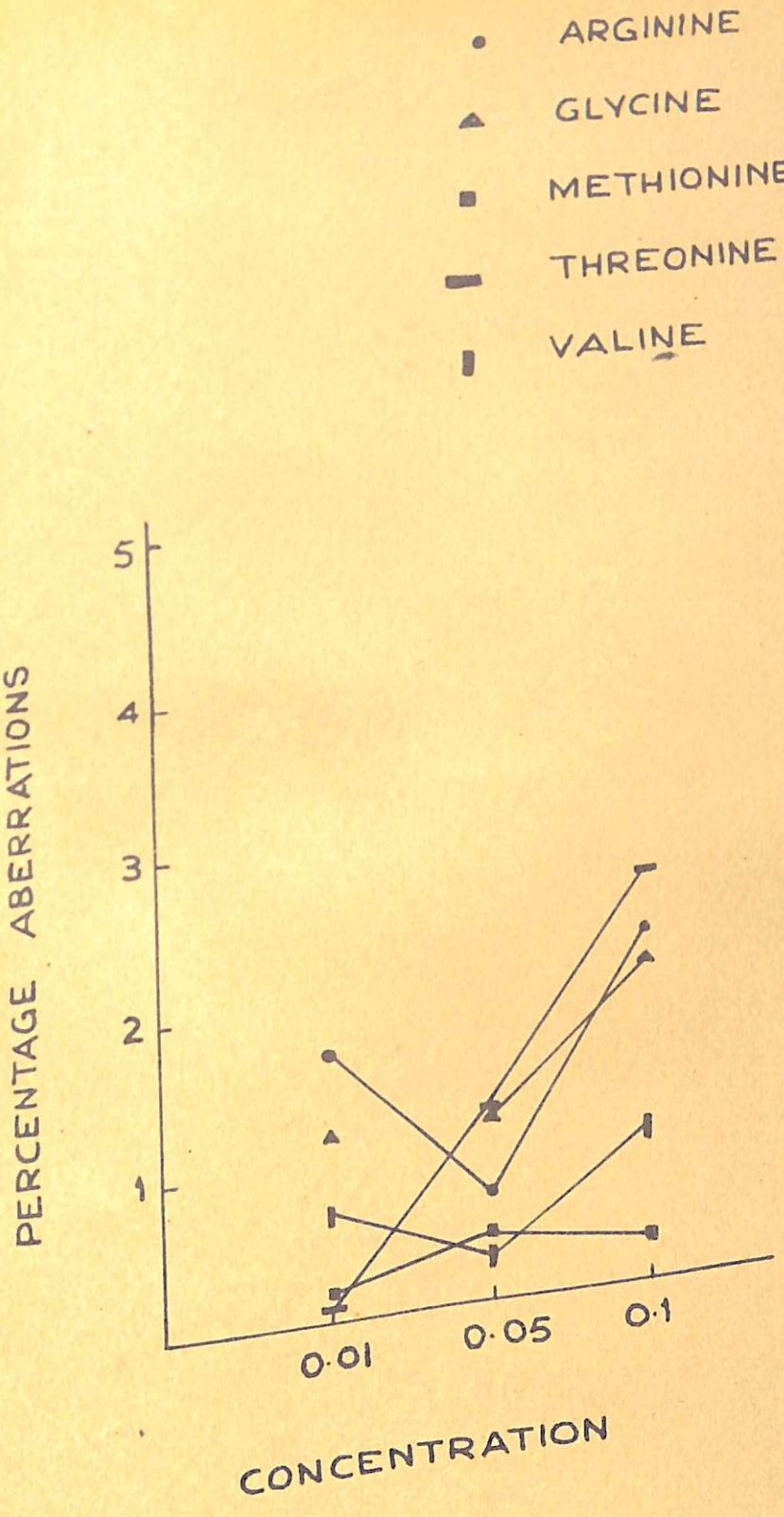



FIG. IV. 1 INFLUENCE OF AMINOACIDS ON ABERRATION FREQUENCIES

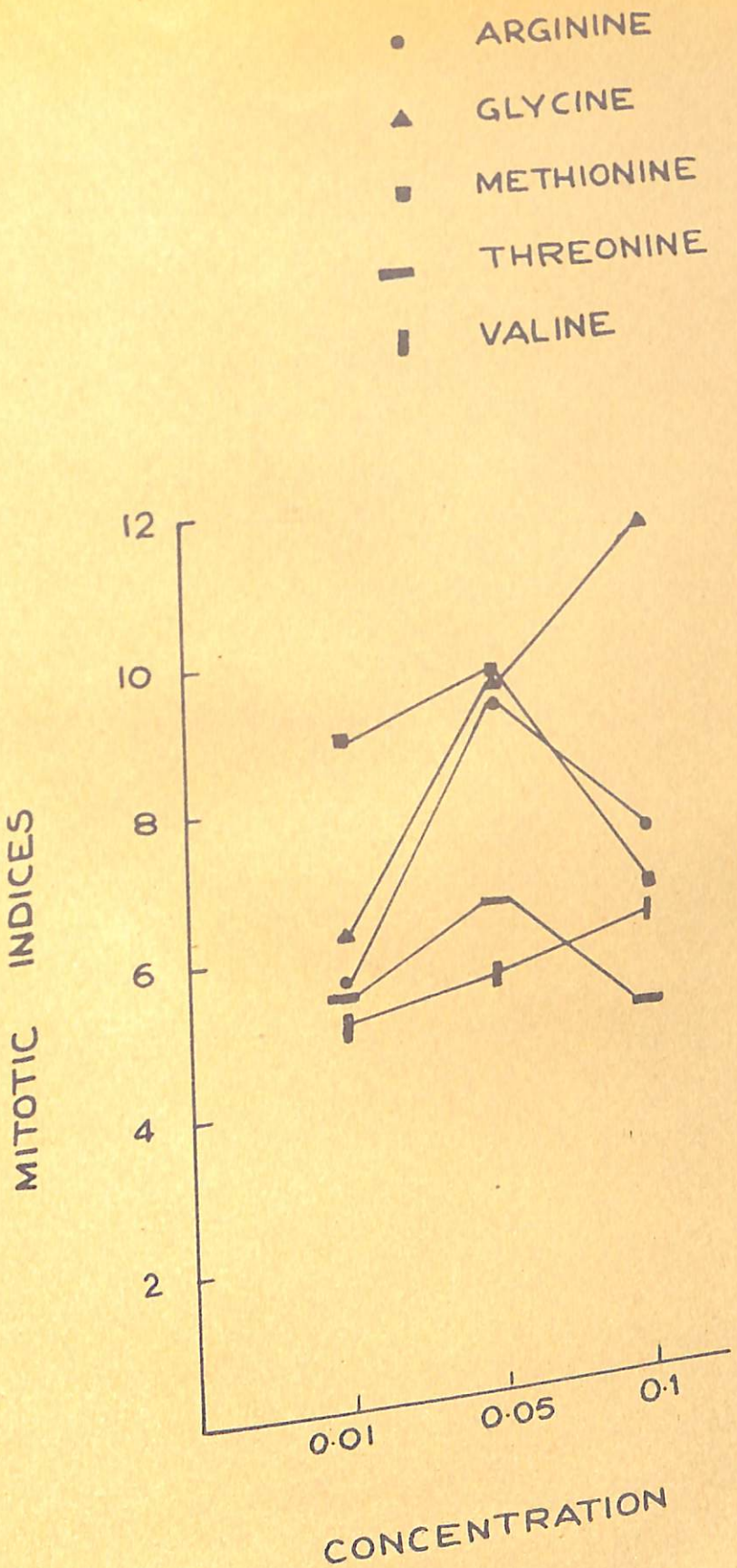



FIG. IV·2 INFLUENCE OF AMINOACIDS ON MITOTIC INDICES - CONCENTRATION

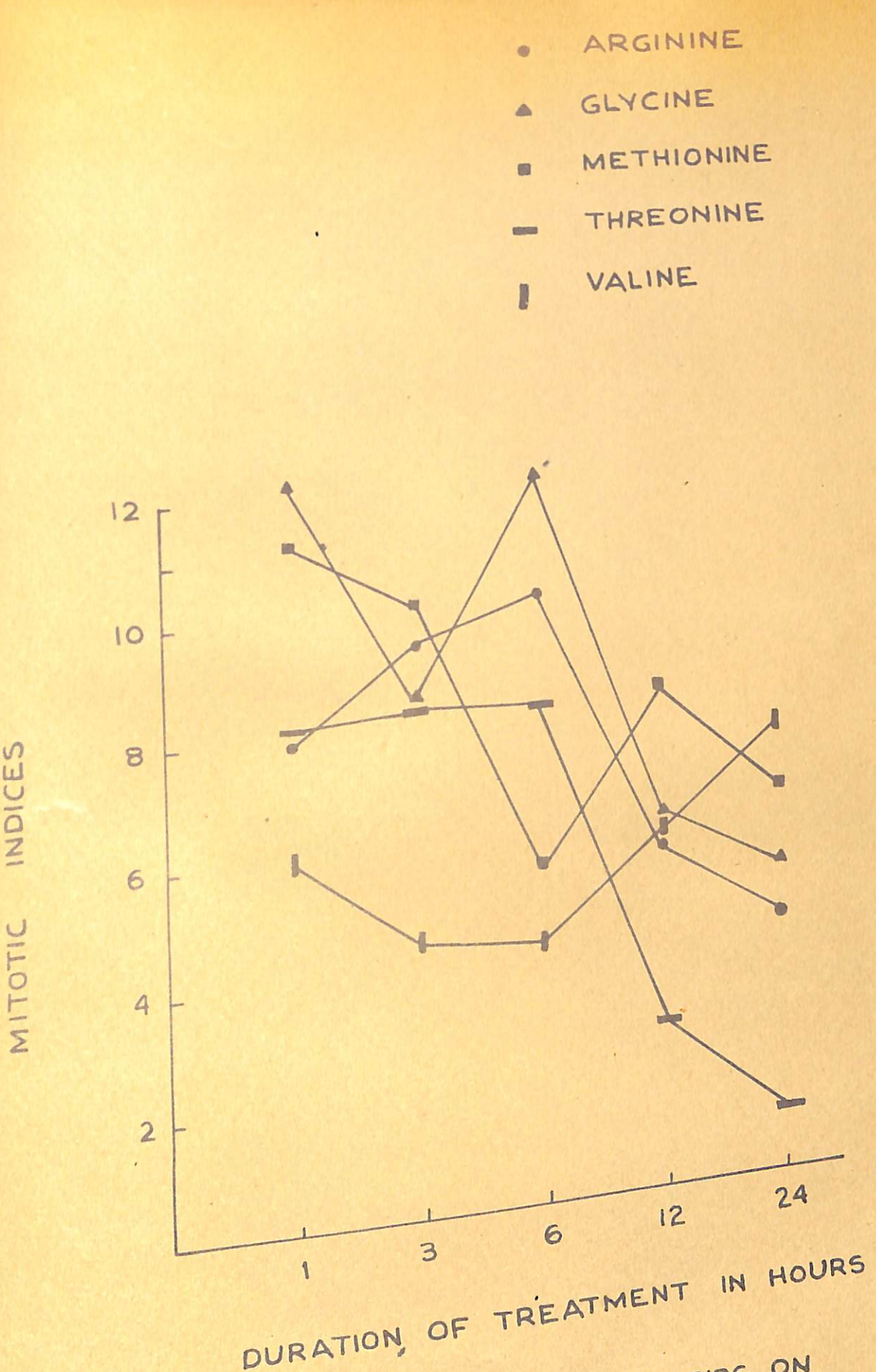



FIG. IV. 3 INFLUENCE OF AMINOACIDS ON MITOTIC INDICES — DURATION OF TREATMENT.

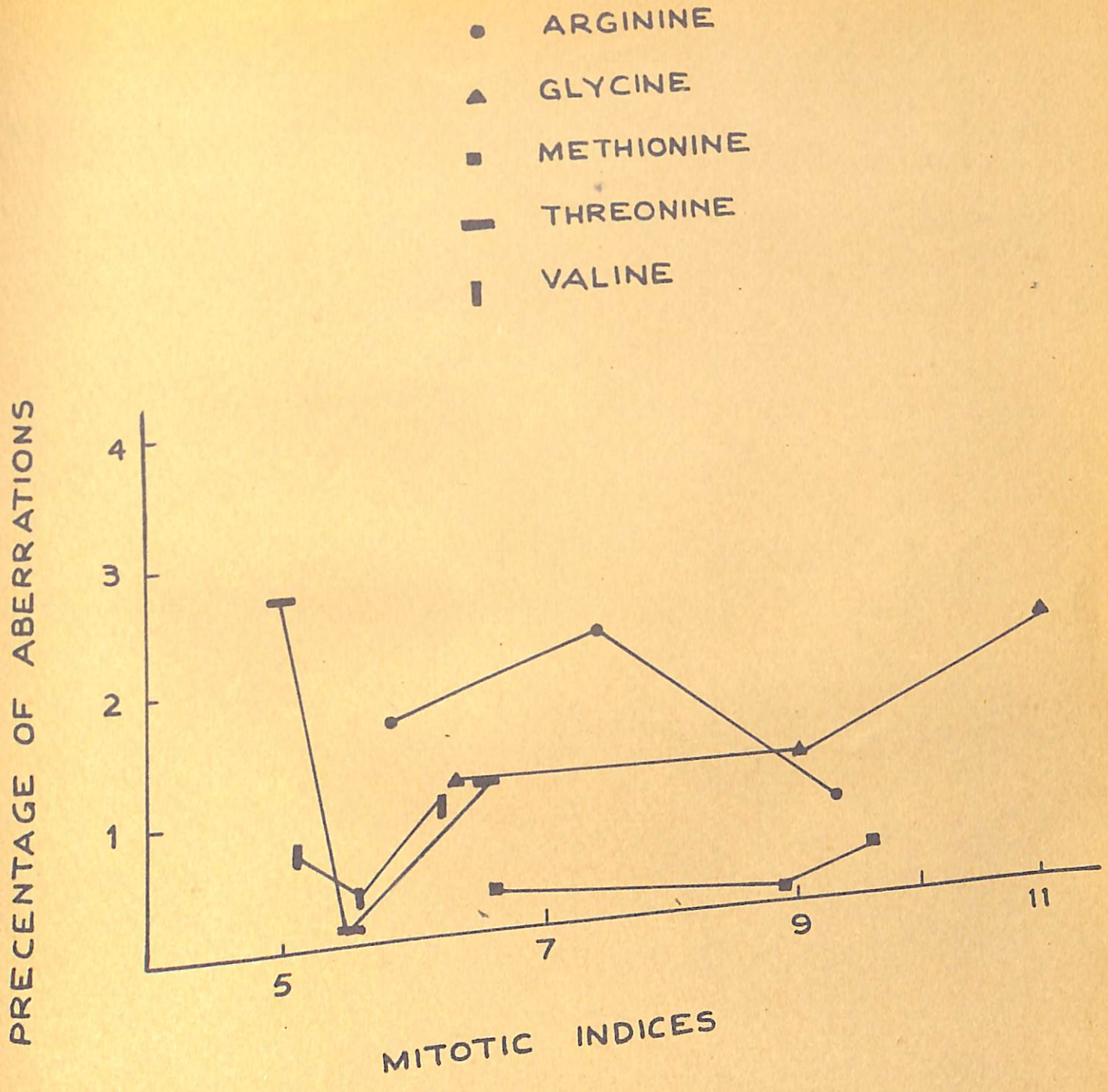



FIG. IV·4 INFLUENCE OF MITOTIC INDICES ON ABERRATION FREQUENCIES IN AMINOACIDS

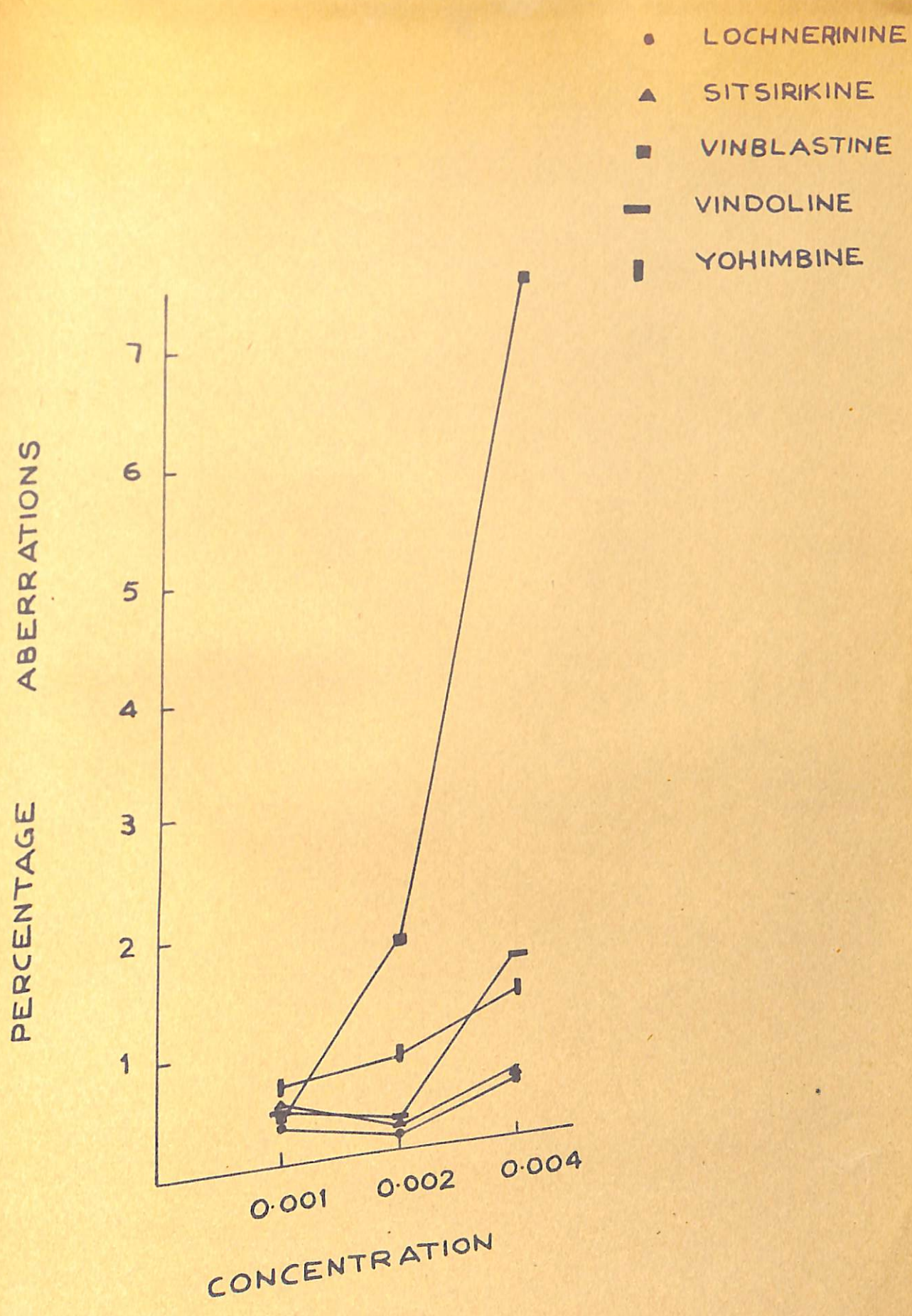



FIG. IV.5 INFLUENCE OF ALKALOIDS ON ABERRATION FREQUENCIES.

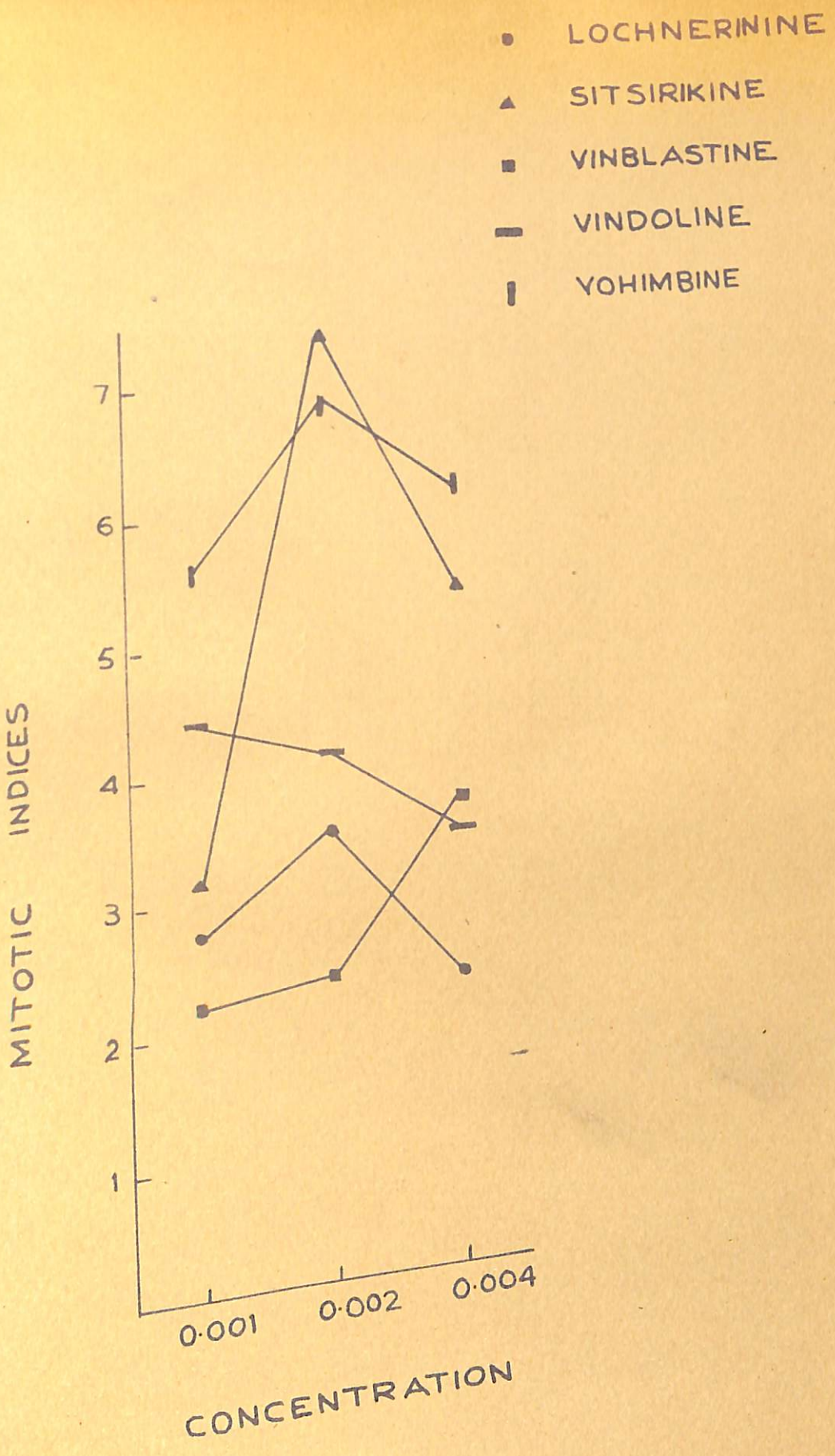



FIG. IV·6 INFLUENCE OF ALKALOIDS ON MITOTIC INDICES – CONCENTRATION

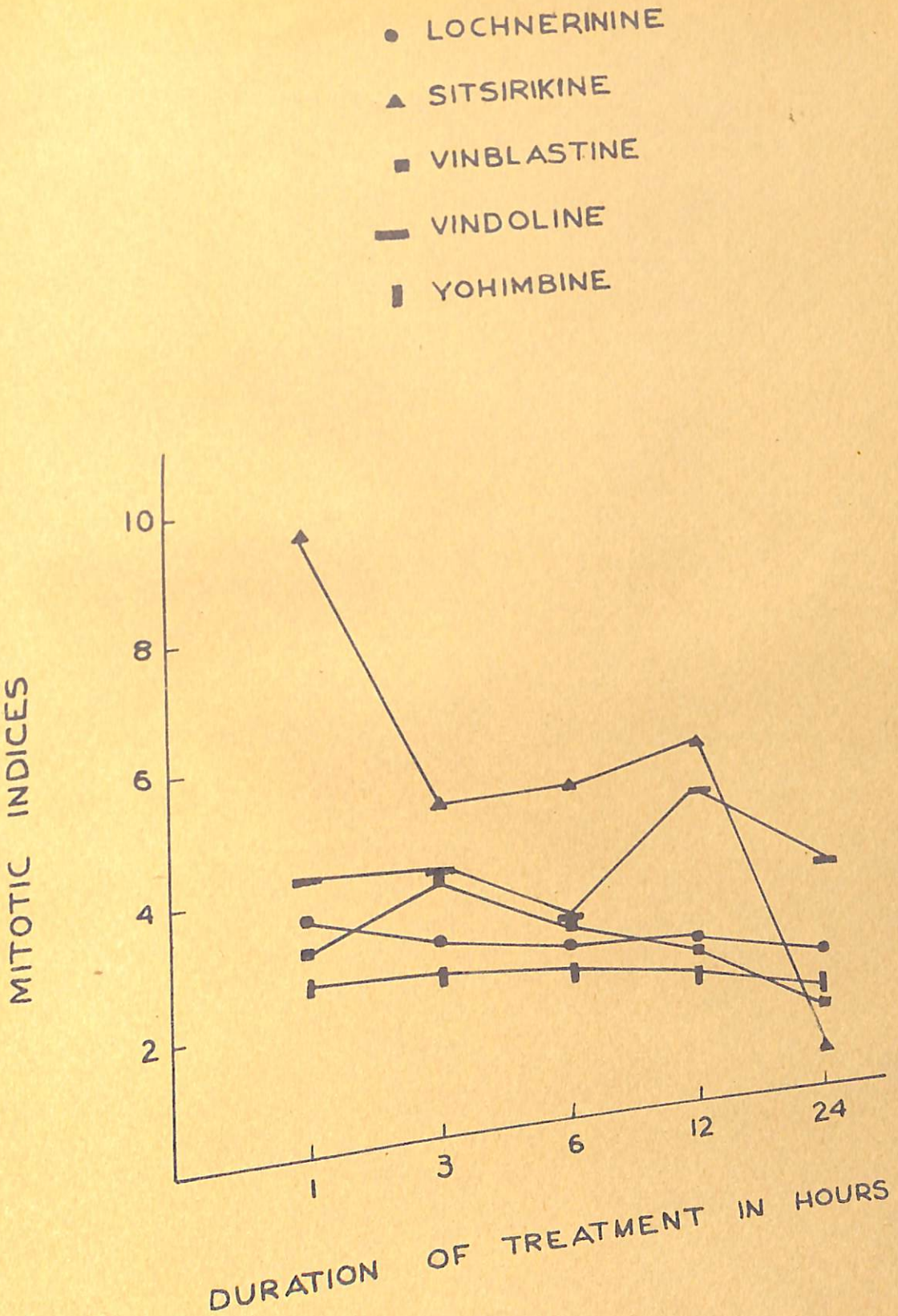



FIG. IV·7 INFLUENCE OF ALKALOIDS ON MITOTIC INDICES — DURATION OF TREATMENT.

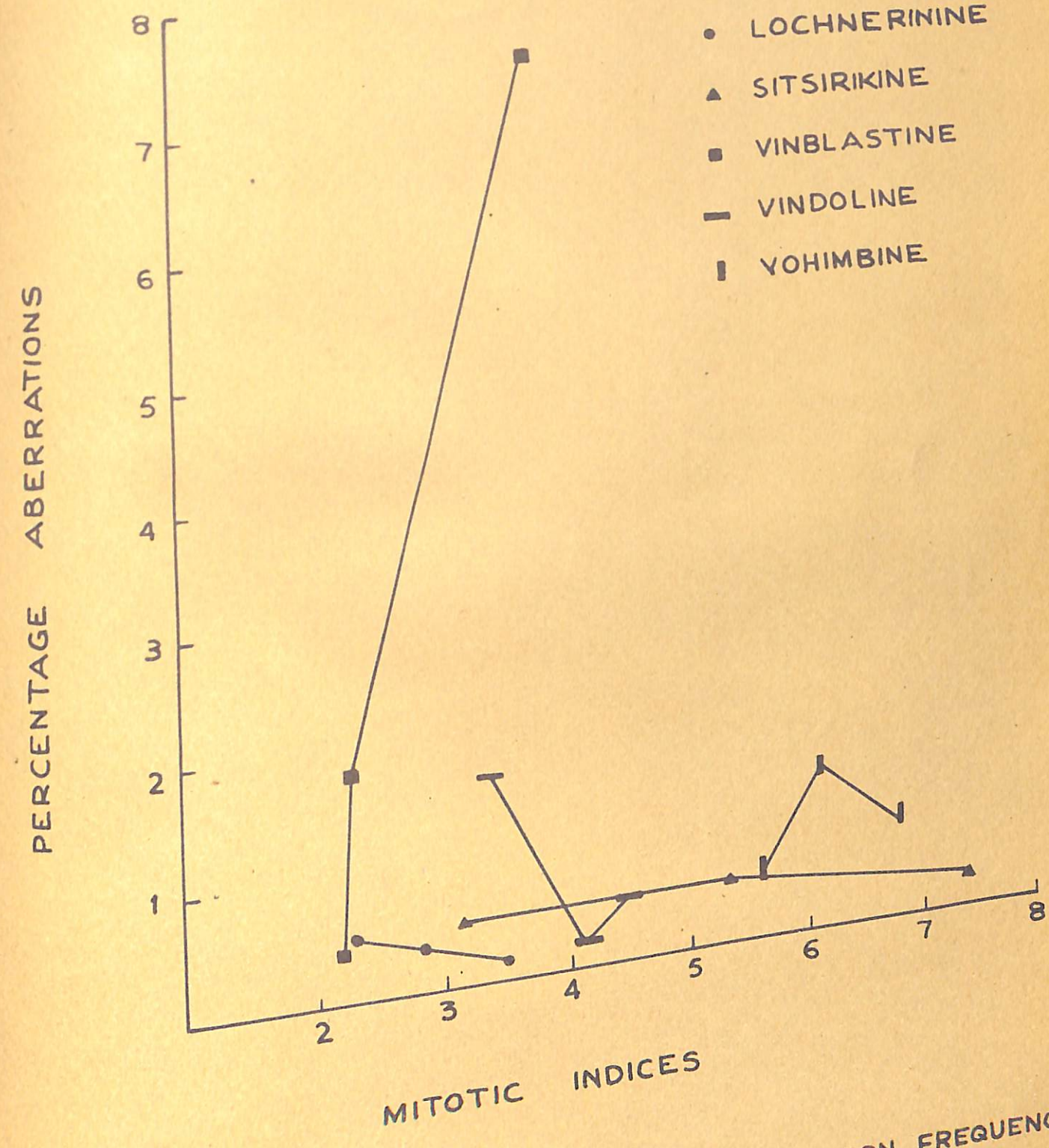



FIG. IV. 8 INFLUENCE OF MITOTIC INDICES ON ABERRATION FREQUENCIES IN ALKALOIDS

# DISCUSSION

## Contents

# I. DIURNAL MITOTIC PERIODICITY

## Cell number and mitotic indices:-

One of the most important aspects of the present study pertains to cell number and mitotic indices. In low temperature, the mitotic index is high but the cell number is low, indicating that the treatment initially induced cell growth and subsequently promoted cell division. The same is true under light where it is even more pronounced. Darkness appears to induce cell division initially which causes an increase in the number of cells. Later, the treatment initiates the growth phase, decreasing the mitotic indices (Table I.10). Brumfield (1942) reported that the process of cell division does not influence the rate of cell elongation in the roots of Phleum pratense. But Sinnot (1960) observed "most increase in cell size, however, comes after the final division", implying that some size increase of cells follows every division. The mutual impact of the two phenomena (cell division and cell elongation) which alternate with each other seems rather imperative, although they were shown to be independent of one another (D' Amato,1949). What is surprising, however, is how both the phases of growth activity can be the outcome of the same environmental conditions, unless the determinant is primarily endogenous.

Peaks, falls and preparatory periods:

The mitotic indices of the controls are almost diurnally uniform and are interrupted only by periods of minimal mitotic activity, which are termed preparatory periods (Sarma and Anand, 1966). But, the long preparatory periods in controls gradually disappear in treated roots. The two preparatory periods of 6 hrs in controls are reduced to a period of an hours duration under low temperature. In darkness, it is marked by a single occassion of negligible mitotic activity. Even this completely disappears in light (Figs I.2; I.4; I.6; I.8).

As the preparatory periods vanished, mitoses started showing peaks and falls in their activity. Such peaks have been earlier reported in the normally grown roots of Allium cepa (Friesner, 1920; Jensen and Kavaljian, 1958), Vicia faba, Zea mays (Karsten, 1915) and a host of other species (Friesner,1920). Such peaks and falls are very prominent in low temperature and to some extent in light. The indices run a wavy [besides course,] being [in low,] darkness. In all these treatments, two of the peaks occur at the same time (8.0 am and 2.0 pm). Three of the falls, similarly occur but only in low temperature and light (9 am, 12 am and 5 pm). Yet the dark grown roots exhibit an interesting phenomenon, the peaks and falls occur exactly at the same hours of the diurnal cycle (Figs. I.3,I.5,I.7).

The influence of darkness is marked not only by the absence of preparatory periods but also by the relative closeness

of phase indices (Table I.11) which might have led to the fall in mitotic indices and to their wavyness. The low mitotic indices may also be due to early differentiation, indicated by elongated cells and relatively higher anaphase indices such as prevailing in this material has been shown to be a symptom of cell differentiation (Melander,1963) in some animal tissues.

In case of low temperature and light the roots apparently respond similarly to a great extent. But similarity of appearance may come through different channels. The high mitotic indices in light and low temperature with peaks and falls must be viewed from the fact that the plant is a desert inhabitant where solar radiation is more, and that it grows vegetatively well only in winter season.

Mitotic cycles:

The phenomena of mitotic cycles occurring in all the treatment, appear totally unconcerned with the peaks and falls. Thus, in controls, where no such trend is known, there are five distinct cycles. In low temperature, where there are six peaks, also there are five cycles. In darkness also, there are five cycles, when the peaks are only four. But in light there are only three cycles while the peaks are seven. Thus it is obvious that cyclic mitotic events continue irrespective of the frequency of cells taking part in them.

The overall cyclic pattern has to be ascribed to the preparatory periods which appear to serve the purpose of nuclear

growth, which by itself is phasic (Taylor,1961). While a shift of this growth by different treatments appear to cause peaks and falls, the cyclic behaviour of mitoses continues indicating that its control is essentially endogenous. Thus the main impact of treatments is indirect - through the gradual loss of preparatory periods, followed by increased mitotic duration and as under light, a decrease in the number of cycles (Figs. I.4, I.6, I.8).

Mitotic durations:

Time taken for the beginning (high index) and end (low index) of prophases is regarded as mitotic cycle duration. It must, however, be remembered that prophases maintain an inverse relationship with telophases at both beginning and end of a mitotic cycle. In controls, the average duration of mitotic cycle has been calculated to be 3.6 hrs in low temperature; 4.6 hrs in darkness 4.8 hrs and in light 8 hrs. Thus mitotic duration correspondingly increases with the gradual disappearance of preparatory periods (Table I.11).

Although the cycles are overlapping, the duration of each phase has been approximately calculated by index method, evolved by the present author. It is based on the assumption that the duration of a phase is directly proportional to the relative number of cells in that phase, (the phase index) - longer the duration, more the cells in that stage, and so higher the indices, and so on. However, it must be admitted

that this can only be tentative approximation and the exact durations are likely to be lesser than those determined by this method.

On the whole, prophase takes maximum time and anaphase minimum. In low temperature and darkness the relative phase durations are similar, although in the latter prophases are accelerated and meta - and anaphases delayed. In light, prophase duration is increased and that of anaphase decreased to a considerable extent (Table I.12).

In dark, the mitotic indices are low and in low temperature and light they are high. But in light the duration also is more. All these indicate that in darkness the main channel of growth is elongation while in the other two, it is cell multiplication. Further, in low temperature the mitotic rate is faster, while in light more cells take part in division. That is how growth is accomplished.

Phasic behaviour:

The normal inverse relationship of prophases and telophases is quite often altered. In low temperature, during the second cycle, prophases suddenly fall instead of rising. It is called "wallow" and specifically termed "Prophase depress wallow" as it concerns the depression in the prophases. Similarly, other wallows like prophase spurt wallow, telophase spurt wallow and telophase depress wallow also occur under other treatments. All these contribute to the lengthening of mitotic

duration, but the causatives governing them are obscure. Number of wallows increase in darkness, reaching maximum in light, corresponding to the gradual decline of preparatory periods (Figs. I.4, I.6 and I.8).

The second type of aberrant phasic behaviour is the overlap i.e., the prophases and telophases either run parallelly (parallel overlap) or one obliterates the other (Obliterate). No overlaps are observed in controls and light. In low temperature they are parallel during 3 to 4 pm and 8 to 9 pm. when the telophases suddenly increase along with the prophases i.e., parallel ascending. But during 1 to 2 pm telophases come down precociously i.e., parallel descending (Fig. I.4). These are not present in any other treatment. The ascending overlap is supposed to be due to prolongation of telophases, while the descending one is due to their accelrated completion. The former increases the mitotic duration while the latter reduce it.

In the dark an overlap occurs during 11 am to 1 pm when telophases numerically dominate the prophases. So this is called obliterate overlap (Fig I.6). This also results in the prolongation of mitotic cycle by detaining the cells in telophases. In the light of the low mitotic indices in this material, it is likely that telophase index of the tissue exercises control over initiating newer cells into mitosis.

General considerations : Rhythmicity and synchrony

It has been stated earlier that mitotic cycles are

independent of peaks and falls and that preparatory periods serve the purpose of nuclear growth, which is phasic. The progressive disappearance of preparatory periods under different environmental conditions caused fluctuations in peaks and falls, and mitotic cycles and their duration. In such an eventuality it has to be assumed that nuclear growth has shifted to the interdivision periods of each of the individual cells. As a matter of fact, the origin of cycles is traceable to the sequential chemical reactions of the interdivision period of cells. Thus all the mitotic rhythms are endogenous as was first stated by Friesner (1920) although a contrary opinion has been maintained by Laughlin (1919) and Erickson (1964).

Besides the sequentiality of nuclear growth, there may be other factors influencing rhythmicity. Zeuthen (1964) opines that preparation for division is along several independent channels which perhaps may meet at division. Further, it is tempting to suggest that a trigger mechanism associated with the finishing course of the cycle, exists.

Mitoses are randomly distributed in time because in their cyclic activities cells are independent units. By definition, any deviation from the fully asynchronous state indicates a degree of unbalanced growth and extrapolates towards a partial synchrony.

The sudden spurts and falls in prophases and telophases (wallows) and the absence of inverse relationship between them

(overlaps) testifying to the lengthening of mitosis and its partial synchronisation. This is further strengthened by the wavy mitotic nature under darkness and the longer mitotic duration and continuous domination of prophases under light (Figs. I.5 and I.7). This partial synchronisation is an effect produced by the changed environment, which acts as a prolonged shock over all the mitotic phases, accelerating some and retarding others. Thus partial synchronisation is due to the differential interference of environment with the rate of progress of mitotic phases. As it naturally acts on the interdivision period also, the partial synchronisation may also be attributed to the differential set back in time of cells in interphase. It is known that internal conditions can be influenced by environment, since these, if unfavourable, can prevent them happening altogether (Fogg,1963). Finally, we may recall Mazia's (1961) observation that "the time of the day when the divisions occur may be shifted by means of a period of exposure to low temperature or by artificial reversal of the diurnal cycle, of light and darkness," and conclude that some sort of temperature - light control mechanism may steer the whole division process towards the desired synchrony besides the colchicine tagging (Van't Hof, et al.,1960).

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## II. KARYOTYPE ANALYSIS

Mehra (1946) considered the haploid chromosome number of *Ephedra foliata* to be 7 while Mulay (1941) regarded it to be more than that. The present study confirms the former, while at the same time reports the occasional somatic chromosome numbers of 13,15 and 16.

Although Mehra (1946) credited aneuploidy with no importance it appears significant owing to its mode of origin and the consequences it may trigger. These different chromosome numbers may be due to nondisjunction of a particular chromosome and its postponed duplication resulting in monosomics and trisomics. Laggards and forwards have considerable role in these numerical variations, for, their elimination or restoration is bound to affect the somatic number. The extent of impact depends upon the number and nature of such aberrent chromosomes. The role of laggards and forwards, especially the latter is further suggested by the specific participation of the 5th, the submetacentric, and the 7th, the acrocentric (Table II.1 and Fig.II.2) chromosomes in lagging, precocity as well as aneuploidy.

Variations within 14 type appear to be mainly due to fragmentation and elimination or repatterning (Khoshoo,1962). Natural aberrations, like micronuclei and bridges strongly suggest

the occurrence of elimination while operation of repatterning is inferred from the relative constancy of total chromatin (Fig.II.1) and variations in the number of metacentric, submetacentric and acrocentric chromosomes (Table II.2).

Among the various kinds within 14 type, 14a, 14b, and 14d appear to have been derived from 14c type, through deletions in the segments of the submetacantrics. This is based on the karyotype formulae of 14a, b and d types, which appear deducible from 14c formula. (Table II.4). This contention is strengthened by the higher average total length and arm ratio of the 14c type when compared to the others. In the rest types the total length has gradually decreased and so also the arm ratios (Table II.2).

In view of higher prevalence 14a type is considered to be the typical karyotype of the species, which at haploid level consists of 4 metacentrics, 1 submetacentric and 2 acrocentrics (Fig.II.3). This is broadly in conformity with Mehra (1946) and Hunziker (1955). According to them the basic karyotype of the genus consists of 5 long metacentrics and 2 short acrocentrics. The karyotype fixed for Ephedra foliata in the present study is as follows:

Chromosome 1 : Metacentric; Total length : 18.75 microns (9.8+8.95); Arm ratio : 1.1

Chromosome 2 : Metacentric; Total length : 17.1 microns (9.05+8.05); Arm ratio : 1.12

Chromosome 3 : Metacentric; Total length : 15.9 microns (8.45+7.45); Arm ratio : 1.15

Chromosome 4 : Metacentric; Total length : 14.49 microns (7.66+6.83); Arm ratio : 1.13

Chromosome 5 : Submetacentric; Total length :14.5 microns (8.51+5.99);Arm ratio :1.42 occasionally observed to bear a satellite on the short arm. May be deficient in some cells resulting in a somatic number of 13. May be extra in other contributing to the somatic number,16.

Chromosome 6 : Acrocentric; Total length : 13.6 microns (9.9+3.7);Arm ratio : 2.79.

Chromosome 7 : Acrocentric; Total length : 10.45 microns. (7.75+2.7). Arm ratio : 3.07. May be deficient in some cells resulting in a somatic number of 15, and contributing to the 16 type.

Mehra 1946 reported secondary constrictions in E. foliata which are not observed in the present work. Satellites also are not observed except very occasionally on the short arm of the chromosome 5. The significance of the elusive nature of satellites and secondary constrictions is not known. Probably they may be somehow connected with nucleolar variations spontaneously observed in the present material, for, satellites and secondary constrictions are generally associated with the organisation of nucleoli. However, it may be noted that some species of Ephedra have as many as 12 chromosomes with either satellite or secondary constrictions (khoshoo,1962) but barely two of them being nucleolar organisers. So it is quite reasonable to assume that nucleolar organiser can be with no apparent morphological distinction. And this may exactly be the case in E. foliata.

Comparison with other species of Ephedra show that 14a type of E.foliata is very similar to the karyotypes of E.chreata, E.breana and E.frustillata; 14c type to E.triandra and 14 d type to E.rupestris and E.americana. But the similarity

pertains to only to the position of centromere and arm ratios (Tables II.3,4 ). All the other species have either satellites or secondary constrictions. In E. foliata the latter are absent and the former inconsistent.

Similarity of karyotypes, in this genus, also found among the larches (Simak,1964) indicates that karyotype alterations are cryptic. Hunziker (1955) states. "The uniformity of the karyotypes in most Ephedra species suggests that perhaps in most cases the barriers of isolation between populations might not have been due to gross structural chromosome rearrangements, but to small or cryptic structural rearrangements or other mechanisms of isolation.

As far as the present species is concerned, karyotype alterations concerning both number and morphology and the average chromosome length (Khooshoo,1962) may be signs of specialisation and pointers to impending newer possibilities.

## III. NATURAL ABERRATIONS

### Micronuclei:

Khoshoo (1957), and Gopinath and Subramaniam (1963) explained the formation of micronuclei as due to laggards. Abraham and Smith (1966) considered them as due to chromosome fragmentation also. Nelson - Rees, et al (1966) found a proportionate increase of micronuclei with bridges implying thereby the role of fragments. While the present study broadly agrees with their explanations, new possibilities are suggested.

Firstly, the precociously moving chromosomes might coalesce at the pole to form an accessory nucleus (Photo 20). Secondly, they may be due to budding or erosion of the main nucleus, the reasons for which are obscure (Photo III.1E). Variations in the number and size of micronuclei depend upon the distances between the abnormally behaving chromosomes (or chromosome groupings) and their number, or to the amount of chromatin pieced out of the main nucleus.

When the micronuclei are present in late telophase, it is possible to speculate on their origin. If they are in between the two groups, they are due to laggards, and if above one of the groups, due to forwards. If they are present on both sides of a nonkinetic nucleus they are obviously due to both laggards as well as forwards (Photo III.1B and 1C).

Concerning their fate, the micronuclei may be restored to the main nucleus or eliminated altogether. Elimination would naturally trigger a chain of aberrations, the most primary being karyotype alterations as indicated in the preceding chapter.

In the present material, the frequency as well as number of micronuclei are more in the body cells which take part in histogenesis. Further, there is an increase in the individual sizes of micronuclei corresponding to their number. The micronuclear index which indicates the relative participating of chromatin is more in body cells and directly proportional to the micronuclear number (Table III.17).

A good percentage of micronuclei present in the columella cells may lead to the belief that the aberration is not of much consequence as they do not take part on cell lineages of histogenetic significance. But higher percentages are encountered in body cells and both restoration as well as persistence of micronuclei have also been observed. So it appears that restoration as well as elimination of chromatin curiously go hand in hand in this species.

Bridges:

There is not much to add to our knowledge of bridges, which spontaneously occur in many plants and are also produced by almost all chemical treatments.

In the present material they are mostly one or two and are of chromatid type (Photo III.2A-2D). Their persistence

till telophase and higher frequency in histologically important body cells (Table III.7) render bridges an aberration of some consequence.

Forwards and laggards:

All chromosomes of a complement mostly behave synchronously at all stages of nuclear division including anaphase segregation of the daughter chromosomes. Occasionally, however some chromosomes behave unusually. These include laggards and forwards. The factors behind their unusual behaviour and their impact are important besides being interesting. It is believed that the same forces are involved in both precocity as well as lagging; for, both are deviations from the normal. Further, what will be a forward or laggard, would be determined by the chromosome itself.....its DNA.

It is suggested that synthesis of DNA and hence the chromosome duplication occur sooner in one or two chromosomes than in the rest. To this effect, there is some evidence in literature (Taylor 1960). So they are precociously ready for poleward movement and do so. This obviously means that both the daughter chromosomes will be at different poles, but only if the centromere has divided. While this is imperative, a still more interesting case has been observed where .... diplo-chromosomes (with undivided centromere) or both the daughter chromosomes of the same homologue are present at the same pole(Photo IV.9A). The occurrence of intact diplochromosomes with undivided centromeres gives credence to the theory of autonomous chromosome

movement. While the role of spindle cannot be underrated, it is apparent that chromosomes may be moved poleward even without the centromere division which is generally thought to be an essential prerequisite for anaphase movements involving the spindle mechanism. As to the precocious synthesis of D N A reported by Taylor (1960), Prescott (1964) explained that "Chromosomes may enter G2 at different time points, some entering G2 while others are still completing S". It is pertinent to recall, in this connection, D'Amato's (1964) statement that "the rigid time relations between D N A synthesis and mitosis which insure chromosome number constancy in meristems (and initial cells generally), is lost at the end of active division in a cell lineage. Although we can try to explain the loss of time relation between D N A synthesis and mitosis as a nonadaptive concomitant of the cessation of cell division with differentiation, as Mather (1964) has suggested, its basic mechanism remains obscure. Perhaps, some light will come from a better knowledge of the time relations in mitosis and of the factors capable of disturbing or upsetting them."

In the same vein, the laggards also can be explained as due to delayed synthesis of D N A. By the time duplication of these aberrent chromosomes is over, the rest of the complement will have reached the pole and the spindle will have faded away. Consequently the chromosome (or chromosomes, if duplicated) has to depend on its autopropulsion if it has to be restored to the main nucleus. The distance between the equatorial region and pole

which depends on the size of the cell also, is a determinant in such a restoration.

What controls the differential D N A synthesis (precocious in some and delayed in others) in the same chromosome complement, is not known. However, concerning the D N A replication, which is equivalent to chromosome duplication, Prescott (1964) states. "The provision of adequate nucleotide pools, the presence of D N A polymerase(s) and the conversion of D N A from a nonpriming condition to a priming condition are all necessary for the initiation of D N A synthesis but are not sufficient. Some element possibly involving the feed back of each D N A molecule upon itself ------ perhaps through the intermediacy of R N A and protein ----- must be involved in the biological regulation of D N A replication."

It is very interesting to note that the frequency of forwards is almost twice to that of laggards, and that the aberrent chromosomes are mostly acrocentric. (Table III.7).

Concerning their fate, if they are not restored to the main nucleus, they restitute into micronuclei. However, these phenomena of lagging and precocity and their prevalence in histogenetically consequential body cells (Table III.7) indicate a certain imbalance in the genetic set up of the tissue, by disturbing the karyotype.

Nucleoli:

Nucleolus is one of the main organelles of the nucleus.

It is chemically different from the remainder in being rich in RNA and RN Protein. Various workers have confirmed the constancy of nucleolar number per nucleus. Some of them (Duncan and Ross 1950) suggested it to constitute an index of the ploidy level of the nucleus. This is based on the assumption that each genome carries one nucleolar chromosome.

In the present material nucleoli vary from 1-5, (Photo III.5A-5F) the most common being 2. Such variations are known in Zephyranthes (Tandon and Kapoor, 1962) Phleum (Lowary and Avers, 1965) and some grasses (Lewis and Rothwell, 1964). Several reasons had been advanced from time to time for explaining this numerical variation in various mitotic tissues. Firstly, this may bedue to variability in the number of nucleolar organisers per cell (aneuploidy is known in this species) or due to fusion and fragmentation (Lewis and Rothwell, 1964). In this vein it is interesting to speculate whether this tendency is in some way be connected with the persistence of nucleoli during certain phases of mitosis as was earlier observed by Brown and Amery (1957) Lewis and Rothwell (1964), and Gopinath and Subramaniam (1963) in some plants.

It is suggested that some of the numerical variations, may be due to the absence of partial or complete failure of nucleolar organisers. According to Sirlin (1960) all the chromosomes in a complement are capable of forming nucleolar material called prenucleolar bodies or nucleolar precursors, and the role of nucleolar organisers is merely their assembly

and organisation. In the light of all this, it is suggested that the nucleoli observed here actually correspond to prenucleolar bodies which may fuse and bring about changes in their number and size.

Although the individual nucleolar sizes have decreased with increased number, interestingly the total sizes showed the exact opposite of it in both columella as well as body cells, especially in the latter. Further, in the body cells, when the cell sizes are maximum, the N-n as well as C-n ratios are very low unlike in columella cells (Table III. 5 and III.6). This probably may have some connection with differentiation as nucleoli contribute to cytoplasmic protein synthesis. It appears that in the smaller body cells the nucleoli and their total size are more as an initial response to the enlargement phase of differentiation (Lowary and Avers 1965). The sharp decrease in nucleoli and C-n ratios in large body cells is hence due to their generally lower metabolism towards the tissue differentiation. In view of the intimate relationship, which exists between the nucleolus and R N A metabolism (Perry, Hell and Errera, 1961) it would seem that these nucleolar variations correspond to metabolic differences that would normally exist between cells of different maturation potentials. Thus, it seems reasonable to assume that nucleolar variations may be used as an additional index of synthetic activities in relation to cellular differentiation. And cellular differentiation is a function of individual cells as part of a whole; it is what it is because it is where it is (Street and Henschaw, 1963).

## IV. INDUCED ABERRATIONS

Since the discovery that chemicals can induce mitotic aberrations (Kostoff,1938), a large number of them have been tried for their effects on mitosis and cell division. In so doing, the search has always been for a specific substance with limited and desired effect and for obtaining information about the structure and chemistry of nuclear material. The practical implications of the chemically induced aberrations, in different types of cells, may quite often be farreaching.

### CLASSIFICATION:

For an adequate understanding, it is necessary that these aberrations are classified appropriately. Mehra (1961) categorised all aberrations into (i) Primary ( i.e., occurring immediately after treatment) and (ii) Secondary (i.e.,occurring later during mitosis). The former were also referred to as physiological and the latter, as structural. The nature of origin only has been considered in this classification. But Kihlman (1950-66) classified them into (i) Chemical and (ii) Biochemical, depending upon their mode of origin, and into (i) Delayed and (ii) Nondelayed depending upon the time of occurrence. From a cytological point of view, both these classifications are not satisfactory unless the type of aberration is initially considered. Thus, Rao (1961) classified

aberrations into (i) Cytotoxic, (ii) Nucleotoxic and (iii) Spindle derangements. It appears that no visible aberrations occur in nondividing nuclei and that they all are toxic. But neither is completely true and hence a new treatment of aberrations is proposed which is as follows:

1. Karyokinetic
   a. Chromosomal
   b. Spindle
2. Cytokinetic
3. Nonkinetic

This is only a working scheme based on cytological criteria, mainly the type and appearance of aberrations. The nature of origin is considered only for the Nonkinetic type, where the aberrations may be physical (i.e.,altering the appearance) or chemical (i.e.,altering the chemical nature). The chemical aberrations may be immediate in appearance or delayed uptil the following karyokinesis.

1. KARYOKINETIC:

a. Chromosomal:

Throughout karyokinesis as the chromosomes are exposed to a different chemical environment many aberrations are produced depending upon the chemical and the cell responses.

Somatic reduction of chromosomes in prophase has been induced by various agents. Among the more important ones are sodium nucleate (Allen *et al* 1950), Phosphates (Galinsky,1949),

and Penicillin and other antibiotics (Levan and Tjio, 1951; and Wilson 1950). In metaphase, the diplochromosomes may be arranged in two more or less equivalent groups. Such distributiv metaphase have been observed by the action of colchicine and other substances like Sulfanilamide (Mehra,1949) and antibiotics like actidione and streptothricin (Wilson,1950). Huskins (1948) and Kodani (1948) first noticed this phenomenon induced by the action of Sodium ribose nucleate on Allium root tips and interpreted it as "reductional mitosis." Often, the distribution of the chromosomes may be highly uneven, or there may be formed more than two groups with irregular number of chromosomes. Recently, similar reductional mitoses had been induced in Sorghum with far reaching consequences by Chen and Ross (1963) and Simantel and Ross (1964).

Chromosome clumping has been induced by higher concentrations of mercurials by MaCfarlane (1954). Molten metaphases have been caused by DDT, and Phenethyl alcohol by Vaarama (1947), and Bammi and Jura (1966) respectively. Chromosome diminution was observed by Vig (1965) in the root tips of Aloe vera under natural conditions, while it has been caused by higher concentrations of several chemicals (Levan,1949, Levan and Tjio,1951). Laggards and bridges of various types have been extensively reported along with many other aberrations. (Kaufman and Das, 1954; Sharma and Chaudhari,1959). They are known to occur spontaneously also in several organisms, including the present plant of investigation.

The most important of all aberrations are the chromosome breaks, which are often referred to as radiomimetic because they mimic radiation effects by being random and non-specific. The first chemical that has been found to mimic radiation-produced breakage, was urethane employed on the root apices of Vicia faba (Oehlkers, 1943). Ever since, a number of others like sulfur mustard (Darlington & Koller, 1947), nitrogen mustard (Bhaduri and Natarjan, 1949, 1949; Ford 1949; Revell 1953, phenols (Levan and Tjio, 1948), pyrogallols (Tjio, 1951) maleic hydrazide (Darlington and McLeish 1951; McLeish, 1953) acridines (D' Amato 1954b, D' Amato and Avanzi, 1954) Coumarin (Sarma, 1968), vegetable oils (Swaminathan and Natarajan, 1956) are known to have similar effects. But most of our recent knowledge is due to Kihlman (1950-1966) who employed a wide variety of chemicals like nitrose compounds, alkylating agents purine derivatives, antibiotics etc. in order to induce breakages.

Depending upon the concentration, and duration of treatment many chemicals are known to have more than one effects which are collectively known as "zonation effects" or "multiple effects". Of the numerous references on this aspect, only a few more important ones are recalled. Sharma and Chaudhari (1959) brought forth varying effects in Lens esculentum by employing Chloral hydrate, caffeine, acenaphthene and gammexane. Venkateswarlu and Srinivasan (1961) and Rao (1961) produced multiple effects in Allium cepa and poecitocera picta (grass hopper) by alkaloids and Urethane respectively. D' Amato (1954b), and Tanaka and Sato (1952) used acridines and streptomycin for

producing similar effects. An interesting work is that of Matsuura and Iwabuchi (1962a, 1962b) who induced a wide range of aberrations by employing several inorganic salts.

Relative impact of aminoacids and alkaloids:

Among the aminoacids arginine is found to be the most potential inducer of chromosome aberrations, while threonine has no effect at all. Forwards are the most widespread and laggards the least, even lesser than in controls. An interesting observation is that methionine produces only chromosome diminution and forwards. Secondly, chromosome dots and laggards are specifically produced byglycine and arginine respectively (Tables IV.11,12).

Among the alkaloids, maximum aberrations are produced by vinblastine, and minimum by lochnerinine. Bridges occur most frequently and molten metaphases, least. Lochnerinine specifically produces only bridges, while sitsirikine produces breakages also. Chromosome dots and laggards are totally absent in alkaloid treatments. Molten metaphases and chromosome diminution are specifically produced by vinblastine and yohimbine respectively (Tables IV.15,16). With glycine and yaline a direct relationship exists between the aberration frequency and concentration, while arginine exhibits no such ralationship. Among the alkaloids, the frequency of aberrations is strictly proportional to concentration only in vinblastine (Figs.IV.1,5).

Mechanism of aberrations:

The manner in which the chromosomal aberrations occur have been understood to quite an appreciable extent, although doubts persist. The aberrations originate mainly from discrepancies among themselves which may be physical, chemical or biochemical involving enzyme-controlled biosynthetic activities.

Chromosome diminution is clearly due to excessive coiling of chromonemata. Chromosome dots probably could be telomeric fragments, as they are observed to be mostly heteropyknotic. Breakages, whether in prophase, metaphase or anaphase, whether of chromosomal or chromatid type, are very consequential. Some of the breaks may result in the formation of bridges if appropriate reunion takes place, the extent of bridges being dependent on the degree of breakage and reunion (Photo IV.6A). However, when the bridges are numerous, they all may not be of the same type. Some of them are sticky resulting probably from incomplete protein synthesis and/or incomplete separation telomere at the region of (Photo IV.6c,6d).

Similar imbalances pertaining to DNA and protein appear to be responsible for the molten appearance of metaphases as well as clumping of chromosomes. However, Bammi and Jura (1966) consider them as mere chromation 'erosions' due to interference with the coiling mechanism. Somatic reduction in Allium cepa (Royan-Subramaniam,1963), Sorghum Chen and Ross, 1963; Simantel and Ross,1964) and the present observations on E.foliata clearly indicate the role of more than one causative factors. Thus,

chromosome duplication, centromeric division and spindle mechanism, together or separately may be the influencing factors. However, according to Simantel and Ross(1964) somatic reduction consists of either a series of divisions involving non-disjunction and selection or genome segregation.

Forwards and laggards are supposed to occur due to their different velocities of movement which was regarded to be a function of their position at the equatorial region. Those farthest from the central interpolar axis move 25% faster than the central chromosomes (Nicklas,1965). Probably this explains the phenomenon of lagging and precocity. While the orientation at the periphery or the centre, of a particular chromosome may be a matter of chance, it is significant that most of the laggards and forwards observed in the present work are acrocentric. Thus it appears that size also is a contributing factor. Submetacentrics also had been observed with similar behaviour, occassionally. Thus, while somatic reduction, forwards and laggards give credence to the concept of autonomous movement of chromosomes, in the light of Rickards' (1965) theory of coorientation, centromere and spindle also appear to have considerable influence on these aberrations.

b. Spindle:

Disruption of spindle material is likely to effect the grouping of chromosomes and may lead to many attending consequences. Kaufman and Das (1954 & 1955) disturbed the spindle mechanism by means of ribonuclease incidentally proving the presence of RNA in the spindle. Barthelmess (1957) reported

induction of multipolar spindles by the action of athnol on Allium root tip cells resulting in the irregular chromosome segregation. Sharma and Chaudhari (1959) reported similar observations in the root tip cells of Lens esculentum treated with acenaphthene. Some chlorophycean pigments (Sharma & Datta, 1961) and Streptonigrin (Kihlman,1964) are some of the important chemicals causing spindle disruption. Rao (1961) alos observed an array of spindle derangements in the grasshopper,Poecilocera picta, treated with urethane.

The most important spindle aberration is its complete inhibition. A very large number of organic and inorganic chemicals are now known to have this kind of effect on the spindle mechanism and can cause polyploidy. Ostergran (1944) enumerated 80 such substances. To mention a few, ether,chloral hydrate, carbon disulphide, naphthalene, chloroform (Steineggar and Levan, 1947) acenapthene (Kostoff 1938; Levan, 1940), hydroxyquinoline (Prakken and Swaminathan,1951), counarin and its derivatives (Cornman, 1947, D'Amato, 1954a, Sarma,1968), acridines (D' Amato and Avanzi, 1954), sulfanilamide (Mehra,1949 and Sarma,1969)*, gammexane (Nybom and Knutson,1947), potassium cyanide (Levan and Wangenheim,1952) etc. may be cited. Yet, the most well known and efficient spindle inhibitor is colchicine, an alkaloid from Colchicum autumnale and C. luteum. Podophyllin, a resinous mixture obtained from Podophyllum peltatum, and P. erodi has been found to be effective in much greater dilutions than colchicine. The activity of podophyllotoxin, its

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therauptically active constituent is even greater (Cornman and Cornman,1951).

It is a fact that the situation of chromosome groups and the orientation of the spindle are correlated. References concerning the progressive shifting of the spindle apparatus from longitudinal to transverse axis of the cell, through oblique and diagonal orientations appear to be lacking. The spindle orientations are recognised by the appearance of chromosome groups in metaphase, anaphase and telophase.

Relative impact of aminoacids and alkaloids:

Among the aminoacids,tried here, glycine is found to be the most effective and threonine, the least, the former causing only spindle shifting and the latter spindle disruption. It is significant to note that the sulphur-containing methionine, is the only aminoacid causing all types of spindle aberrations including its complete cessation. Among alkaloids, vinblastine is the most effective and sitsirikine, the least. Spindle shifting is more widespread. Lochnerinine and sitsirikine cause only spindle shifting and spindle inhibition, respectively while vinblastine and vindoline produce all the three spindle aberrations (Tables IV.11,15).

In all amino acids but methionine there is a direct relationship between concentration and spindle shifting and inverse relationship with spindle disruption. In all alkaloids, the spindle shifting either occurs only in higher concentrations or directly increases with concentration.

Mechanism of aberrations:

The spindle mechanism, is naturally subject to minor variations like convergence and elongation (Bajor 1965). Thus the orientation of the whole apparatus is conditioned by the physiological state of the cell, which if altered would naturally bring about its attendant results. The spindle shifting may beexplained by the co-orientation theory of Rickards (1965) who suggested that two principal factors govern the orientation of mitotic chromosomes. The first factor is that orientation is achieved by the presence of two independently acting centromeres linked to one another. The second factor stems from the capacity of the centromere to become influenced and oriented to one of the spindle poles. Although this scheme obviously credits the centromere with major role, it seems that the orientation of chromosome groups is enough proof for the coordinated angular shifting of the spindle apparatus. Probably the physical nature, concerning the bundling of protein fibers is altered.

Other spindle derangements, like spindle divergence, spindle furcation etc. commonly referred to as spindle disruption, are mainly due to the changes in the normal convergent and bipolar nature of the spindle.

Although a vast amount of literature on spindle inhibition especially by colchicine (Eigsti and Dustin, 1955) has accumulated, the exact mode of its action is little understood. Brachet (1957) gave an account of various researches on the chemical nature of colchicine action which holds good for explaining

the mechanism of spindle inhibition. Mazia and Dan (1952) showed the absence of fibrillar orientation of the spindle proteins when treated with inhibitors. Mazia (1955,1956,1956b) contends that the -SH groups, responsible for parallel fibrillar oreintation of spindle proteins are affected through oxidation.

2. CYTOKINETIC:

Cytokinesis occurs along the equatorial region of the cell. Shifting of it from that place is not known earlier. But reports of its complete inhibition exist. Simonet and Guinochet (1939) found that the halogenated derivatives of benzene and toluene have this kind of effect on the cells of Linum. A similar response was produced by adenine and methylated purines in Allium by Kihlman (1950). Gonzalez - Fernandez et al (1964) also inhibited the formation of phragmoplast by caffeine treatment. An already formed phragmoplast has been reported to be dissolved by 2- hydrozinotropone and 7 - bromo 2- hydrozinio-tropone by Wada (1952).

Relative impact of amino acids and alkaloids:

Aminoacids and alkaloids affect cytokinesis differently. The shifting of phragmoplast from its equatorial region, produced mainly by aminoacids, is being reported for the first time. Alkaloids are found to inhibit mainly the phragmoplast formation, of course in higher concentrations, and after longer duration of treatment. Glycine and vinblastine are the only chemicals to cause both shifting as well as inhibition of phragmoplast.

Methionine, Lochnerinine and Vindoline have no such effects. On the whole, aminoacids are more active. Threorine is specific for phragmoplast shifting. In general, there appears to be a direct relationship between the aberrations and the concentration of the alkaloids and an inverse relationship in case of amino-acids (Table IV.11,12,15,16).

Mechanism of aberrations:

The mechanism of cytokinetic aberrations is slightly understood. Concerning the nature of phragmoplast, it originates from golgi vescicles and serves as a precursor of the cell wall. Chemically, it consists of carpohydrates and urenoides which later polymerise (Frey-Wyssling et al,1964) to give rise to cell wall. The profiles that establish the phragmoplast are said to be derived from the nuclear envelope (Underbrink and Olah,1965). Further, existence of certain relationship between the spindle and phragmoplast has been stressed by Bajor (1965) and Olah (1965); the latter even observed a secondary phragmoplast usually associated with one of the groups of chromosomes. In the light of all these, the phragmoplast shifting may be explained as due to either dislocation of golgi vesicles from their normal position, or to disruption of polar equidistance of spindles and chromosome groups. In this connection, it may berecalled that elongation of spindle, and disrupting its polar equidistance has already been observed by Bajor (1965). Mehra (1961) states "no sharp line of distinction can be drawn between chemicals that are responsible for spindle callapse and those which cause

cytokinetic inhibition, for, at different concentrations the same substance may be found to show both the effects." Thus it is clear that a close relationship exists between the spindle and the phragmoplast. Complete inhibition of phragmoplast has been considered to be due to destruction of cell plate by Wada (1952). But it is more probable that the phragmoplast precursors produced by golgi vesicles are disturbed. On the otherhand as Belousova et al (1966) suggested that the oxidative phosphorylation in the mitochondria may be inhibited, thus affecting the ATP pool, - the major energy source of cellular syntheses.

3. NONKINETIC:

Some of the aberrations occur in the nonkinetic nucleus. Of these, micronucleus has been reported to occur spontaneously in Pisum sativum (Gopinath and Subramaniam, 1963) Cephalotaxus (Khoshoo, 1957) and also in the present material. Barthelmess (1957) and Jain (1963) induced them in Allium and Lolium respectively by athnol and high temperature. Nucleic acid starvation or prevention of its synthesis has also been obtained earlier (Kihlman 1962,1963).

Relative impact of aminoacids and alkaloids:

The frequency of aberrations is more in aminoacids than in alkaloids. Four types of aberrations are present in the former and only two in the latter, the only aberration common for both being micronuclei (Tables IV.11,15).

Among aminoacids, threonine is more potential and

methionine, the least. Nuclear atrophy is more widespread, caused mainly by threonine. Persistent nucleolus is less frequent, but produced by all aminoacids, except valine. Methionine and valine appear to specifically produce persistent nucleolus and micronuclei respectively (Table IV.11,12).

Among alkaloids, vinblostine is the most powerful and no aberrations are produced by sitsirikine and yohimbine. Nucleic acid starvation is more pronounced. Lochnerinine specifically produces micronuclei, while the only chemicals causing nucleic acid starvation are viniblastine and vindoline, especially the former. These had been employed earlier in animal tissues (Palmer et al 1960, Nuess et al 1964) where micronuclei were reported (Table IV.15,16).

In aminoacids, aberrations occur either in higher concentrations or increase with concentration, only in case of threonine. With arginine, glycine and valine, the trend is just the opposite, while methionine does not exhibit any sort of relationship at all. Persistent nucleolus is always produced by lower concentrations, or decrease with increase ~~with increase~~ in concentration. Among alkaloids, direct relationship of aberrations with concentration exists only in case of vinblastine. Nucleic acid starvation in vinblastine increases with concentration.

Mechanism of aberrations:

Nelson-Rees et al (1963) found a strange relationship between bridges and micronuclei implying that the latter may have originated from the chromosome fragments. However, micronuclei

are generally formed by the lagging (Khoshoo 1957; Gopinath and subramaniam 1963, Jain 1963) or precoceously moving chromosomes. They may also be due to budding of the main nucleus (Photo IV.15). On the other hand, the reasons behind the persistence of nucleoli during mitosis are obscure. It is likely that aminoacids so alter the chemical state of nucleolus that it does not dissolve into cytoplasm during mitokinetics. Nucleic acid starvation, as indicated by the differential staining of nucleus is obviously due to degradation of DNA (Kihlman, 1962,1963) or repression in the D.N.A synthesis (Richards et al 1963). Nuclear atrophy and nuclear ejection are merely physical effects caused by high concentrations of chemicals.

MITOTIC INDICES:

Cell multiplication, an aspect of growth, is indicated by the mitotic indices. In all aminoacids there is an increase of mitoses - more by glycine and less by threonine. In glycine and valine mitotic indices are directly proportional to concentration. In the rest of aminoacids, there appears to be an optimum limit because in lower as well as higher concentrations, indices are lesser than in the medium concentration(Table IV.14 Fig.IV.2)

With alkaloids, in general, the mitotic indices are low. But this decrease from control is less, unlike the increase in aminoacids which is quite large. Further, two of them sitsirikine and vindoline even exhibit a slight increase. With vinblastine the mitotic indices are directly proportional to concentration

where as in vindoline they are inversly proportional, the solitary case of this type.(Table IV.18 Fig.IV.6).

The duration of treatment, with some of the chemicals as expected, has a considerable effect on the mitotic indices. Among the aminoacids, with arginine and threonine mitotic indices increase initially with duration and later fall. However, with valine, mitotic indices fall with increase in duration for some time and then rise. Among alkaloids vinblastine behaves like arginine and threonine. Lochnerinine and yohimbine maintain an inverse relationship with duration (Fig.IV.3,7).

Mitotic indices and aberration frequency:

Mitotic indices appear to have a certain corelation with the extent of aberrations. For example with glycine aberrations proportionately increase with mitotic index and thus with concentration. In case of methionine, a significant relation exists i.e., mitotic indices are directly proportional to the frequency of aberrations without at the same time maintaining concurrent relationship with the concentration of the chemical. In case of arginine also a somewhat similar relation is found (Fig IV.4). Thus, while in aminoacids the higher aberration frequencies are due to increased population of mitotic cells, in alkaloids, they are due to increased duration of mitotic cycle.

Mode of action:

The exact mode of action of aminoacids can be explained only by the knowledge of their general biochemistry as earlier

literature on this aspect appears negligible. Aminoacids are primarily nutritive in nature, constituting the "Nitrogen pool" of the cell. Directly or indirectly, they take part in the metabolism of carbohydrates, fatty acids, nucleic acids, etc., because of their dynamic reactivity among themselves and with others. Of course their major role is in protein synthesis. At the same time, some amino acids like valine and methionine have certain roles in the metabolism of cyanogenic glucosides, mercaptons and ephedrine the alkaloid generally present in several species of Ephedra. Synthesis of cell wall materials like pectin and lignin are influenced by methionine. It appears that nearly all alkoloids are related structurally to aminoacids or aminoacid derivatives through methylation, decarboxylation etc.(Fruton and Simmonds,1958; White et al 1959; Davies et al 1964).

Thus aminoacids, while increase mitotic indices through increased synthesis of proteins, may also deviate into aberrent pathways and produce substances (alkaloids,mercaptans etc.) which are likely to be toxic to nuclear kinetics. Hence, their action may be direct or indirect.

Among the alkaloids, only in case of vinblastine aberrations proportionately increase with mitotic indices which in turn increase with concentration. But with Lochnerinine and sitsirikine, the aberration frequency increases with decrease in mitotic index (Fig.IV.8)

As already stated in aminoacids aberrations increase

with mitotic indices, which generally increase with concentration. Increase in mitotic indices indicates that (i) the rate of divi-sion is more i.e., the duration of cycle is less or (ii) induction of mitosis, i.e., neweer (differentiated) cells are initiated into division because of increased DNA Synthesis (Davidson, 1966). Vant Hoff and Sparrow (1963) have shown that aberration frequencies increase with mitotic cycle duration. So, the first explanation for the increased mitotid indices, is unlikely, as it assumes that the duration of the cycle is less. Thus the increase in mitotic indices is due to induction of mitosis which hence accelerates the aberration frequency by exposing more cells to treatments. Similar induction of mitosis by aminoacids has been reported by Waidyasekera (1955). Otherwise, only auxins and similar other substances are known to possess this effect (Levan,1939; Huskins and Steinetz,1948).

The lesser mitotic indices in alkaloid treatments indicate inhibition of cell division, probably due to inhibition of DNA synthesis, which is also caused by several other substances like parascorbic acid (Cornman, 1947), acridines (D'Amate 1954b), Protoanemonin (Erickson and Rosen 1949), coumarin (Sarma,1968) etc. But the aberration frequencies also are more, implying thereby an increased duration of mitotic cycle (Van't Hoff and Sparrow, 1963).

Concerning the action of alkaloids, only that of vinblastine has so far been studied. They might act on respiration affecting the cell energy production required for mitosis

(Hunter, 1963). Richards et al (1963) found that vinblastine depresses the specific activity of DNA, especially the incorporation of ademine and guanine nucleotides. This appears to be more probable in the light of the cytological observation of nucleic acid starvation (differential Fuelgen staining of nuclei) in the present material. Nuess et al (1964) on the otherhand suggest the possibility of some effect on polymerases. However, possible effects on proteins cannot be ruled out.

## SUMMARY

I. Diurnal mitotic periodicity:

Diurnal periodicity of mitotic indices, nature of mitotic cycles and the behaviour of the component phases within the cycles have been studied under natural(controls), low temperature, dark and light conditions. The mitotic indices are not fluctuating in controls, but exhibit peaks and falls under other conditions to varying degrees. Five cycles of mitotic activity are present within a span of 24 hours in controls, low temperatue as well as darkness, but with varying durations. Under continuous light, they are reduced to 3. The phasic behavious is complementary in controls but disturbed to a greater or leasser extent under the other conditions. They exhibit "Wallows" and "Overlaps". The significance of peaks, falls, preparatory periods, wallows and overlaps has been discussed in the context of cell multiplicatiom and synchronisation.

II. Karyotype analysis:

The mitotic karyotype of the plant has been studied taking the variations in the number and morphology of chromosomes into consideration. Satellites and secondary constrictions are usually absent. Aneuploid chromosome numbers occur. The

karyotype is compared with earlier reports in other species of Ephedra.

III. Natural aberrations:

Variations concerning nucleoli are recorded. Their relative frequencies in the columella and body cells of the root apices have been scored. The possible significance of these spontaneous aberrations has been discussed. Specific involvement of only some chromosomes in producing aberrations has been observed.

IV. Induced aberrations:

The material was treated in different concentrations of five amino acids, for different durations: Arginine, Glycine, Methionine, Threonine and Valine, and of five alkaloids, namely, Lochnerinine, Sitsirikine, Vinblastine, Vindoline and Yohimbine. Their effects on the mitotic indices have been recorded. Cytokinetic nonkinetic and karyokinetic aberrations produced concern both spindle as well as the chromatic material. In all, about 19 aberrations have been recorded. The relative frequencies of these aberrations in different variables of concentration and treatment have been scored and discussed. The possible nature of aberrations and the general mechanism of action of aminoacids and alkaloids are discussed. Various correlations have also been made.

# CONCLUSIONS

From the foregone study, the following conclusions are tentatively drawn:

1. Mitosis exhibits circadian rhythms, influenced by external factors. The influence is through accelerating or retarding the mitotic indices and the duration of karyokinesis.

2. Spontaneous aberrations and karyotype variations indicate that a sort of specialisation and microevolution is taking place through chromatin elimination and repatterning.

3. The 'nutritive' aminoacids, under higher concentrations can disturb mitosis, by producing 'toxic' substances through aberrent biosynthetic pathways.

4. The antineoplastic properties of Vinca alkaloids is probably due to induction of aberrations in the nuclear behaviour.

5. In addition, several aspects of cellular behaviour have been amplified:

   (a) autonomous chromosome movement in anaphase probably connected with loss of synchrony of DNA replication (forwards, a laggards and somatic reduction).

   (b) histologically significant impact on cell division of cytoplasmic factors connected with spindle and phragmoplast (Spindle and phragmoplast shifting).

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