EFFECT OF CERTAIN CHEMICALS ON THE CYTOLOGY OF THE PLANT CELLS

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This thesis has not been previously submitted
for a degree at this or any other university.

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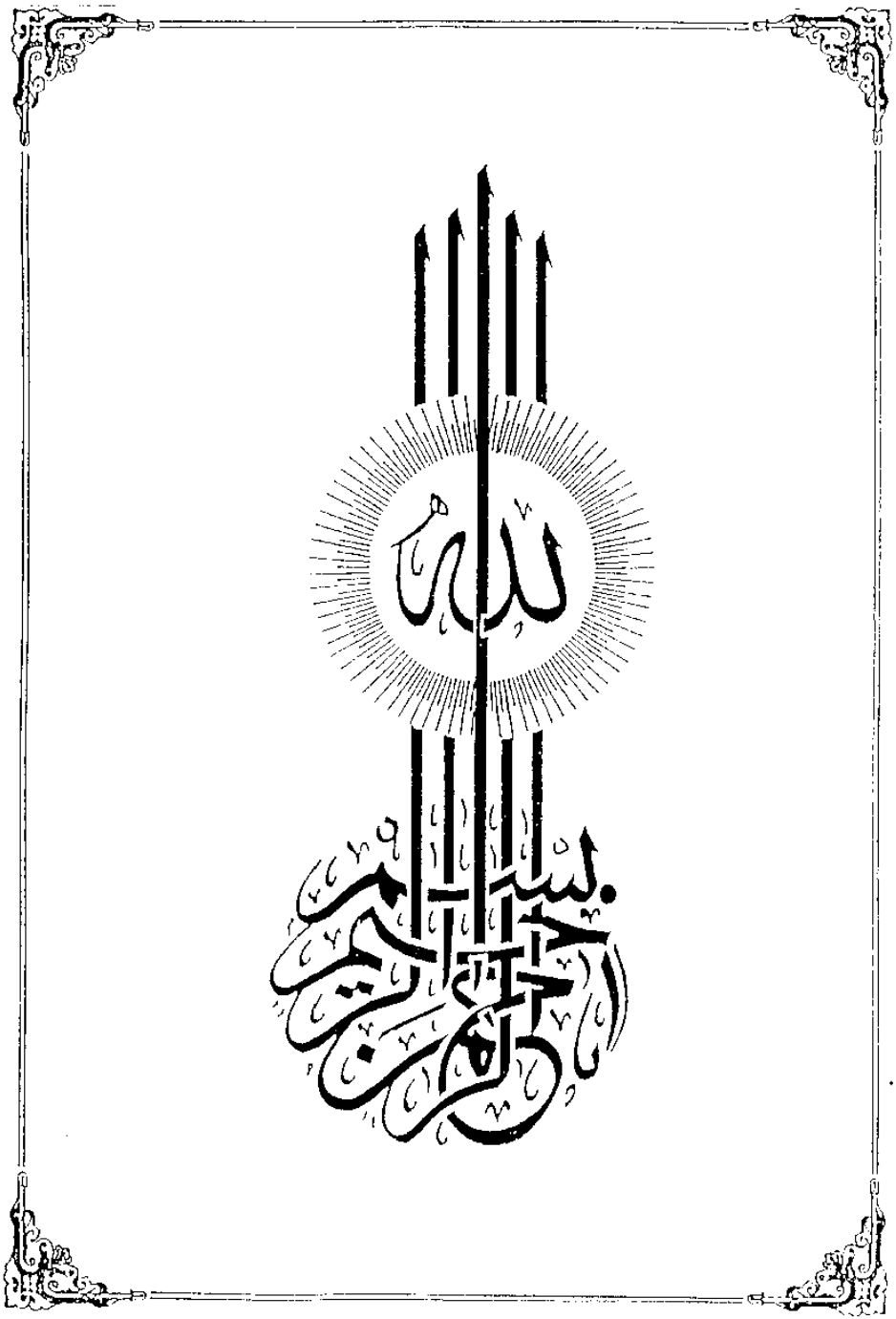

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INTRODUCTION

The well known stathmokinetic chemicals affect cells undergoing mitosis in two ways:

1- they block cells at metaphase and suppress anaphase.
2- They prolong the duration of metaphase.

The consequences of these effects are an increase in the mitotic index, due to the accumulation of cells in metaphase i.e. these chemicals affected the transition of the Karyotypes from metaphases to anaphases. The chromosomes in anaphases did not change their site in comparison to metaphase stages. The centromeres, after splitting, allowed the chromatids or the so-called daughter chromosomes to be absolutely separated from each other and to lie neighbouring each other, forming what is known as parallel-anaphase. Later, in telophase and interphase stages, these daughter chromosomes form a nucleus in the area occupied by the chromosomes and thus acquire an irregular shape. The newly formed nucleus (restitution nucleus) contains double the number of chromosomes and hence is larger than the parent nucleus. Sometimes the daughter chromosomes lie in separate groups in such a way that the whole chromosome set will not be coated by one nuclear membrane. Consequently each separate group of chromosomes acquires a nucleur membrane of its own

and thus many heteroploid nuclei are formed in each cell (Kabarity 1968). Though dividing cells show a prolonged metaphase stage. They are not arrested permanently in that stage; they undergo restitution and may re-enter mitosis after an interphase period.

On the other hand B-indol-3-acetic acid (IAA) appears to affect cells during interphase, it can stimulate dividing cells to enter mitosis e.g. Pericycle cells (Goldacre 1959) and mature cells of roots (Levan 1939; D'Amato 1945) and it is necessary for mitosis and DNA synthesis in tissue explants (Patau, Das and Skoog 1957). In fully formed meristems, however, IAA reduces the number of meristematic cells (Hughes and Street 1960) and has been shown to inhibit mitosis (Mangenot 1942; Stern 1956). But though stathmokinetic substances as colchicine and IAA affects mitosis in different ways it must be remembered that they do so over different periods; Colchicine e.g. produces detectable effects on mitosis more rapidly than IAA.

IAA is present in many plants (Leopold 1955; Audus 1959) including Vicia faba (Bennet-Clark and Kefford, 1953). IAA usually inhibits growth of normal meristems and roots. In roots previously treated with Colchicine, however, IAA stimulates growth (Davidson, Macleod and Taylor 1965).

This effect leads Davidson and Macleod (1966) to suggest that roots treated with colchicine undergo a change in the level of growth factors. Such a change could be the basis both of the increase in mitotic index (Macleod 1965, Davidson, Macleod and O'Riordan 1966)and of the abnormalities, such as aberrant patterns of xylem formation, seen after colchicine treatments (Davidson 1963).

2-Chloroethyl trimethyl ammonium chloride (cycocel) is a plant growth retardant and it has been reported to exert marked effects on plant growth. The most striking influence of ccc is shortening of plant height. Cotton plant is one among these crops which are greatly affected by ccc application even when it was used at low concentrations (El-Fouly, M.M., Salib, J.G. and El Baz, F.K. (1969), Hamawi, H. (1967), Leukopoulou, S. (1966), Shaffer, N.E. (1969) and Thomas, R.O. (1964) under Egyptian conditions, retardation of stem elongation of cotton plant by low and moderate concentrations was not accompanied by positive effects on yield (El-Fouly, M.M., Salib, J.G. and El Baz, F.K. (1969) and Hamawi, H. (1967) while it was reduced when higher concentrations were applied.

Application of ccc as growth retardants measurably inhibited root formation or delayed root development

(Cathey et al. 1960). Wittwer and Tolbert (1960) suggested that the root systems of treated plants were less developed than those observed on untreated ones and the ratios of tap-root were reduced.

Since IAA stimulates the growth, and ccc acts as a retardant for the growth of the roots we set out to determine whether there is any interaction between ccc and IAA in the effects they produce on mitosis.

One approach to this problem is to check the effect of these chemicals separately on the cytological behaviour of cells and together in different concentrations and at different intervals.

MATERIAL AND METHODS

The experimental plant used in this investigation was broad bean (Vicia faba var. Kobrosy).

Pure strains of seeds of the above mentioned plant was obtained from Egyptian Ministry of Agriculture.

The growth retarding chemical cycocel (ccc) and Indol 3-acetic acid were obtained from the department of physiology of cotton Research, Ministry of Agriculture of Arab Republic of Egypt.

Broad beans (Vicia faba var. Kobrosy) were soaked in tap water for 24 hours, and then planted in pots, seeds were let to germinate 10 days, and after the roots (lateral roots) were 4-6 cm long they were placed in solutions which contained the test substances for the treatment time given in the text. The root tips were then fixed in carnoy (3:1 absolute alcohol:acetic acid glacial) for 24 hours. At first the lethal concentration and the subthreshold concentration were determined then a number of concentrations intermediate between these two limiting doses were selected for further experiments. These concentrations were expressed in the tables in terms of p.p.m. A large number of roots were immersed in each of the test solutions and at least 20 root tips of

(lateral roots) from each incubation were fixed and prepared by using Feulgen's squash method (Morin 1960).

Feulgen-squash method :

a) After fixation and washing the root tips were macerated by hydrolysis in HCl at 60°C for 12 minutes.

b) Root tips were then left in the stain (leucobasic Fuchsin) for 2-3 hours.

c) Root tips were teased out on a slide with a drop of 45% acetic acid with the blunt end of abone needle holder.

d) After filming the cover slip, it was placed in position and pressure was applied under several thickness of blotting paper allowing no side way movement of the cover slip.

e) The slide was then heated over a spirit flame 4 or 5 times (not boiling).

f) The slide was turned face down in a smearing dish containing distilled water, after 3-10 minutes the cover slip fell off.

g) The slide and cover slip were dehydrated through a series of alcohols (30% , 50%, 70%, 96%, 100%), then passed through a mixture (1:1) of xylol and absolute alcohol, cleared in xylol and mounted in canada balsam, section were placed in a hot air oven (35-40°C) for 1-2 days.

Permanent squash preparations were then made of the lateral roots of Vicia faba, 5 preparations being made for each concentration at each time of fixation. From each slide 1000 cells were scored, giving a total of 5000 cells for each treatment. The photomicrographs were taken by the silver eosin plate 12 Din with the aid of Zeiss Camera. The reagents used in this reaction were:

a- The hydrolysing solution :

This is merely a normal solution of hydrochloric acid prepared as follows :

Hydrochloric acid (sp. gr. = 1.19	82.5 c.cs.
Distilled H_2O	1000 c.cs.

This dilute acid solution was placed in a dropping jar and brought to a temperature of 60°C, a temperature which should be determined constant throughout hydrolysis.

A water bath was used for this purpose. However, it was found out that the variation of a degree or two had no effect on the final result.

b- The staining solution :

Modified formula after Coleman (1938) was used. 19 m basic fuchsin was dissolved by pouring over it 200 ml. boiling distilled water, after filtering add 30 cc NHCl and 3 gm. $K_2S_2O_5$. The solution was allowed to bleach for 24 hours in a tight stoppered bottle in the dark, 0.5 gm decolarizing carbon was added, after which the solution was rapidly filtered and stored in a tightly stoppered bottle in the dark.

1- Long treatment experiment using (ccc) :

Vicia faba seeds were soaked in distilled water for 24 hours. The testas were then removed and the beans were germinated in moist sand. After about 10 days the beans with lateral roots about 6 cm long were washed free of sand.

Roots were treated with the following concentrations of ccc: 30,000, 20,000, 10,000, 5,000, 4,000, 3,000, 2,000. p.p.m. in ccc. Each treatment was for 4 hours, 12 hours and successive treatments were separated by 1 day