Effects of IAA with Maleic Hydrazide and Colchicine on Root Tip Mitosis

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Mutachromosomic chemicals not only cause structural changes in the chromosomes but also affect the rate of mitosis (Darlington and McLeish 1951, Bowen and Wilson 1954, Rossi 1955, Kihlman 1956, McManus 1959, Davidson 1964, Prasad and Das 1977). These active chemicals belong to divergent groups and vary in their action. Continuous attempts are being made to discover certain general principles of action among these numerous and divergent chemicals. A fruitful method in this field is to study their comparative effects under similar conditions. This paper reports the results of an experiment designed to study the effects of three chemicals of varying concentration and duration on the mitotic behaviour of *Allium cepa* Linn. root tips. While the literature on MH and colchicine is large, work with IAA is still scanty. A comparative study of the effects of IAA with MH and colchicine was thus thought to be fruitful.

Materials and methods

Three chemicals namely, maleic hydrazide, MH (an antiauxine), indole-3 acetic acid, IAA (a growth substance) and colchicine (a mitotic poison) were used for the treatment. The bulbs of *Allium cepa* Linn. were placed on the mouth of the specimen tubes filled with the aqueous solution of each chemical of 25, 50 and 75 ppm concentrations for 4, 8 and 12 hours and it was ensured that the base and roots of bulbs were dipping in the solution during the treatment. After this treatment the bulbs were allowed a recovery period of 24 hours in distilled water. The root tips were fixed in 3:1 alcohol-acetic acid and stored in 70 per cent alcohol in a refrigerator. For cytological preparations 0.5 per cent haematoxylin was used.

Results

The effects were studied in two groups namely, the effects on mitotic index and the induction of abnormalities in dividing nuclei. The mitotic index and cell metabolism were greatly affected by all the three chemicals. Data relating to comparative effects of the treatments on cell division and chromosomal abnormalities

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Table 1. Mitotic index and frequencies of abnormal cells after treatment with the three chemicals

Chemicals	Duration (hours)	Number of cells examined			Percentage of division			Percentage of abnormal cells		
		25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm	25 ppm	50 ppm	75 ppm
MH	4	3521	2732	2942	13.0	12.9	10.1	14.8	13.9	12.4
	8	2583	3713	2525	12.7	9.0	7.1	16.1	17.8	14.1
	12	2726	3045	2278	10.6	7.5	5.1	18.3	18.9	21.8
IAA	4	2592	2757	2922	18.3	11.6	—	2.6	9.0	—
	8	3214	3109	2463	20.1	6.3	—	4.6	16.4	—
	12	2723	2518	2625	26.5	5.1	—	15.0	16.7	—
Colchicine	4	2899	2276	2567	17.0	14.9	10.0	5.1	9.0	11.5
	8	2937	1948	2868	11.6	10.5	5.8	11.4	15.5	19.4
	12	2513	3242	2619	10.4	6.7	4.8	15.3	17.6	23.8
Control		11552	—	—	17.5	—	—	—	—	—

are shown in Tables 1 and 2 and are diagrammatically represented in Figs. 1 and 2.

The inhibition of mitosis was brought about in each case except with 25 ppm IAA which in fact stimulated the mitosis and was directly proportional to the duration of the treatment. The mitotic indices recorded were 18.3, 20.1 and 26.5 per cent for 4, 8 and 12 hour durations respectively in contrast to 17.5 per cent in control condition. The inhibition of mitosis due to MH and colchicine and higher concentrations of IAA was found to be increased with the increasing concentration of chemicals and duration of treatments. Maximum inhibition occurred at 75 ppm of IAA and the mitosis completely ceased (Table 1). A minimum of 4.8 and 5.1 per cent division occurred at 75 ppm of MH and colchicine respectively for 12 hour duration. Data in terms of frequency of cells at different stages of mitosis were also taken but they did not show any relationship neither with the concentration of chemicals nor with the treatment duration.

All the three chemicals induced several types of abnormalities in dividing nuclei. The abnormalities were grouped in four categories as shown in Table 2 and portrayed in Fig. 2.

Chromosome breaks

Chromosome and chromatid breaks were found at all the treatments with different frequencies (Table 2, Fig. 3A). The frequency of breaks increased with the increase in duration. MH caused more breaks at metaphase while IAA and colchicine at anaphase. The breaks caused by MH were mostly located in heterochromatic regions. The fragments were found lying in cytoplasm at anaphase and telophase (Fig. 3B) and suppressed the cell plate formation resulting in binucleate cells.

Bridge formation

Bridges were limited only to MH. Single, double and triple bridges were

Fig. 1. Frequency of cells in mitosis after treatment at 25, 50 and 75 ppm with 4, 8 and 12 hour durations.

Fig. 2. Frequency of abnormal cells induced by the three chemicals at 25, 50 and 75 ppm concentration with 4, 8 and 12 hour durations.

Fig. 3. Some abnormalities induced by the three chemicals. A, fragments at anaphase. B, fragments at anaphase in cell plate area. C, bridges at telophase. D, stickiness at metaphase. E, micronucleus. F, dicentric bridges at anaphase. G, spindle inactivation at anaphase. H, polyploid cells. I, erosion at metaphase. J, lagging at anaphase.

observed (Fig. 3C). Chromosomes at metaphase showed stickiness (Fig. 3D) and formed bridges at anaphase which persisted till telophase. They were broken by the cell plate formation leading to micronuclei (Fig. 3E). Dicentric bridges were also observed due to the union of the broken ends (Fig. 3F).

Inactivation of spindle fibres

Inactivation of spindle fibres leading to the polyploid cells was observed in varying frequencies due to IAA and colchicine treatments (Figs. 3G and 3H). Highest frequency (11.6%) was observed when treated with 25 ppm of IAA for 12 hours (Table 2). Colchicine caused 8.7 per cent spindle inactivation at 75 ppm for 12 hours. Lagging of chromosomes due to inactivation of spindle fibres in one or two chromosomes was also observed (Fig. 3J).

Other abnormalities

Chromosome erosion (Fig. 3I), swelling of the nucleus, multipolar cells, binucleate cells and chromosome condensation were also observed. These abnormalities were not found to be related to any specific chemical.

The frequency of total abnormal cells caused by MH did not show any relationship with duration of the treatment at lower concentration but it increased with the increase in duration at higher concentration. Increasing concentration of both IAA and colchicine and the increase in treatment duration caused increase in the frequency of abnormality. Highest frequency (23.8%) of abnormality was found at 75 ppm of colchicine for 12 hour duration (Table 2).

Discussion

The cell division was stimulated by IAA at lower concentration and was strongly inhibited at higher concentration but both MH and colchicine inhibited it under all the treatments. Interphase nuclei were restrained from entering the mitosis and if somehow entered they returned to interphase. The stimulation by IAA might be due to catalase and peroxidase activity. Fan and MacLachlan (1966) found cellulase activity due to IAA. Huskin and Steinitz (1948) found temporary stimulated cell division in differentiated regions of the roots when treated with IAA. Antimitotic results obtained with IAA treatments were also observed by McManus (1959) and Davidson *et al.* (1964) in their studies. This result with IAA might be due to inhibition of glycolysis. The results obtained with MH treatments are similar to those obtained by Greulach and Atchison (1950), Greulach (1953) and Rossi (1955). Muir and Hansch (1953) and Hughes and Spragg (1958) are of the opinion that MH reacts with the sulfhydryl groups of the protein in the cells. The results with colchicine treatments are also similar to those obtained by Davidson (1964) and Herich (1966).

The chromosome and chromatid breaks caused by MH were localised in heterochromatic regions because heterochromatin is more susceptible than euchromatin as reported by Revell (1952). Kihlman (1952) and McLeish (1953) are of the opinion that MH being the structural isomere of uracil has some selective action.

The breaks caused by MH might be attributed to the error in chromosome duplication occurring during DNA synthesis. The bridges induced might have been due to the stickiness as reported by Sawamura (1965) or due to the formation of dicentric. Dahmen and Dahmen (1962) have reported that MH causes pseudo-bridges which do not persist after the removal of the chemical. Prasad and Das (1977) have observed dicentric bridges due to the rejoining of the broken ends of chromosomes in *Vicia faba* when treated with some growth substances.

The breakage caused by IAA resembles with that observed by Sharma and Mookerjee (1954) and the breakage induced by colchicine resembles with those obtained by Venkateswarlu and Shriniwasan (1964). It might be due to greater synthesis of nucleic acids. Chakraworty and Biswas (1965) found two three fold increase in the synthesis of nucleic acids and protein due to colchicine treatment.

The spindle suppression observed here by IAA agrees with the findings of Chouinard (1956) and with colchicine treatment it agrees with those obtained by Levan (1954), Eigsti and Dustin (1954) and Venkateswarlu and Shriniwasan (1964). The lagging of chromosomes at anaphase might be due to the fact that spindle fibres of the lagging chromosomes were inactivated. Brachet (1957) assumed that under the influence of colchicine the protein molecules which built up the spindle fibres were altered in their composition.

Erosion caused by IAA and MH appeared before the breakage. It might be due to the fact that certain chromosome sites became devoid of the full quota of nucleic acids and appeared thinner than the rest of the segments. As regards the chromosome condensation Amar and Farah (1974) also observed it with Rogor treatment. In *Vicia faba* chromosome condensation was observed with some growth substances by Prasad and Das (1977). From the above facts it may be concluded that chemicals which are either antimitotic or growth substance or mitotic poison can affect the mitotic index causing hazards in the biological phenomena of the cell and can induce chromosome breakage without stickiness or both breakage and stickiness and can suppress the spindle fibres along with the breakage. The general mode of their action is that they act through different means producing some active radicals which finally create imbalance in the nucleic acid and protein metabolism resulting into the induction of chromosomal abnormalities.

Summary

The effects of maleic hydrazide MH, indole-3 acetic acid IAA and colchicine were studied on the mitotic division and induction of chromosomal abnormality in *Allium cepa* root tips treated with 25, 50 and 75 ppm concentrations for 4, 8 and 12 hour durations. Inhibition of mitosis was brought about under all treatments except IAA which at lower concentration stimulated the division. For the inhibition of mitosis IAA was most effective and MH was least effective. The capacity to produce chromosomal abnormality was evidenced by the presence of chromosome and chromatid breaks, bridge formation, spindle fibre inhibition resulting in the formation of polyploid cells; chromosome erosion, lagging of chromosomes, micronuclei, stickiness and swelling of the nucleus.

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