Interaction between the Plant Growth Retardant Cycocel and the Plant Growth Stimulant Indol Acetic Acid (IAA)

A. Kabarity and S. Khodary

Botany Department, Faculty of Science, Kuwait University, Kuwait

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Since 2-chloroethyl trimethyl ammonium chloride (cycocel) acts as a retardant for the growth of the roots and there are substances like indol acetic acid which stimulate the growth we set out to determine whether there is any interaction between cycocel and indol acetic acid in the effects they produce on mitosis. The cytological effects on cycocel and indol acetic acid separately were already examined (Kabarity and Khodary 1974 a, b, c).

In the present investigation the cytological behaviour of *Vicia faba* root tips treated with both together was studied.

Materials and methods

The experimental plant used in this investigation was broad bean (*Vicia faba* var. Kobrosy). Pure strains of the above mentioned plant was obtained from Egyptian Ministry of Agriculture. The growth retarding chemical cycocel (CCC) and indol acetic acid (IAA) were obtained from the department 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 time given in text. The root tips were then fixed in Carnoy (3: 1 absolute alcohol; glacial acetic acid) for 24 hours. The concentrations of the test substances were expressed in the tables in terms of part per million (ppm). At least 20 root tips from each incubation were fixed and prepared by using Feulgen's squash method.

Results

1. Interaction between different concentrations of IAA and 12,000 ppm CCC for 4 hours treatment

The difference between the effects produced by IAA and CCC on mitosis makes it easy to determine whether any interaction occurs between them when they are used together to treat roots.

After 4 hours combined treatment of IAA and 12,000 ppm CCC, there was a depression in mitotic indices in all of the applied concentrations. Fig. 2 represented

the mitotic analysis of 800, 600, 400, 200 and 100 ppm IAA, 12,000 ppm CCC and the above mentioned concentrations of IAA and CCC together. Concentration 400 ppm IAA with 12,000 ppm CCC showed the highest mitotic index (3.2) decreased gradually till reached (2.1) in the weakest solution. The high value of MI (3.2) was due to the accumulation of metaphase stage as its frequency reached (95.6) and there was an inhibition in both prophase and anaphases. Normal metaphases and anaphases were absent in the last two concentrations although it was found by using IAA alone (Table 1 and 2). The cytological effect of (800 or 600 IAA + 12,000 ppm CCC) was characterised by stickiness in all phases examined, meanwhile c-metaphases were represented in the other concentrations (400 ppm IAA + 12,000 ppm CCC), (200 ppm IAA + 12,000 ppm CCC), (100 ppm IAA + 12,000 ppm CCC).

Table 1. Numbers of cells in interphase and in mitosis in cells of *Vicia faba* roots treated with different conc. of IAA

	800 IAA		600 IAA		400 IAA		200 IAA		100 IAA		Control.	
Interphase norm.	5213		5551		5931		5484		5639		5522	
micro. and multi.	—		—		—		—		—		—	
Prophase												
Total no. norm.	182	—	140	—	96	—	57	44	84	78	113	—
Metaphase												
C.m.	—		7		86		13		6		56	
Tetrapl. stick.		182	—	133	10	—	—	—	—	—	—	57
Ana + telophase												
Total no. norm.	39	12	54	32	70	42	55	50	38	34	39	39
C. anaph. multip.	—	—	—	—	—	—	—	—	—	—	—	—
Lagg. bridge		27	—	27	—	28	—	5	—	4	—	—

2. *Interaction between different concentrations of IAA and 12,000 ppm CCC for 4 hours treatment followed by 1 hour recovery*

After 1 hour recovery mitotic index had been risen in all concentrations used. MI of highest and lowest concentration was similar (5.8 and 5.7) which showed an increase than those in control value (3.7) (Fig. 2).

Concerning the abnormalities found in the divided cells after the combined treatment and fixed 1 hour later, the abnormal sticky metaphase figures first seen after 4 hours treatment in the first 2 concentrations were changed to c-metaphases. Tetraploid cells were often observed in cells treated with IAA and CCC and fixed 1 hour later. 33 tetraploid cells per 248 divided cells were found in concentration (400 ppm IAA + 12,000 ppm CCC). Also this concentration showed a high number of multipolar anaphase 48 cells among 248 divided cells. This number of multipolar anaphases and tetraploid cells decreased reaching 39, 17 respectively in 100 ppm IAA + 12,000 ppm CCC (Table 2).

3. *Interaction between different concentrations of IAA and 12,000 ppm CCC for 4 hours treatment followed by 2 hours recovery*

After 2 hours recovery to these combined treatment, MI fell in all of the applied concentrations except the highest which showed an increase in MI from 5.8

Figs. 1-2. 1, frequency of multipolar anaphase after treating *Vicia faba* root cells with different concentrations of IAA + 30000 ppm Cycocel for 4 hours and fixed 1 and 2 hours later. 2, mitotic index in *Vicia faba* root cells treated with different concentrations of IAA + 12000 ppm Cycocel for 4 hours, and fixed at once and after 1, 2 and 4 hours later.

Table 2. Numbers of cells in interphase and in mitosis in cells of *Vicia faba* roots treated with different conc. of IAA and 12000 CCC in combined treatments for 4 h, 4 h and fixed 1 h later, 2 h later, 4 h later

	800 IAA +12000 CCC	600 IAA +12000 CCC	400 IAA +12000 CCC	200 IAA +12000 CCC	100 IAA +12000 CCC	Cont.
Fixed immediately						
Interphase						
Norm.	5116	5267	5684	5165	5037	5522
Micro and multi.	—	—	—	—	—	—
Prophase						
Norm. abn.	25 —	33 —	4 2	39 —	15 —	137 —
Metaphase						
Total no. norm.	134 —	133 —	180 —	89 —	94 —	70 70
C.m.	—	—	180	88	91	—
Tetrap. stick.	— 134	— 133	— —	1 —	3 —	— —
Ana+telophase						
Total no. norm.	4 —	3 —	2 —	7 4	4 2	39 39
C. ana, multip.	— —	— —	— —	— —	— —	— —
Lagg, bridge	— 4	— 3	— 2	— 3	— 2	— —
Fixed one hour later						
Interphase						
Norm.	5471	5454	4370		5361	5304
Micro and multi.	—	10	—	—	—	—
Prophase						
Norm. abn.	73 —	53 —	52 —	— —	98 —	77 —
Metaphase						
Total no. norm.	214 —	165 —	133 —	— —	197 —	58 58
C.m.	213	160	100	—	180	—
Tetrap. stick.	1 —	5 —	33 —	— —	17 —	— —
Ana+telophase						
Total no. norm.	5 2	24 —	63 9	— —	39 —	33 33
C. ana, multip.	— —	— 22	— 48	— —	— 39	— —
Lagg, bridge	— 3	1 1	3 3	— —	— —	— —
Fixed two hours later						
Interphase						
Norm.	4196	4638	4643	4714	4285	5206
Micro and multi.	—	72	122	13	110	—
Prophase						
Norm. abn.	41 —	43 —	29 —	32 —	65 —	97 —
Metaphase						
Total no. norm.	230 —	52 —	58 —	149 —	82 —	37 37
C.m.	228	52	53	145	62	—
Tetrap. stick.	2	— —	5 —	4 —	20 —	— —
Ana+telophase						
Total no. norm.	5 —	44 41	28 17	19 9	26 —	49 49
C. ana, multip.	— 5	— 3	— 10	7 7	— 26	— —
Lagg, bridge	— —	— —	— 1	— 3	— —	— —
Fixed 4 hours later						
Interphase						
Norm.	4343	5214	4749	4088	4640	3941
Micro. and multi.	—	96	7	79	282	—
Prophase						
Norm. abn.	— —	30 —	25 —	16 —	9 —	50 —
Metaphase						
Total no. norm.	151 —	61 40	62 38	52 12	44 —	48 48
C.m.	151	21	24	40	44	—
Tetrapl. stick.	— —	— —	— —	— —	— —	— —
Ana+telophase						
Total no. norm.	12 12	25 25	26 24	36 32	14 8	78 78
C. ana, multip.	— —	— —	— 1	— 2	— 6	— —
Lagg, bridge	— —	— —	1 —	2 —	— —	— —

Figs. 3-8. 3, c-metaphase in *Vicia faba* roots after treatment with 800 IAA+12,000 CCC ppm for 4 hours. 4, c-metaphase after the treatment with (200 IAA+12,000 CCC) ppm for 4 hours. 5, c-metaphase after the treatment with (100 IAA+12,000 CCC) ppm for 4 hours. 6, c-metaphase after the treatment with (800 IAA+12,000 CCC) ppm for 4 hours and fixed one hour later. 7, c-metaphase in *Vicia faba* roots with (600 IAA+12,000 CCC) ppm for 4 hours and fixed one hour later. 8, abnormal metaphase after treatment with (800 IAA+12,000 CCC) ppm for 4 hours and fixed 2 hours later.

to 6.1 (Fig. 2). Number of tetraploid cells and multipolar anaphase reached 20 and 26 respectively among the total divided cells 173 in the lowest concentration, then this number decreased as the concentration increased. Normal anaphases started to appear earlier in concentration (600 ppm IAA+12,000 ppm CCC) as represented by 41 cells showing gradual decrease towards the lower concentration (Table 2).

After 1 and 2 hours recovery to the roots of *Vicia faba* previously treated with different concentrations of IAA+12,000 ppm CCC for 4 hours, the number and percentage of multipolar anaphase was strikingly high (Table 2), thus the percentage of multipolar anaphase had been plotted against concentrations (Fig. 1). It can be seen that high percentage of multipolar anaphase was found at the lowest concentration after 1 and 2 hours recovery (100%) as all the anaphases was found from the multipolar type and the same observation was also recorded at the concentration of 800 IAA+12,000 ppm CCC after 2 hours recovery (Table 2).

4. Interaction between different concentration of IAA and 12,000 ppm CCC for 4 hours treatment followed by 4 hours recovery

The trend in the fall in MI that is first seen after 2 hours recovery continues in the followed experiment (4 hours recovery). The highest mitotic index (3.6) was found in the highest concentration and MI of lowest value (1.3) was found in the lowest concentration which remained below that of control value (4.2) (Fig. 2). The high value of MI (3.6) was attributed to the high frequency of metaphases (92%) although it is accompanied with completely inhibition of the prophase stage. Normal metaphases started to appear after (4 hours recovery) in the root cells treated with different concentrations of IAA and 12,000 ppm and normal anaphases were found in all concentrations.

Micronuclei and multinucleate cells which previously seen after 2 hours recovery with this combined treatment was also observed after treatment of *Vicia faba* root cells with different concentrations of IAA+12,000 ppm CCC followed by 4 hours recovery, reaching a maximum of 282 micro- and multinucleated cells per 5,000 cells at the lowest concentration (Table 2).

Figs. 3-8 represent different forms of c-metaphases after combined treatment with different concentrations of IAA+12,000 ppm CCC for 4 hours treatment and for different periods of recovery. 12 chromosomes were clearly observed. The chromosomes do not form a metaphase plate. They are distributed at random all over the cell and each chromosome divides into two distinct chromatids which are attached only at their centromere. Fig. 6 represents c-metaphase in which the chromosomes were highly contracted after 1 hour recovery to the root cells treated with (800 IAA+12,000 ppm CCC). Treatment of *Vicia faba* root cells with IAA+12,000 ppm CCC and fixed few hours later showed different types of abnormal anaphases. Multipolar anaphases were very numerous, the number of poles ranging from three poles to several poles (Figs. 11, 12, 13, and 14). Chromosome bridge

+12,000 CCC) ppm for 4 hrs and fixed 1 hr later. 15, lagging of chromosome after treatment with 400 IAA+12,000 CCC ppm for 4 hrs and fixed 2 hrs later. 16, multinucleated cell after treatment with 400 IAA+12,000 CCC ppm for 4 hrs and fixed 2 hrs later.

Figs. 9-16. 9 and 10, doubling of chromosome number (c-anaphases) after treatment with (IAA +12,000 CCC) ppm for 4 hours (Fig. 11), the same treatment but fixed one hour later (Fig. 12). 11 and 12, multipolar anaphases after treatment with (400 IAA+12,000 CCC) ppm for 4 hours treatment. 13, multipolar anaphase after treatment with (800 IAA+12,000 CCC) ppm for 4 hours treatment and fixed one hour later. 14, multipolar telophase after treatment with (400 IAA

Table 3. Numbers of cells in interphase and in mitosis in cells of *Vicia faba* roots treated with (different concentrations of IAA) and 4000 ppm CCC in combined treatments for 4 h, 4 h and fixed 1 h later, 2 h later, 4 h later

	800 IAA + 4000 CCC		600 IAA + 4000 CCC		400 IAA + 4000 CCC		200 IAA + 4000 CCC		100 IAA + 4000 CCC	
4 hours treatment and fixed immediately										
Interphase										
Norm.	5188		5949		5825		5535		5330	5522
Micro. and multi.	12		22		28		—		—	—
Prophase										
Norm. abn.	12	—	81	—	98	—	104	—	77	137
Metaphase										
Total no. norm.	315	—	177	24	100	42	146	15	77	68
C.m.	—	—	—	—	58	—	129	—	9	—
Tetrapl. stick.	—	315	—	153	—	—	2	—	—	—
Ana + Telophase										
Total no. norm.	32	2	87	30	84	35	92	19	66	42
C. ana. multip.	—	—	—	35	—	40	—	68	—	19
Lagg. bridge	—	30	—	22	2	7	5	—	—	5
Fixed one hour later										
Interphase										
Norm.	5532		5743		5806		5958		5117	5287
Micro and multi.	7		14		10		35		5	—
Prophase										
Norm. abn.	65	—	116	—	137	—	148	—	114	197
Metaphase										
Total no. norm.	203	26	107	59	70	61	72	48	66	25
C.m.	177	—	48	—	9	—	24	—	41	—
Tetrapl. stick.	—	—	—	—	—	—	—	—	—	—
Ana + telophase										
Total no. norm.	42	35	134	75	111	94	115	76	77	40
C. ana. multip.	—	4	—	34	—	12	—	34	—	37
Lagg. bridge	1	2	19	6	3	2	5	—	—	—
Fixed 2 hours later										
Interphase										
Norm.	5517		5291		5917		5657		5702	5020
Micro and multi.	—		—		16		143		6	—
Prophase										
Norm. abn.	3	—	44	—	178	—	122	—	90	170
Metaphase										
Total no. norm.	238	11	80	71	66	66	83	53	103	99
C.m.	227	—	9	—	—	—	30	—	4	—
Tetrapl. stick.	—	—	—	—	—	—	—	—	—	—
Ana + Telophase										
Total no. norm.	50	33	38	22	79	79	66	38	101	80
C. ana. multip.	—	—	—	3	—	—	—	21	—	19
Lagg. bridge	—	17	12	1	—	—	7	—	2	—
Fixed 4 hours later										
Interphase										
Norm.	5357		4998		5410		5691		4740	4412
Micro and multi.	2		110		104		132		9	—
Prophase										
Norm. abn.	47	—	38	—	66	—	66	—	84	92
Metaphase										
Total no. norm.	41	10	30	17	39	27	70	40	67	37
C.m.	31	—	13	—	12	—	30	—	30	—
Tetrapl. stick.	—	—	—	—	—	—	—	—	—	—
Ana + telophase										
Total no. norm.	158	152	60	59	72	71	96	96	76	74
C. ana. multip.	—	—	—	—	—	—	—	—	—	1
Lagg. bridge	3	3	1	—	1	—	—	—	—	—

was also observed in few phases, represented by Fig. 15. Multinucleated cells and micronuclei were represented in Fig. 16.

5. *Interaction between different concentrations of IAA and 4,000 ppm CCC for 4 hours treatment*

Concerning MI, it is clear that its value was higher in roots treated with 4,000 ppm CCC, alone, or with IAA than in roots treated only with IAA (Fig. 17). According to the normal and abnormal phases, treatment of *Vicia faba* root cells with 4,000 ppm CCC resulted in the generation of c-metaphases (all metaphases were of abnormal type). Treatment with IAA alone showed no normal metaphases, treatment of *Vicia faba* root cells with 4,000 ppm CCC resulted in the generation of c-metaphases (all metaphases were of abnormal type). Treatment with IAA alone showed no normal metaphases at 600 and 400 ppm CCC. But, in the present experiment, addition of 4,000 ppm CCC to different concentrations of IAA, specially at the previously mentioned two concentrations of IAA (600 and 400 ppm) resulted in the generation of normal metaphases which attained the highest number of 42 cells at concentration of (400 ppm IAA+4,000 ppm CCC). This observation was not met on using either IAA or CCC alone in the previous mentioned concentrations (Kabarthy and Khodary 1974a,c).

Finally we come to the conclusion: using combined treatment of (600 or 400 ppm IAA+4,000 ppm CCC) led to an antagonistic effect. And also addition of CCC at this concentration to IAA at different concentrations induced an increase in the abnormal anaphases as well as induced multipolar anaphase (Table 3).

6. *Interaction between different concentrations of IAA and 4,000 ppm CCC for 4 hours treatment followed by 1 hour recovery*

After 1 hour recovery to the root cells previously treated with different concentrations of (IAA+4,000 ppm CCC), the MI showed a slight increase at all concentrations, except at the highest concentration which showed a decrease from 6.5 to 5.4 (Table 3). The number of normal phases started to appear earlier at the first concentration after 1 hour recovery, i.e. the roots do a recover. Number of normal phases which previously found at 4 hour treatment showing an increase after 1 hour recovery and increased by 2 hours and 4 hours recovery. Concentration 200 ppm IAA+4,000 ppm CCC represented always the highest number of micronuclei and multinucleate cells.

7. *Interaction between different concentrations of IAA and 4,000 ppm CCC for 4 hours treatment followed by 2 hours recovery*

After 2 hours recovery, MI began to decrease and continued this decrease by further recovery for 4 hours. This decrease is due to the gradual fall in the number of total divided cells. The number of normal cells showed an increase and that of abnormal cells was decreased as the intervals of the recovery increased.

8. *Interaction between different concentrations of IAA and 4,000 ppm for 4 hours treatment followed by 4 hours recovery*

Figs. 17-18. 17, the effect of 4000 ppm CC+IAA in different concentrations on the mitotic index of *Vicia faba*. 18, the effect of 400 ppm IAA+CC in different concentrations on the mitotic index of *Vicia faba*.

After 4 hours recovery to the root cells of *Vicia faba* treated with this combined treatment, the highest frequency was reached with anaphase as compared with the other phases at all concentrations applied. The maximum frequency of anaphase (68.2%) was found at the first concentration, then decreased gradually as the concentration decreased till 33.4% in the weakest solution (Table 3).

9. *Interaction between different concentrations of CCC and 400 ppm after 4 hours treatment*

In order to confirm the previously mentioned conclusion: "There is an antagonistic effect between concentration of 4,000 ppm CCC and 400 ppm IAA", this experiment has been carried out: concentration of 400 ppm IAA was added to each of different concentrations of CCC (12,000, 8,000, 4,000 and 5,000 ppm CCC). The antagonistic effect was observed 4 hours after the experiment began at the same concentration (4,000 ppm CCC+400 ppm IAA) just as was expected, and could be detected by the appearance of the same number of normal cells in metaphase stage beside that of c-metaphase i.e. 55 normal metaphase and 49 c-metaphase. This number of either normal metaphase and c-metaphase is similar to those found in the previous experiment at the same concentration (Tables 3 and 5).

Table 4. Numbers of cells in interphase and in mitosis in cells of *Vicia faba* roots treated with different conc. of CCC

	12000 CCC		8000 CCC		4000 CCC		2000 CCC		400 IAA		Control	
Interphase												
Norm.	5280		5250		5689		5010		5931		5014	
Micro. and multi.	—		—		—		—		—		—	
Prophase												
Norm. abn.	158	6	176	—	107	—	87	—	131	9	176	—
Metaphase												
Total no. norm.	134	—	83	—	120	—	46	30	96	—	80	80
C.m.	—		27		120		16		86		—	
Tetrapl. stick.	— 134		— 56		— —		— —		10 —		— —	
Ana + telephase												
Total no. norm.	—	—	—	—	42	14	35	20	42	—	86	—
C. ana. multip.	—	—	—	—	—	22	—	6	—	—	—	—
Lagg. bridge	—	—	—	—	—	6	2	—	—	28	—	—

Normal metaphase was also found by using (20,000 ppm CCC+400 ppm IAA) but in a less number, (11 cells per total divided cells). This observation was not shown in those concentrations of either CCC or IAA.

Anaphases of high number (41) were observed at the lowest concentration, then fell gradually till reached (6) at the highest one.

There was a depression in the mitotic index of root cells treated with different concentrations of CCC due to the addition of IAA (Fig. 18). The highest value of MI (4) was found at the lowest concentration (2,000 ppm CCC+400 ppm IAA).

Metaphase with high frequency of 80% was found at the highest concentration after 4 hours treatment, then decreased gradually to 70.7, 45.6 till reached 30.2 at the lowest concentration used. This lowest concentration also possessed the highest frequency of prophases 51.0%. Frequency of prophase and anaphase was

Table 5. Numbers of cells in interphase and in mitosis in cells of *Vicia faba* roots treated with different concs. of CCC and 400 IAA for 4 hours and fixed immediately and after 1 h, 2 h, 4 h later

	12000 CCC + 400 IAA		8000 CCC + 400 IAA		4000 CCC + 400 IAA		2000 CCC + 400 IAA		Control	
Fixed immediately										
Interphase	5075		5041		5531		5460		5014	
Norm.	—		—		—		—		—	
Micro. and multi.	—		—		—		—		—	
Prophase	31		23		103		118		176	
Norm. abn.	—		—		—		—		—	
Metaphase	146		104		104		69		80	
Total no. norm.	—		11		55		44		80	
C.m.	143		92		49		25		—	
Tetrapl. stick.	3		1		—		—		—	
Ana + telophase	6		20		21		41		86	
Total no. norm.	—		12		20		38		86	
C. ana. multip.	— 1		— 6		— —		— —		— —	
Lagg. bridge	4 1		1 1		— 1		1 2		— —	
1 hour later										
Interphase	5450		5456		5547		5563		5711	
Norm.	—		—		—		—		—	
Micro. and multi.	—		—		—		—		—	
Prophase	31		33		29		75		137	
Norm. abn.	—		—		—		—		—	
Metaphase	146		45		101		73		71	
Total no. norm.	4		4		55		59		71	
C.m.	137		39		44		13		—	
Tetrapl. stick.	5		2		2		1		—	
Ana + telophase	18		37		32		36		35	
Total no. norm.	12		24		18		31		35	
C. ana. multip.	— 6		2 5		1 3		— 1		— —	
Lagg. bridge	— —		3 3		5 5		1 3		— —	
2 hours later										
Interphase	5027		5991		5356		5230		5409	
Norm.	—		—		—		—		—	
Micro and multip.	—		—		—		—		—	
Prophase	18		32		47		136		153	
Norm. abn.	—		—		—		—		—	
Metaphase	74		27		52		97		53	
Total no. norm.	33		18		37		85		53	
C.m.	39		9		15		12		—	
Tetrapl. stick.	2		—		—		—		—	
Ana + telophase	47		77		56		54		23	
Total no. norm.	41		77		53		40		23	
C. ana. multip.	— —		— —		— 2		1 —		— —	
Lagg. bridge	5 1		— —		— 1		2 2		— —	
4 hours later										
Interphase	5352		5004		5054		5711		5595	
Norm.	36		27		—		—		—	
Micro and multi.	—		—		—		—		—	
Prophase	—		35		72		137		92	
Norm. abn.	—		—		—		—		—	
Metaphase	3		6		60		71		29	
Total no. norm.	3		6		41		53		29	
C.m.	— —		— —		19 —		18 —		— —	
Tetrapl. stick.	— —		— —		— —		— —		— —	
Ana + telophase	7		18		40		35		38	
Total no. norm.	7		18		37		29		38	
C. ana. multip.	— —		— —		— —		— —		— —	
Lagg. bridge.	— —		— —		1 2		— 6		— —	

very low at the highest concentration, it was progressively raised with the decrease in the concentration. This was inversely proportional with the frequency of metaphase attained.

10. *Interaction between different concentrations of CCC and 400 ppm IAA after 4 hours treatment followed by 1 hour recovery*

At 1 hour recovery there was an increase in anaphase frequency at all concentrations applied. This increase continued at 2 and 4 hours recovery (Table 5). At the same time there was a decrease in metaphase frequency. At all concentrations, till the minimum value was reached at 4 hours recovery. Table 5 indicates that the same number of normal and c-metaphase was found in the concentration with antagonistic effect (4,000 ppm CCC+400 ppm IAA). There was a great fall in the prophase number from 103 to 29 at this concentration (Table 5). This led to a decrease in number of total divided cells from 228 to 162 and consequently a depression in MI from 3.8 to 2.8. In general, there was a slight decrease in MI with all of the applied concentrations.

11. *Interaction between different concentrations of CCC and 400 ppm IAA after 4 hours treatment followed by 2 hours recovery*

Further recovery, for 2 hours showed a rapid increase in anaphase frequency (Table 5) whereas the frequency of metaphases, at the same time decreased. At all concentrations used, MI was decreased except at treatment with the lowest concentration of which MI was markedly increased from 3.2 to 5.4 above control figure (4.6). This might have been the result of an increase in the number of prophases from 75 to 136 as well as in the number of total divided cells from 184 to 278 (Table 5). The roots do a recover this could be detected since, the number of normal phases was increased and that of abnormal type was decreased.

12. *Interaction between different concentrations of CCC and 400 ppm IAA after 4 hours treatment followed by 4 hours recovery*

After 4 hours recovery, there was a reduction in the total divided cells which led to a decrease in the mitotic index. There was a complete inhibition of the prophase stage, accompanied by the existence of a very few number of cells at metaphase and anaphase stages and consequently MI fell to 0.18 at the highest concentration. MI was found to increase gradually till reached the maximum value (4.8) at the lowest concentration a value which was higher than that of control (2.7). At the first two concentrations, a complete absence of abnormal divided cells has been obtained accompanied by the appearance of micronuclei. At the next concentrations, few numbers of abnormal phases at metaphases and anaphases were observed (Table 5).

Discussion

A partial antagonistic effect was reported in the present investigation as no abnormal metaphases were recorded in the combined treatment of 600 and 400 ppm IAA

and 4,000 ppm CCC.

A confirmation of this finding was found in the result of Davidson and Macleod (1966) and many other investigators. Davidson and Macleod showed that IAA can reverse the changes that follow colchicine treatment. Mesoinositol, 6-hexachlorocyclohexane (gammexane) a widely used insecticide, has been reported by several authors to induce c-mitosis in *Allium* and other plant cells (D'Amato 1949, 1951, 1952, Kostoff 1938, 1940, 1948, and Northen 1950). Both the γ and the α isomers have been found to be active (Carpenter and Fromageot 1950), while the first only is of use as an insecticide. Polyploidy and chromosome fragmentation have also been recorded. Gammexane is probably antagonist of a naturally occurring substances, mesoinositol, having the same stereoisomeric structure as this sugar, the biological significance of which appears from its presence in many types of cells. It was not surprising that in 1948 it was announced that mesoinositol, but neither d-inositol nor sorbitol prevented, in proper concentrations, the c-mitotic activity of gammexane in *Allium cepa* (Chargaff, Stewart and Magasanik 1948). It was, however, more surprising and most interesting that mesoinositol was claimed to prevent also the spindle effect of colchicine. Inositol alone did not interfere with mitosis. A confirmation of these findings was found in the observation that colchicine and podophyllotoxine effects were antagonized in the egg of the sea urchin *Lytechinus variegatus* by glucose (Cornman 1941, 1942, 1950). *The antagonism was never total*; it was suggested that inositol may become changed into glucose in all cells.

In tissue cultures of rabbit heart fibroblasts, l-ascorbic acid was found to prevent, to a certain extent, the action of colchicine (Baucher 1940, 1947). An antagonism between colchicine and soluble 'prontosil' (Sulfanilamide) has been reported in plants (Baucher 1946, 1949) but the effective concentrations of the sulfa drug were about a hundred times those colchicine, and solubility effects were unavoidable.

An extract from hearts of embryonic warm blooded animals has been reported to delay the cytotoxicity of colchicine in fibroblast and myoblast cultures. Another more recent observation is that glycosidic substances endowed with cardiotonic activity decrease the action of colchicine in tissue cultures of chick heart fibroblasts (Landschutz 1949).

It appears evident from these data that no true antagonism has yet been found between any substance and colchicine, on a molar basis, and that the only effects observed depend on the presence of substances either of unknown chemical nature or in concentrated solutions.

Summary

1. Since IAA stimulates the growth and cycocel acts as retardant for the growth of the roots we set out to determine whether is any interaction between CCC and IAA (indol 3-acetic acid) in the effects they produce on mitosis.

2. After 4 hours combined treatment of IAA in the previously mentioned concentrations and 12,000 ppm CCC, there is a depression in the mitotic indices. Cells fixed one hour later after the above mentioned combined treatment show an

increase in MI. On the other hand cells fixed 2 hours later showed a decrease in MI. This trend in decrease the MI continues in the following 4 hours.

3. Sticky in the highest concentrations, C-anaphases-micronuclei and multi-nucleated cells are the types of abnormalities which were observed after the combined treatment of IAA with 12,000 ppm CCC.

4. The treatment of *Vicia faba* root cells with 400 ppm IAA and 12,000 ppm CCC, causes the formation of tetraploid cells and multipolar anaphases in relatively high frequency.

5. The data from roots treated with cycocel indicates that this treatment induce alternation in the duration of mitotic cycles of cells. We have concluded that these alternations occur from changes in the numbers of cells in prophase and metaphase and the numbers of tetraploid metaphases seen in treated roots. On the other hand this change can be counteracted by supply different concentrations of IAA to a relatively moderate concentration of cycocel.

6. In addition a partial antagonistic effect was reported after treating *Vicia faba* roots with 600 and 400 ppm IAA and 12,000 ppm CCC. This finding agrees with the results of other investigations who concluded that antagonism between any two substances was never total.

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