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CHANGES IN MITOTIC INDICES IN ROOTS OF VICIA FABA
I. ANTAGONISTIC EFFECTS OF COLCHICINE AND IAA

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Abstract. Roots of *Vicia faba* were treated with solutions of colchicine or IAA or both. Mitotic indices and the frequencies of the different stages of mitosis were determined immediately after a three hour treatment or following a 24 hour period of recovery. Roots scored after treatment with colchicine for three hours showed several effects, none of which were reversed by simultaneous treatment with IAA. Treatment with IAA for three hours had little detectable effect on mitotic index (MI) on the frequencies of the various stages of mitosis. After a recovery period, following a three hour treatment, of 24 hours, colchicine treated roots showed a significant increase in their MI; this was due largely to an increase in the number of metaphases but it was also due in part to the presence of tetraploid cells in division. IAA treated roots revealed an inhibition of mitotic activity, which was most marked at 3.13—6.26 $\times 10^{-4}$ M IAA. The results from roots treated with mixtures of colchicine and IAA for three hours and fixed 24 hours later showed: 1) the increase in MI induced by colchicine is reversed by IAA, the intensity of the reversal increasing with increasing concentrations of IAA; 2) reductions in the total numbers of cells in prophase or in metaphase occur after treatment with different concentrations of IAA; 3) IAA leads to a reduction in the number of tetraploid cells seen in division.

It appears that colchicine induces a change in the pattern of mitotic activity 24 hours after the end of treatment and its effects are reversed by IAA. At 4.2×10^{-4} M IAA a balance occurs between the opposing effects of colchicine and IAA and the MI is not significantly different from that of the controls. It is suggested that one result of a treatment with colchicine is a change in the level of growth factors in root meristems. This change, which appears to result in a temporary increase in MI is reversed by the addition of IAA. Thus one of the growth factors, the level of which has been affected, is replaceable by exogenous IAA.

Introduction

Colchicine affects cells undergoing mitosis in two ways: 1) it blocks cells at metaphase and suppresses anaphase and 2) it prolongs the duration of metaphase. The consequences of these effects are an increase in the mitotic index (MI), due to the accumulation of cells in metaphase, and the formation, following restitution, of polyploid nuclei. 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.

Colchicine has other effects on roots and they are similar to changes induced by IAA (β-indole-3-acetic acid). Both compounds inhibit root growth and, by altering the polarity of cell expansion, (BURSTRÖM, 1943;

Mohr, 1956) lead to the formation of apical tumours (Levan, 1938; Hawkes, 1942). While colchicine affects cells actually undergoing mitosis, IAA appears to affect cells during interphase; it can stimulate non-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 colchicine and IAA affect mitosis in different ways it must be remembered that they do so over different periods; colchicine produces detectable effects on mitosis more rapidly than IAA.

Unlike colchicine, which occurs in few plant species, 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 us 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).

Since IAA stimulates the growth of colchicine treated roots we set out to determine whether there is any interaction between colchicine and IAA in the effects they produce on mitosis. A high mitotic index is found in roots 24 hours after treatment with colchicine (MacLeod, 1965). This increase is suppressed, however, if roots are treated with IAA at the same time as they are treated with colchicine (Davidson, MacLeod and O'Riordan, 1966). We have determined and we will report here the concentrations of IAA that lower the increase in MI induced by colchicine. Part of the effect of treatment with IAA appears to be due to a reduction in the extent of metaphase delay; and part appears to be due to a delay of entry of cells into prophase. The former effect occurs after treatment with lower concentrations and the latter effect after treatment with higher concentrations of IAA. Both these effects lead to a reduction in MI, 24 hours after the end of treatment. The effect of IAA in reversing a change induced by colchicine provides further evidence that changes in the levels of auxins, i.e. IAA-like growth factors, occur in roots previously treated with colchicine.

Material and Methods

Beans were soaked in distilled water for 24 hours. The testas were removed and the beans planted in moist sand. When the radicles were about 6 cms long the beans were washed and grown in water containing a complete Hoagland's nutrient

solution. The beans were illuminated continuously. The culture solution was aerated and changed every 36 hours. All treatments were carried out at 20° C.

Roots were treated with solutions of colchicine or IAA or both. All solutions of colchicine contained 0.025 %. The solutions of IAA varied from 0.329×10^{-4} M to 6.26×10^{-4} M.

Lateral roots were fixed either at the end of a 3 hour treatment or after a recovery period, following treatment, or 24 hours. The fixative was $^1/_3$ v/v (acetic acid — absolute ethyl alcohol) with 1 drop of formalin. They were hydrolysed in 1 N HCl at 60° C for 8 minutes, stained in Feulgen and prepared as permanent squash preparations. Ten slides were made for each treatment. From each slide 600 cells were scored, at random, giving a total of 6,000 cells scored for each treatment.

Results

1. Treatment for 3 hours

a) IAA. Roots were treated with solutions of IAA, 0.329×10^{-4} M to 6.26×10^{-4} M, for 3 hours. The mitotic index varied slightly following treatment with the different concentrations of IAA (Table 1); the difference between MI in control and treated roots does not, however, appear

Table 1. *Numbers of Cells in Interphase and in Mitosis in Cells of Vicia faba Roots Treated with Colchicine and, or, IAA for 3 hrs. In all Treatments with Colchicine the Final Solution Contained 0.025 %. The values for IAA are from 0.329 to 6.26 $\times 10^{-4}$ M. Each Determination is Based on 6,000 cells; 600 Cells Were Scored from each of 10 Roots*

Colchicine	IAA*	Interphase	Prophase	Metaphase	Anaphase	Telophase	MI
—	—	5,724	178	42	8	48	4.6
+	—	5,527	148	325	—	—	7.9
+	0.329	5,531	223	246	—	—	7.8
+	0.625	5,594	129	277	—	—	6.8
+	1.043	5,450	128	422	—	—	9.2
+	2.083	5.466	156	377	—	1	8.9
+	3.13	5.493	197	310	—	—	8.5
+	4.2	5,566	157	277	—	—	7.2
+	4.7	5,564	163	273	—	—	7.3
+	5.2	5,645	116	239	—	—	5.9
+	5.6	5,642	144	214	—	—	6.0
+	6.26	5,618	145	237	—	—	6.4
—	0.329	5,685	213	50	7	45	5.3
—	0.625	5,799	137	30	9	25	3.4
—	1.043	5,695	200	54	5	46	5.1
—	2.083	5,789	137	46	8	20	3.5
—	3.13	5,770	124	82	8	16	3.8
—	4.2	5,800	126	52	5	17	3.3
—	4.7	5,767	141	50	2	40	3.9
—	5.2	5,797	121	40	18	26	3.4
—	5.6	5,769	145	44	7	35	3.9
—	6.26	5,698	208	40	6	48	5.0

* $\times 10^{-4}$ M.

to be significant. Similar variations were found in the relative frequencies of cells in the various stages of mitosis (Table 2). It appears that with the concentrations used here, a 3 hour treatment had no inhibitory effects on either the entry of cells into mitosis or on the rate at which cells pass through the various stages of mitosis.

Table 2. *Percentages of Cells in Different Stages of Mitosis in Roots of V. faba Treated with 0.025% Colchicine and, or, IAA for 3 Hours*

Colchicine	IAA*	Prophase	Metaphase	Anaphase	Telophase
—	—	64.5	15.2	2.9	17.4
+	—	31.2	68.9	—	—
+	0.329	47.5	52.5	—	—
+	0.625	31.8	68.2	—	—
+	1.043	23.3	76.7	—	—
+	2.083	29.2	70.6	—	0.2
+	3.13	38.9	61.1	—	—
+	4.2	36.2	63.8	—	—
+	4.7	37.4	62.6	—	—
+	5.2	32.7	67.3	—	—
+	5.6	40.2	59.8	—	—
+	6.26	38.0	62.0	—	—
	0.329	67.7	15.9	2.1	14.4
—	0.625	68.2	14.9	4.5	12.4
—	1.043	65.6	17.7	1.6	15.1
—	2.083	64.9	21.8	3.8	9.5
—	3.13	54.0	35.7	3.4	6.8
—	4.2	63.0	26.0	2.5	8.5
—	4.7	60.5	21.5	0.9	17.2
—	5.2	59.0	19.5	8.8	12.7
—	5.6	62.8	19.1	3.0	15.2
—	6.26	68.9	13.3	1.9	15.9

* $\times 10^{-4}$ M.

Roots of only one treatment, 2.083×10^{-4} M, showed a visible response to IAA. Their chromosomes were highly contracted and were between $^1/_8$ and $^1/_{16}$th of the length of normal chromosomes. This effect of IAA on spiralization was not found, however, in other treatments.

b) Colchicine. A 3 hour treatment with colchicine produced the expected changes in the cells; they were arrested at metaphase thus leading to the disappearance of anaphase and telophase stages and producing an increase in the number of metaphases (Table 1). This build up of metaphases reflects the temporary delay, induced at this stage, by colchicine and is largely responsible for the increase in MI from 4.6 to 7.9. In these experiments the increase in MI following treatment with colchicine has not led to a significant difference between control and treated roots ($p = > 0.1$).

c) Colchicine and IAA. The difference between the effects produced on mitosis by IAA and colchicine make it easy to determine whether any interaction occurs between them when they are used together to treat roots. If we consider MI, for example, it is clear that it is always higher in roots treated with colchicine, alone or with IAA, than in roots treated only with IAA. However, differences between pairs of mitotic indices show no consistent trend associated with an increase in the concentration of IAA. The greatest difference between pairs of mitotic indices (5.4) occurs in the middle of the range of IAA concentrations used (2.083×10^{-4} M IAA, Table 1); with the highest (6.26×10^{-4} M) and lowest (0.329×10^{-4} M) concentrations of IAA the differences are small (2.5 and 1.4 respectively). Thus a 3 hour treatment with IAA does not suppress the increase in MI induced by colchicine.

The failure of IAA to alter the increase in MI induced by colchicine reflects its inability, within 3 hours, to reduce the extent of metaphase delay (Table 1). Furthermore, IAA does not restore the ability of cells to undergo anaphase, indicating that the effect of colchicine on the mitotic spindle is not due to an inhibition of some process normally dependent upon IAA.

Thus it appears that IAA, in a 3 hour combined treatment does not modify any of the changes induced by colchicine and roots continue to show an increased MI, metaphase delay and absence of anaphase and telophase stages.

2. Treatment for 3 hours and 24 hours recovery

a) IAA. Mitotic index has been plotted against concentration of IAA to show its effect 24 hours after treatment ended (Fig. 1). It can be seen that only the lowest concentrations of IAA used do not produce an effect on MI; IAA solutions of 3.13×10^{-4} M and stronger reduce the MI. In three groups of roots the MI was less than 1, compared with a control value of 6.95 (Table 3). One of the low values of MI, in roots treated with 4.2×10^{-4} M IAA was compared with the control and the difference in their mitotic indices was very significant ($p = 0.001$). Accompanying the fall in MI fluctuations occur in the relative frequencies of the different stages of mitosis (Tables 3 and 4), probably due, at least partially, to the decreasing numbers of dividing cells in the samples scored. Thus, though there is no detectable effect on MI immediately following a 3 hour treatment with IAA, there is an effect eventually: concentrations of IAA from 3.13×10^{-4} M to 6.26×10^{-4} M lead, within 24 hours, to an inhibition of mitosis (Fig. 1).

b) Colchicine. The mitotic index in roots that had been treated with colchicine 24 hours earlier had risen to 17.2 %. The difference between this value and that of the controls, 6.95 %, is highly significant ($p =$

< 0.001). This experiment has been repeated and the result confirmed (MACLEOD, 1965; O'RIORDAN, 1965). The increase is not due solely to a delay in metaphase, since tetraploid cells were seen in division, nor is it the result solely of a wave of synchronized tetraploid cells entering mitosis together (Table 5). This appears to be a genuine stimulation of cells during the period of interphase that follows the colchicine treatment.

c) IAA and Colchicine. The effect of IAA in reducing the MI (Table 3; Fig. 1) was repeated in roots that had been treated with a mixture of

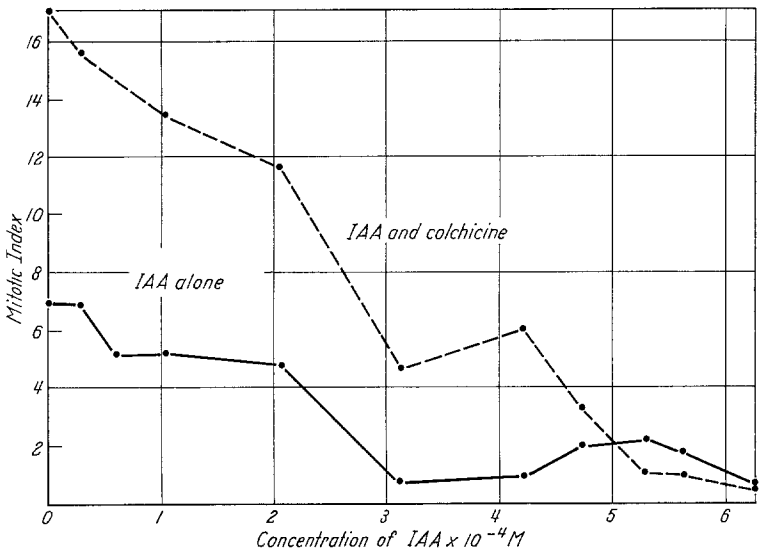

Fig. 1

IAA and colchicine. This effect of IAA was found to increase with increasing concentrations of IAA until, at the highest concentrations ($5.2—6.26 \times 10^{-4}$ M), the MI was 1 % or less.

The extent of the effects of colchicine and IAA on MI can be seen by comparing pairs of mitotic indices from roots treated as follows:

a) controls (MI = 6.95) or roots treated with 0.025 % colchicine (MI = 17.2),

b) 0.025 % colchicine (17.2) or the mixture containing 0.025 % colchicine and 4.2×10^{-4} M IAA (6.0),

c) control (6.95) or 4.2×10^{-4} M IAA (0.9),

d) control (6.95) or the mixture containing 0.025 % colchicine and 4.2×10^{-4} M IAA (6.0).

The differences between these pairs of mitotic indices were significant ($p = 0.001$) in a), b) and c) but not d) where the values are similar (6.95

and 6.0). It is clear that colchicine results in an increased MI and that this increase is suppressed by treatment with 4.2×10^{-4} M IAA. However, it is equally clear that IAA alone depresses the MI and that this depressive effect is reversed by colchicine. Over the range of concentrations of IAA we have used there is a change in their effects at 3.13×10^{-4} M IAA. Lower concentrations of IAA (0.329 to 2.083×10^{-4} M) have little effect

Table 3. *Numbers of Cells in Interphase and in Mitosis in Cells of Vicia faba Roots Treated with Colchicine and, or, IAA for 3 hrs. and Fixed after 24 hrs. Recovery. 600 Cells were Scored from each of 10 Roots*

Colchicine	IAA*	Interphase	Prophase	Metaphase	Anaphase	Telophase	MI
—	—	5,583	231	84	36	66	6.95
+	—	4,969	212	818	—	1	17.2
+	0.329	5,059	284	654	—	3	15.7
+	1.043	5,194	125	677	—	4	13.4
+	2.083	5,297	143	559	—	1	11.7
+	3.13	5,719	138	143	—	—	4.7
+	4.2	5,639	198	163	—	—	6.0
+	4.7	5,805	63	131	—	1	3.3
+	5.2	5,937	33	30	—	—	1.1
+	5.6	5,940	47	13	—	—	1.0
+	6.26	5,970	20	10	—	—	0.49
—	0.329	5,582	262	80	36	40	6.97
—	0.626	5,687	179	70	18	46	5.2
—	1.043	5,683	193	59	18	47	5.3
—	2.083	5,713	182	60	13	32	4.8
—	3.13	5,958	30	10	1	1	0.7
—	4.2	5,944	31	21	1	3	0.9
—	4.7	5,880	74	18	6	22	2.0
—	5.2	5,860	75	41	3	21	2.3
—	5.6	5,897	52	24	6	21	1.7
—	6.26	5,962	3	31	—	4	0.63

* $\times 10^{-4}$ M.

on MI when given alone but they reduce the MI of roots also treated with colchicine (Table 6). Higher concentrations of IAA (3.13 to 6.26×10^{-4} M) reduce the MI significantly when given alone and their effect is counteracted, to some extent, by colchicine. It appears that 24 hours after a 3 hour treatment IAA inhibits and colchicine stimulates mitotic activity. With about 3 to 4×10^{-4} M IAA the inhibitory effects are balanced by the stimulatory effects of colchicine; the result is that the MI of roots treated with 0.025 % colchicine and 4.2×10^{-4} M IAA have a MI that is similar to the controls. The implications of these results will be considered later (see Discussion).

Table 4. *Percentages of Cells in Different Stages of Mitosis in Roots of V. faba Treated with 0.025% Colchicine and, or, IAA for 3 Hours and Fixed 24 Hours Later*

Colchicine	IAA*	Prophase	Metaphase	Anaphase	Telophase
—	—	55.4	20.1	8.6	15.8
+	—	20.6	79.3	—	0.1
+	0.329	30.2	69.5	—	0.3
+	1.043	15.5	84.0	—	0.5
+	2.083	20.3	79.5	—	0.1
+	3.13	48.0	52.0	—	—
+	4.2	54.8	45.2	—	—
+	4.7	32.3	67.2	—	0.5
+	5.2	52.4	47.6	—	—
+	5.6	78.5	21.5	—	—
+	6.26	66.7	33.3	—	—
—	0.329	62.7	19.8	8.6	8.9
—	0.626	57.2	22.4	5.8	14.7
—	1.043	60.9	18.6	5.7	14.8
—	2.083	63.4	20.9	4.5	11.2
—	3.13	71.4	24.0	2.4	2.4
—	4.2	55.4	37.5	1.8	5.4
—	4.7	61.7	15.0	5.0	18.3
—	5.2	53.6	29.3	21.1	15.0
—	5.6	50.5	23.3	5.8	20.4
—	6.26	8.0	81.6	—	10.5

* $\times 10^{-4}$ M.

Table 5. *Percentage Frequencies of Tetraploid Interphases and Metaphases in Roots of V. faba Treated with Colchicine and/or IAA for 3 Hours and Fixed 24 Hours Later*

Colchicine	IAA*	Interphases		Metaphases	
		Total	% Tetraploid	Total	% Tetraploid
—	—	—	—	300	—
+	—	500	15	1000	10.6
+	0.329	500	11.6	1000	13.9
+	1.0			1000	7.2
+	2.1			1000	8.4
+	3.1			800	0.75
+	4.2	500	1.6	700	0.57
+	5.2			214	—
+	6.26			147	—

* $\times 10^{-4}$ M.

MI, in these results, is based solely on numbers of prophases and metaphases, since anaphase and telophase stages were absent. Their frequencies also provide evidence of effects induced by colchicine and IAA.

Table 6. *Numbers of Cells in Prophase and Metaphase; Mitotic Indices; P:M Ratios and Frequencies of Tetraploid Metaphases in Roots of V. faba Treated with Colchicine and/or IAA for 3 Hours and Fixed 24 Hours Later*

IAA ($\times 10^{-4}$M)	0.025% colchicine	Numbers/6000 cells scored		P:M ratio	MI	% number of tetraploid metaphases
		Prophase	Metaphase			
—	—	231	84	2.75:1	6.95	—
—	+	212	818	0.26:1	17.2	10.6
0.329	+	284	654	0.45:1	15.7	13.9
	—	262	80	3.5:1	6.97	—
1.043	+	125	677	0.18:1	13.4	7.2
	—	193	59	3.2:1	5.3	—
2.083	+	143	559	0.25:1	11.7	8.4
	—	182	60	3.3:1	4.8	—
3.13	+	138	143	0.97:1	4.7	0.75
	—	30	10	3:1	0.7	—
4.2	+	198	163	1.2:1	6.0	0.57
	—	31	21	1.4:1	0.9	—
4.7	+	63	131	0.48:1	3.3	—
	—	74	18	4.1:1	2.0	—
5.2	+	33	30	1.1:1	1.1	—
	—	75	41	1.83:1	2.3	—

3. *Numbers of Cells in Prophase and Metaphase*

a) Prophases. In roots treated with colchicine and IAA the numbers of prophase cells are similar in the range 1.043—4.2 $\times 10^{-4}$ M IAA (Table 3). IAA alone reduces the number of prophase cells from 193 and 182, at 1.043 and 2.083 $\times 10^{-4}$ M IAA, to 30 and 31 at 3.13 and 4.2 $\times 10^{-4}$ M IAA. IAA appears to inhibit prophase or to prolong interphase and this is prevented, in treatments with 4.2 $\times 10^{-4}$ M or weaker solutions of IAA, by colchicine.

b) Metaphases. The numbers of metaphase cells first show a fall after treatment with 3.13 $\times 10^{-4}$ M IAA and are low after treatment with all stronger IAA solutions. After treatment with colchicine and IAA mixtures, the numbers of metaphases per 6.000 cells fall gradually, from 654 to 10, as the concentration of IAA increases (Table 3). But whereas with IAA alone numbers of metaphases are low with 3.13 $\times 10^{-4}$ M IAA or greater, they do not reach comparable values in mixtures with colchicine till the concentration of IAA has reached 5.2 $\times 10^{-4}$ M. As with the prophases, IAA reduces the numbers of cells in metaphase and this reduction is reversed, or delayed, in the range 1.043—4.7 $\times 10^{-4}$ M IAA, by colchicine.

To a considerable extent in the results reported here the decrease in MI induced by IAA is due to a decrease in the number of metaphases.

However, we have also shown that there is also an IAA effect on the frequency of tetraploid cells (Tables 5 and 6) and of prophases, indicating that several phases of the mitotic cycle are influenced by IAA.

4. Prophase: Metaphase Ratios

The prophase: metaphase ratio in normal roots is about 3.5:1 (Tables 1—4,6). After colchicine treatment this ratio changes to around 0.3:1. A simultaneous treatment for 3 hrs. with IAA and colchicine does not affect the changed ratio, which remains at about 0.3:1 (Tables 1—4, 6); that is, IAA does not reverse the metaphase delay induced by colchicine. After a recovery period of 24 hours, however, IAA does affect this ratio in roots treated with colchicine and IAA mixtures.

The P:M ratio is still abnormal 24 hours after treatment with colchicine (Table 6). This change is maintained after treatment with colchicine and IAA until the concentration of IAA has reached 3.13×10^{-4} M IAA. In the range of concentrations of IAA we have used in mixtures with colchicine, the P:M ratio approaches a normal value only at $5.6—6.26 \times 10^{-4}$ M IAA and then only when the numbers of cells in division are very low (M. I. is less than 1).

The prophase: metaphase ratios have been compared in roots treated with the following solutions for 3 hours and fixed 24 hours later:

i) water or 0.025 % colchicine,

ii) 0.025 % colchicine or 4.2×10^{-4} M IAA,

iii) water or a mixture of 0.025 % colchicine and 4.2×10^{-4} M IAA,

iv) 0.025 % colchicine or a mixture containing 0.025 % colchicine and 4.2×10^{-4} M IAA,

v) 4.2×10^{-4} M IAA or a mixture containing 0.025 % colchicine and 4.2×10^{-4} M IAA.

With the exception of the last pair of treatments, the differences in P:M ratio were highly significant ($P = 0.00006$). IAA appears to have no effect on P:M ratios when given alone; its effects appear to occur only in roots also treated with colchicine and only several hours after the end of treatment.

These results (Tables 1—6) indicate that the effects induced in roots by colchicine are modified by simultaneous treatment with IAA. IAA has been found to influence the numbers of prophases and metaphases, the duration of metaphase and the number of tetraploid cells in division; these changes result in alterations of P:M ratios and mitotic indices. It appears that colchicine induces changes in the durations of the various stages of the mitotic cycle and its effects are reversible by IAA.

Discussion

We have shown here that colchicine has both immediate and delayed effects on cells undergoing mitosis. The well-known immediate effects of colchicine, seen within 3 hours, are the inhibition of anaphase and the increase in the number of metaphases (EIGSTI and DUSTIN, 1955). The delayed effects, seen in roots of *V. faba* 24 hours after a 3 hour treatment are an increase in MI, a changed prophase: metaphase ratio and the presence of tetraploid cells undergoing mitosis. All these delayed effects are modified if roots are treated with IAA at the same time as they are being treated with colchicine.

1. Prophase: Metaphase Ratio

Colchicine treatments change the P:M ratio due to the delay they induce at metaphase. After a 3 hour treatment the ratio is changed from 4.2:1 in controls, to 0.45:1 in colchicine treated roots (Table 1) and after 24 hours recovery the ratio is changed from 2.5:1 to 0.25:1 (Table 3). IAA does not change this ratio when used alone but it does affect the ratio in roots also treated, 24 hours earlier, with colchicine. The lowest concentration that induces the changed ratio in roots also treated with colchicine is 3.13×10^{-4} M IAA; it also counteracts the mitotic stimulation induced by colchicine. It would appear that IAA changes the relative durations of either prophase or metaphase, but only 24 hours after the end of treatment and only in roots also treated with colchicine. The results indicate that the change in the P:M ratio induced by 3.13×10^{-4} M IAA is due to a reduction in metaphase delay since the number of prophases remains approximately constant in the lower range of IAA concentrations we have used and it is the number of metaphases that falls as concentration of IAA is increased (Table 3). The higher concentrations of IAA inhibit mitosis severely and the P:M ratios become highly variable because they are based on small numbers of cells.

2. Frequency of Tetraploid Cells at Metaphase

Tetraploid cells are seen in division some time after treatment with colchicine (BLAKESLEE and AVERY, 1937). They may be synchronised in division and occur in high frequency soon after treatment (VAN'T HOFF, WILSON and COLON, 1960; MURIN, 1964; VAN'T HOFF, 1965; DAVIDSON, 1965). The presence of tetraploid cells in *V. faba* roots 24 hours after treatment with colchicine shows that cells arrested at metaphase are not delayed indefinately but undergo restitution and complete a mitotic cycle. When the tetraploid cells enter mitosis they may make a significant contribution to the MI and we have already shown that the high MI seen 24 hours after treatment with colchicine depends to a large extent upon

the presence of tetraploid metaphases (DAVIDSON, MACLEOD and O'RIORDAN, 1966). Different batches of beans would not be expected to behave identically and in the present experiments the maximum frequency of tetraploid metaphases seen was 13.9 % (Table 5).

The percentage number of tetraploid cells seen in division can be reduced by treatment with IAA and our results show that the reduction becomes greater as the concentration of IAA increases (Table 5). These results suggest that the effect of IAA is to delay cells in interphase. From the reduction in the percentage frequency of tetraploid metaphases we conclude that the IAA induced delay occurs in some stage that tetraploid cells must pass through before they can enter mitosis but which some diploid cells in the root had already completed. This suggests that the delay is induced in interphase and though it may occur in G_1, S, or G_2, there is some evidence to suggest that the delay occurs in G_1 or S. Tetraploid cells in G_1 would be indistinguishable from diploid cells in G_2 unless all four nucleoli of the tetraploid cell were evident and this would not be expected in all nuclei. After 4.2×10^{-4} M IAA and colchicine roots appear to contain only 1.6 % tetraploid nuclei in interphase (Table 5). The difference between this frequency and that obtained after colchicine alone would be explained by a delay induced, in tetraploid nuclei in G_1, by IAA.

3. Mitotic Indices

The increase in MI that is usually reported to follow a colchicine treatment is detected in the first few hours of treatment and is due to the accumulation of cells at metaphase (LEVAN, 1938; EIGSTI and DUSTIN, 1955). With a three hour treatment with colchicine we have found an increase in MI from 4.6 to 7.9. The increase in MI due to metaphase arrest continues to be found in subsequent fixations, i.e. 6, 12 hours, etc., and high mitotic indices have been reported after 24 hours continuous treatment with colchicine (EVANS, NEARY and TONKINSON, 1957). The period in which high mitotic indices occur may last up to 36 hours after the end of a three hour treatment (DAVIDSON, MACLEOD and O'RIORDAN, 1966) and the results reported here show that part of the increase is due to metaphase delay. Thus, the number of metaphases per 6.000 cells is higher in colchicine treated roots than in controls and the prophase: metaphase ratio is reduced. However, the increase in MI is also due, at least in part, to the presence of tetraploid metaphases. Subtracting the numbers of tetraploid metaphases from the total number of cells in division produces significant reductions in MI (Table 6; DAVIDSON, MACLEOD and O'RIORDAN, 1966) i.e. a MI nearer control values is obtained if we do not include those cells that we are certain completed one whole mitotic cycle after treatment with colchicine. These results show

that in the period after treatment with colchicine some stimulation occurs in interphase and this accounts for the rise in the number of cells seen in division 24 hours later. This stimulation is reversed by IAA.

With increasing concentrations of IAA the first effect that is noted is a decrease in the number of metaphases; this number falls from 654 to 131 per 6.000 cells as the concentration of IAA increases from 0.329 to 4.7×10^{-4} M. Simultaneously the MI falls from 15.7 to 3.3. This effect indicates that IAA, 24 hours after treatment, can affect the duration of metaphase in roots that were also treated with colchicine; at the same time, as we have seen, there is a reduction in the number of tetraploid metaphases that suggests that these concentrations of IAA are inhibiting some interphase processes in addition to their effect on the duration of metaphases (Table 6). A different effect is seen following treatment with 3.13 and 4.2×10^{-4} M IAA. With these concentrations the number of prophases is 30 and 31 per 6.000 cells, compared with a control value of 231; in roots treated with colchicine and IAA, however, there were 138 and 198 prophases. Thus, IAA has a severe inhibitory effect on the number of cells entering prophase and it reduces the M I to values less than 1. Colchicine reverses this inhibition and the M I is 4.7—6.0 (Table 6) after treatment with 3.13 or 4.2×10^{-4} M IAA and colchicine.

The results suggest (Table 6) that all but the weakest concentration of IAA we have used have an inhibitory effect on the mitotic cycle that leads to a gradual fall in the number of cells entering prophase. The inhibitory effect becomes clear at 3.13 and 4.2×10^{-4} M IAA; it is also at these concentrations that the colchicine reversal of the IAA induced inhibition is clear (Table 6). At these concentrations of IAA, colchicine is also able to reverse the severe fall in the M.I. induced by IAA. The effects induced by higher concentrations of IAA are not reversed by colchicine and the roots have low mitotic indices.

4. The Response of Roots to Colchicine and IAA

The data from roots treated with colchicine or colchicine and IAA indicate that these treatments induce alterations in the duration of the mitotic cycles of cells. We have concluded that these alterations occur from changes in the numbers of cells in prophase and metaphase and in the numbers of tetraploid metaphases seen in treated roots. The general indicator of these changes is the mitotic index. The results show that this is affected by changes in the numbers of prophases or metaphases or both. The relative frequencies of prophases and metaphases is given by the P:M ratio and this also differs following different treatments.

Mitotic index, P:M ratio and the composition of a meristem all change in the 24 hours following treatment with colchicine. IAA given at the

same time as colchicine modifies the responses of the roots; it reduces the MI, reverses metaphase delay and so changes both the MI and the P:M ratio and it reduces the frequency of tetraploid cells seen in division. These results suggest that levels of the growth factors that control both interphase processes and the durations of prophase and metaphase change after treatment with colchicine. It appears that colchicine stimulates interphase cells or removes some restraint on division and the result is an increase in MI, 24 hours later. This stimulus is reversed by IAA, which, since it is used at the same time as colchicine, appears to affect the same population of cells. The inhibition induced by IAA is seen both as an effect on the duration of metaphase which it shortens, and as an effect on the length of the mitotic cycle, which it lengthens. Thus it leads to a reduction in the number of cells in prophase and the number of tetraploid cells in division. Its latter effect suggests an inhibition during interphase since the IAA must be exerting its effect after restitution and before mitosis.

It is suggested that root meristems treated with colchicine undergo a change in their levels of growth factors. This change can be counteracted by supplying IAA. The results reported here show that colchicine induced changes in MI are reversed by IAA and we have previously shown that roots whose growth has been inhibited by colchicine are also stimulated by IAA (DAVIDSON, MACLEOD and TAYLOR, 1965). Furthermore, the long term changes that follow colchicine treatment, such as the eventual drop in the mitotic index (DAVIDSON, MACLEOD and O'RIORDAN, 1966) can also be reversed by IAA (MACLEOD, 1966). The hypothesis that has been proposed here and previously (DAVIDSON, MACLEOD and TAYLOR, 1965; DAVIDSON, MACLEOD and O'RIORDAN, 1966) is general in the sense that it refers to changes in the levels of growth factors, not one factor; though we have shown that IAA can reverse the changes that follow colchicine treatment, it should not be inferred that an auxin is the only growth factor whose synthesis has been reduced. It is probable that other factors are also involved.

These studies have been extended and the changes induced by colchicine and IAA have been followed over a period of 8 days (MACLEOD, 1966); they will be reported elsewhere.

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Zusammenfassung

1. Wurzeln von *Vicia faba* wurden mit Colchicin- oder (IES-) Indolessigsäure-Lösungen einzeln oder kombiniert behandelt. Das Mitoseverhältnis und die Frequenz der verschiedenen Mitosestadien wurde unmit-

telbar nach der dreistündigen Behandlung oder anschließend an die 24stündige Erholungsperiode festgestellt.

2. Wurzeln, die drei Stunden lang mit Colchicin behandelt wurden zeigten verschiedene Effekte:

i) Das Mitoseverhältnis war erhöht,

ii) Die Zahl der Metaphasen war erhöht infolge der Metaphasenverschiebung und der Anaphasenhemmung.

3. Die dreistündige Behandlung mit IES allein oder mit Colchicin hatte wenig Einfluß auf das Mitoseverhältnis oder die Frequenz der verschiedenen Mitosestadien. Die durch Colchicin hervorgerufene Hemmung der Anaphase oder Verspätung die Verschiebung der Metaphase wird nach drei Stunden durch IES nicht rückgängig gemacht.

4. Nach einer 24stündigen Periode der Erholung, zeigten die mit Colchicin behandelten Wurzeln:

A. a) eine bedeutende Erhöhung des Mitoseverhältnisses,

b) eine ansehnliche Erhöhung in der totalen Zahl und relativen Frequenz der Metaphasen,

c) tetraploide Metaphasen.

Dagegen zeigten die mit IES behandelten Wurzeln:

B. einen Hemmungseffekt auf das Mitoseverhältnis, der bei einer Konzentration von 3, 13 bis $6{,}26 \times 10^{-4}$ M am größten war und zu einer Herabsetzung des Mitoseverhältnisses war bedingt durch die Verringerung der Zellenzahl in den verschiedenen Mitosestadien

C. Zeigten die mit IES und Colchicin behandelten Wurzeln:

a) Die Erhöhung des Mitoseverhältnisses ist durch IES rückgängig gemacht, wobei die Intensität dieses rückgängigen Prozesses von der IES-Konzentration abhängig ist.

b) Bis zu einer Konzentration von $2{,}083 \times 10^{-4}$ M IES war das Mitoseverhältnis hauptsächlich wegen der Verringerung der Metaphasenzahl verringert; bei höheren Konzentrationen wurde auch die Zahl der Prophasen reduziert.

c) Daß IES zu einer Verminderung der Zahl der tetraploider Zellen in Teilung führt.

5. Es scheint, daß nach einer 24stündigen Behandlung Colchicin zur Stimulation der Mitoseaktivität führt. Die Stimulation wird aber durch IES umkehrbar gemacht. Ein Gleichgewicht zwischen den entgegengesetzten Wirkungen von Colchicin und IES stellt sich ein bei einer Konzentration von $4{,}2 \times 10^{-4}$ M IES und das Mitoseverhältnis ist nicht bedeutend verschieden von dem der Kontrollen.

6. Es wird vermutet, daß eines der Resultate der Behandlung mit Colchicin die Änderung der Stufe der Wachstumsfaktoren in dem

Wurzelmeristem ist. Diese Änderung welche offenbar das Mitoseverhältnis allmählich erhöht, wird mit IES rückgängig gemacht. Wahrscheinlich wird einer der Wachstumsfaktoren, dessen Menge sich im apicalen Wurzelmeristem durch Colchicin verändert hatte, durch IES ersetzt.

References

AUDUS, L. J.: Plant growth substances. London 1959.

BENNET-CLARK, T. A., and N. P. KEFFORD: Chromatography of the growth substance in plant extracts. Nature (Lond.) **171**, 645—647 (1953).

BLAKESLEE, A. F., and A. G. AVERY: Methods of inducing doubling of chromosomes in plants. J. Hered. **28**, 393—411 (1937).

BURGSTRÖM, H.: The influence of heteroauxin on cell growth and root development. Ann. Agr. Coll. Swed. **10**, 209—240 (1942).

D'AMATO, F.: A brief discussion on „endomitosis". Caryologia **6**, 341—345 (1954).

DAVIDSON, D.: Patterns of differentiation in *Vicia* roots. Trans. bot. Soc. Edinburgh **39**, 437 (Abs.) (1963); — A differential response to colchicine of meristems of roots of *Vicia faba*. Ann. Bot. **29**, 253—264 (1965);

— R. D. MACLEOD, and JANET E. TAYLOR: An IAA induced stimulation of growth of roots inhibited by colchicine. New Phytologist **64**, 393—397.

— — and MAUREEN L. O'RIORDAN: Changes in mitotic index induced by colchicine. Submitted to press (1966).

EIGSTI, O. J., and P. DUSTIN: Colchicine. Iowa: Iowa State University Press 1957.

EVANS, H. J., G. J. NEARY, and S. M. TONKINSON: The use of colchicine as an indicator of mitotic rate in broad bean root meristems. J. Genet. **55**, 487—502 (1957).

GOLDACRE, P. L.: Potentiation of lateral root induction by root initials in isolated flax roots. Aust. J. biol. Sci. **12**, 388—394 (1959).

HAWKES, J. G.: Some effects of the drug colchicine on cell division. J. Genet. **44**, 11—22 (1942).

HUGHES, E. W. D., and H. E. STREET: Effects of inhibitory concentrations of 3-Indolylacetic acid and 3-Indolylacetonitrile on cell division and tissue differentiation in excised tomato roots. J. exp. Bot. **11**, 198—205 (1960).

LEOPOLD, A. C.: Auxins and plant growth. California 1955.

LEVAN, A.: The effect of colchicine on root mitosis in *Allium*. Hereditas (Lund) **24**, 471—486 (1938); — Cytological phenomena connected with the root swelling caused by growth substances. Hereditas (Lund) **25**, 87—96 (1939).

MACLEOD, R. D.: Changes in mitotic index following colchicine and indole acetic acid (IAA) treatment. Trans. bot. Soc. Edinburgh **41**, 142 (abs.) (1965); — Changes induced in mitotic indices of roots of *Vicia faba* L. by colchicine and IAA. Ph. D. Thesis University of St. Andrews, Scotland 1966.

MANGENOT, G.: Recherches sur l'action des substances mitoclassiques I, action de la colchicine sur les racines d'*Allium cepa*. Cyt. et Cytophys. veget. **4**, 1—120 (1942).

MOHR, H.: Die Abhängigkeit des Protonemawachstums und der Protonemapolarität bei Farnem vom Licht. Planta (Berl.) **47**, 127 (1956).

MURÍN, A.: Der Mitosezyklus und seine Zeitparameter in den Wurzelspitzen von *Vicia faba* L. Chromosoma (Berl.) **15**, 457—468 (1964).

O'Riordan, M. L.: The effects of colchicine and indoleacetic acid on mitosis in lateral roots of *Vicia faba*. Hons. Thesis University of St. Andrews, Scotland 1965.

Patau, K., N. K. Das, and F. Skoog: Induction of DNA synthesis by kinetin and IAA in excised tobacco pith tissue. Physiol. Plantarum (Kbh.) **10**, 949—966 (1957).

Stern, H.: The physiology of cell division. Ann. Rev. Plant Phys. **7**, 91—114 (1956).

Van't Hof, J.: Discrepancies in mitotic cycle time when measured with tritiated thymidine and colchicine. Exp. Cell Res. **37**, 292—299 (1965).

— G. B. Wilson, and A. Colon: Studies on the control of mitotic activity. Chromosoma (Berl.) **11**, 313—321 (1960).

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