

## Specific inhibitors of RNA and protein synthesis as suppressors of the IAA— and coumarin—induced growth responses\*

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### I. INTRODUCTION

In spite of numerous experiments the mechanism of growth regulating activity of auxins is still a subject of guesses and controversy. Of the many hypotheses evolved for elucidation the mechanism of the auxin-mediated growth responses (cf. Audus 1959; Van Overbeek 1960; Kefford and Goldacre 1961), the opinion that auxins act on the nucleic acid system controlling synthesis of proteins, essential for the cell elongation (Noodén and Thimann 1963 and 1965; Knypl 1963 and 1965) and differentiation, seems to be the most probable (cf. Partanen 1965; Tuan and Bonner 1964). The validity of this assumption can be partially verified by the study of the effects of specific inhibitors of nucleic acids and protein synthesis on the induced growth. Among such inhibitors, used in the experiments reported here, are: Mitomycin C<sub>1</sub> (MMC), actinomycin C<sub>1</sub> (AMC), puromycin (PMC), chloramphenicol (CHI) and two uracil analogues, thiouracil (TU) and diazouracil (DAU).

Mitomycin interferes with replication of DNA by means of the in vivo occurring covalent linking of the complementary DNA strands (Iyer and Szybalski 1963). Actinomycin C<sub>1</sub>, referred to also as actinomycin D, blocks the DNA-dependent synthesis of ribonucleic acid (Goldberg and Rabinowitz 1962, Goldberg et al. 1962) by binding on to the guanine residues of priming DNA (Hamilton et al. 1963). Puromycin inhibits protein synthesis by substituting for the next incoming aminoacyl-sRNA (Yarmolinski and de la Haba 1959) in the polypeptide chain formed on the mRNA template and leads, thus, to the splitting off peptides linked via their terminal carboxyl groups to the p-methoxyphenylalanyl part of the PMC molecule. Chloramphenicol is known as a specific inhibitor of protein synthesis in microorganisms (Brock 1961). The mechanism of action of CHI on plants is obscure since recently it was suggested that this antibiotic can primarily affect oxidative

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phosphorylation in plant cells (Stoner et al. 1964). Nevertheless, there are strong evidences that CHI really blocks protein synthesis in plants (cf. Noodén and Thimann 1965). Diazouracil (Schlegel and Rawlins 1954) and thiouracil by competing with or displacing uracil can presumably lead either to synthesis of abnormal RNA molecules or to blocking the synthesis (cf. Matthews 1958).

## II. MATERIAL AND METHODS

The experiments were carried out on etiolated plants of sunflower (*Helianthus annuus* L. var. Pastewny) and maize (*Zea mays* L. var. Wir-42 or var. Gibrid Bukovinskiy).

10-mm hypocotyl sections were dissected out about 5 mm below the node of the cotyledon from 7–9 cm sunflower plants grown in the dark at 23–25° C for 6–7 days. The sections were preincubated on distilled water for 2 hrs., thoroughly blotted, weighed on a torsion balance and, finally, in lots of 8 transferred into 5.5 cm Petri dishes previously filled with 10 or 8 ml of the solution of a given inhibitor mixed with indolyl-3-acetic acid (IAA) or coumarin. The controls did not contain any inhibitor. Sucrose was added to each Petri dish to a final concentration of 2 per cent.

The dishes, as a rule, were placed at 24–25° C under the continuous illumination produced by „daily-like” fluorescent tubes and electric bulbs (Knypl 1964). Growth of the sections was measured by rapid weighing. Experiments with application of actinomycin, for a reason of its light-sensitivity, were conducted in the dark; in this case the sections were handled in diffused incandescent light.

In the further series of analysis a specific test for gibberellins (Boyarkin and Dmitryeva 1959) was used in the following modification: 17 mm segments were cut off, 12 mm above and 5 mm below the node, from 7–8 days-old etiolated maize seedlings in such developmental stage that the first leaf protruded outwards a coleoptile sheath to about 1–2 cm. The segments were floated on distilled water for 2 hr. in the dark, divided into groups of 10, and finally placed vertically in small glasses (Ø 3.2 cm) previously filled with 10 ml of a nutrient medium of the following composition: Sucrose 2.0%; 0.01 M citric acid-sodium phosphate buffer, pH 5.2; agar-agar 1.0% (cf. Gamborg 1962). Growth regulators or inhibitors were added to this basal medium to the final concentrations as indicated in the proper tables. After a suitable time of incubation at 26° C in the dark in the atmosphere of about 90 per cent relative humidity, the measurements of length of the first leaf and roots of each cutting were made with a millimeter scale. If incubation lasted longer than 48 hours, the segments were replanted into larger glasses (Ø 5.5 cm) containing 25 ml of freshly prepared nutrient media.

The cuttings were manipulated in diffused incandescent light.

## III. RESULTS

## 1. Action of the inhibitors on IAA- and coumarin-induced growth of sunflower hypocotyl sections

Mitomycin C, even in such physiologically high concentration as 80  $\mu\text{g/ml}$  was found to be inactive both against coumarin- and IAA-induced growth (Table 1). Thus, the inference was made that the cell elongation of sunflower is not directly dependent on the replication of DNA. It is dependent, however, on the DNA-directed RNA synthesis as inferred from a finding that actinomycin (4.8  $\mu\text{g/ml}$ ) reduced both IAA- and coumarin-induced growth as well as decreased the rate of elongation in the control, that is in the sample treated with sole AMC (Fig. 1, thin lines). The inhibition, slight initially, is particularly evident after 8 hours of incubation: Within 8th and 12th hrs. growth rate of the control (Fig. 1 *A*) treated with AMC (Fig. 1 *D*) rapidly decreases, and correspondingly to that drops down the growth

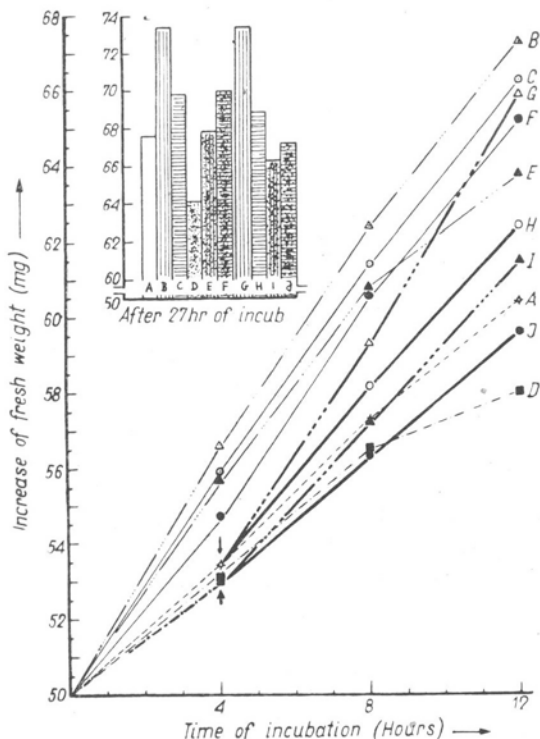


Fig. 1. Effect of actinomycin on the IAA- and coumarin-induced growth of sunflower hypocotyl sections

*A* — Sucrose, 2.0 %; *B* — IAA,  $10^{-5}$  M; *C* — Coumarin, 200  $\mu\text{g/ml}$ ; *D* — AMC, 4.8  $\mu\text{g/ml}$ ; *E* — IAA and AMC; *F* — Coumarin and AMC. Thick lines: Sections pre-treated for 4 hrs. on 2.0 per cent sucrose (controls) or AMC (6.0  $\mu\text{g/ml}$ ) and thereafter transferred into solutions containing growth regulators (controls; thin arrow) or growth regulators and AMC (4.8  $\mu\text{g/ml}$ ; thick arrow). *G* — IAA; *H* — Coumarin; *I* — IAA and AMC; *J* — Coumarin and AMC. Final concentrations of the compounds in mixtures are same as in the case of sole solutions. Incubation was carried out in the dark initial weight of a one section = 50.0 mgm.

rate in the IAA-treated sample (Fig. 1 *B*) containing the inhibitor (Fig. 1 *E*). In the contrary, a slope of the curve representing growth in a (coumarin+AMC) treated sample is unchanged (Fig. 1 *F*). In consequence after 12 hrs. fresh matter of the (coumarin+AMC) section exceeds the weight of the section affected with (IAA+AMC).

Table 1

No effect of mitomycin C on the IAA- and coumarin-induced growth of sunflower hypocotyl sections

Time of incubation, hours	Increment of fresh weight (mgm) under the influence of:						S.E.
	O (Control)	IAA	Coumarin	MMC	IAA +MMC	Coumarin +MMC	
6	54.3	60.7	58.3	54.3	60.8	58.5	$\pm 0.20$
12	58.1	69.9	66.1	58.0	69.7	66.4	$\pm 0.31$
24	64.6	79.1	74.7	63.7	76.7	75.6	$\pm 0.77$

Final concentrations: Sucrose — 2.0 % ; IAA —  $1.2 \times 10^{-5}$  M; Coumarin — 200  $\mu\text{g/ml}$ ; MMC — 80  $\mu\text{g/ml}$ . Initial weight of the section = 50.0 mgm. S.E. = Standard error.

The suppressory action of AMC is more manifested when growth stimulators are applied 4 hrs. after application of the inhibitor (Fig. 1, thick lines). This suggests that IAA and coumarin can act, directly or indirectly, as inductors of RNA synthesis. One can assume that RNA under consideration is the messenger RNA(s), acting as a template for the synthesis of protein(s).

If this conclusion is correct than AMC in higher concentration ought to prevent the induction of growth. As it is seen from the data plotted in Fig. 2, AMC (10  $\mu\text{g/ml}$ ) markedly reduced growth of the sections. It is striking that after 8 hrs. of incubation a curve for (IAA+AMC) treated sample (Fig. 2 *E*) runs parallelly to the analogical curve for the proper control, that is AMC alone treated sample (Fig. 2 *D*): After 8 and 12 as well as after 34 hrs. fresh matter of the former sample is about 3 mg higher in comparison with the weight of the control. Thus, after 8 hrs. of action AMC completely stopped the IAA-dependent promotion of growth. Nevertheless, even in a concentration of 20  $\mu\text{g/ml}$  actinomycin was unable to prevent the stimulatory action of IAA and coumarin applied 4 hours later, despite the fact that AMC in this dose inhibited growth in the control to about 65 per cent. It may be concluded, therefore, that (1) the cell elongation of sunflower hypocotyl section is dependent on the synthesis of RNA. It seems that (2) auxin stimulates this process, and that the induced growth is dependent upon and follows the induced RNA synthesis. Nevertheless, (3) some portion of the net induced growth cannot be explained on the basis of the above assumption.

If really IAA and coumarin stimulate the synthesis of RNA directing the synthesis of essential protein(s), i.e. the template mRNA, then puromycin should affect growth of the treated samples in a manner similar to that of AMC. The data plotted, in Fig. 3 show that this is the case: PMC (58  $\mu\text{g/ml}$ ), applied in a mixture with IAA,

markedly reduced IAA-induced growth between 4th and 8th hrs. of incubation, and almost completely prevented the stimulatory action of the auxin after 8 hours (cf. fresh matter of the sections after 27 hrs.). Again, sections preincubated in

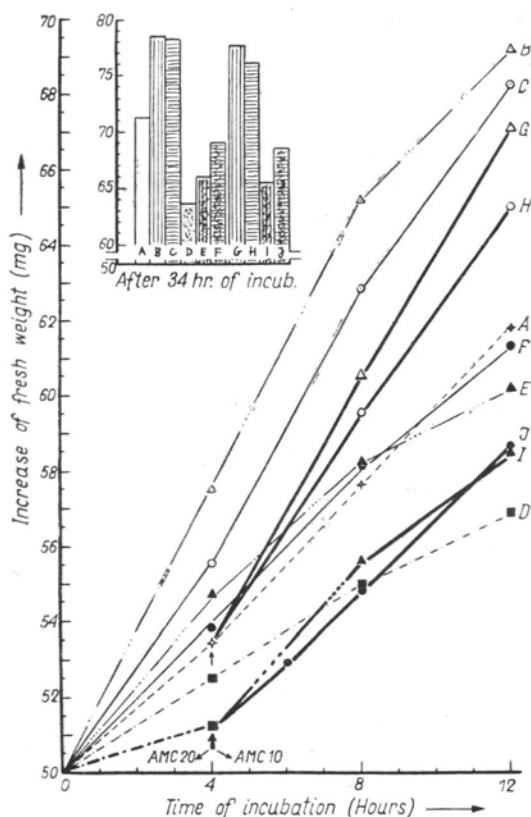


Fig. 2. Effect of actinomycin on the IAA- and coumarin-induced growth of sunflower hypocotyl sections.

*D* — AMC, 10  $\mu\text{g}/\text{ml}$ ; *I* and *J*, sections pre-treated for 4 hrs. in AMC, 20.0  $\mu\text{g}/\text{ml}$ ., and thereafter transferred into solutions containing IAA (*I*) or coumarin (*J*) and AMC, 10  $\mu\text{g}/\text{ml}$ . Concentration of AMC in *E* and *F* = 10  $\mu\text{g}/\text{ml}$ . Other details are the same as described in Fig. 1.

PMC for 4 hrs. continued to respond to the growth stimulators added later, but the magnitude of the response was greatly reduced in comparison with the control which had not been treated with PMC.

More concentrated solutions of PMC produced symptoms of the general toxication when incubation lasted longer than 12 hours (Table 2).

Similar results to those produced by PMC were noted also after application of chloramphenicol in a concentration slightly affecting growth in the control (Fig. 4), though CHI is far less effective than PMC.

It is worth noting that the IAA-induced growth seems to be much more sensitive to AMC, PMC and CHI than is the coumarin-induced one. This is well illustrated by the corresponding values of fresh weight of the sections incubated for

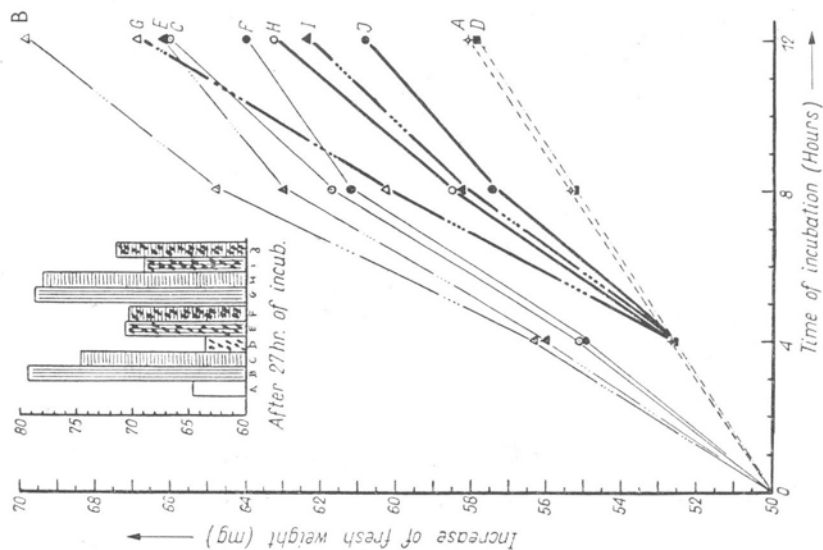


Fig. 3

Fig. 3. Effect of puromycin on the IAA- and coumarin-induced growth of sunflower hypocotyl sections.

A — Sucrose, 2.0 %; B — IAA,  $1.2 \times 10^{-5}$  M; C — Coumarin,  $200 \mu\text{g/ml}$ ; D — PMC,  $58.0 \mu\text{g/ml}$ ; E — IAA and PMC; F — Coumarin and PMC. Thick lines: sections pre-treated for 4 hrs. on 2.0 per cent sucrose (the controls) or PMC ( $58.0 \mu\text{g/ml}$ ) and then transferred into solutions containing growth regulators (controls) or growth regulators and PMC ( $58.0 \mu\text{g/ml}$ ). G — IAA; H — IAA and PMC; I — Coumarin; J — Coumarin and PMC. Final concentrations of IAA and coumarin in mixtures with PMC are the same as in the case of sole solutions. Incubation was carried out in the light.

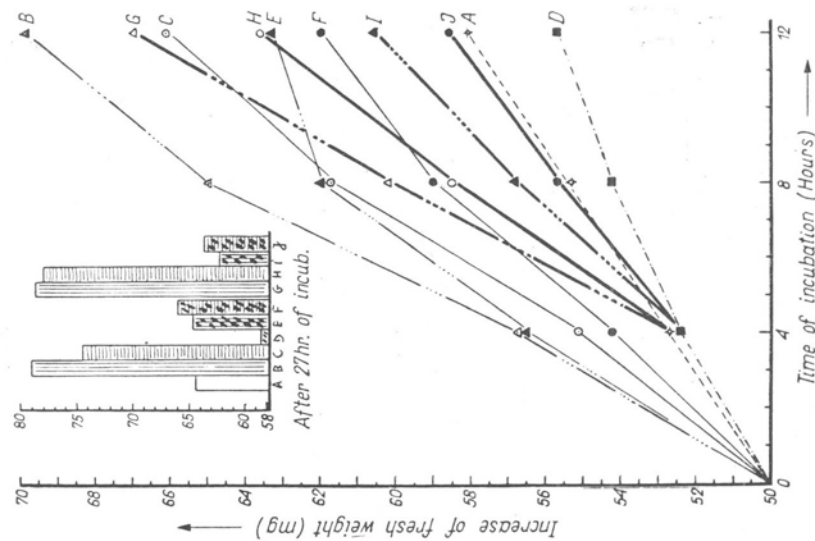


Fig. 4

Fig. 4. Effect of chloramphenicol on the IAA- and coumarin-induced growth of sunflower hypocotyl sections.

Description the same as in Fig. 3; instead of PMC read CHI,  $5 \times 10^{-3}$  M.

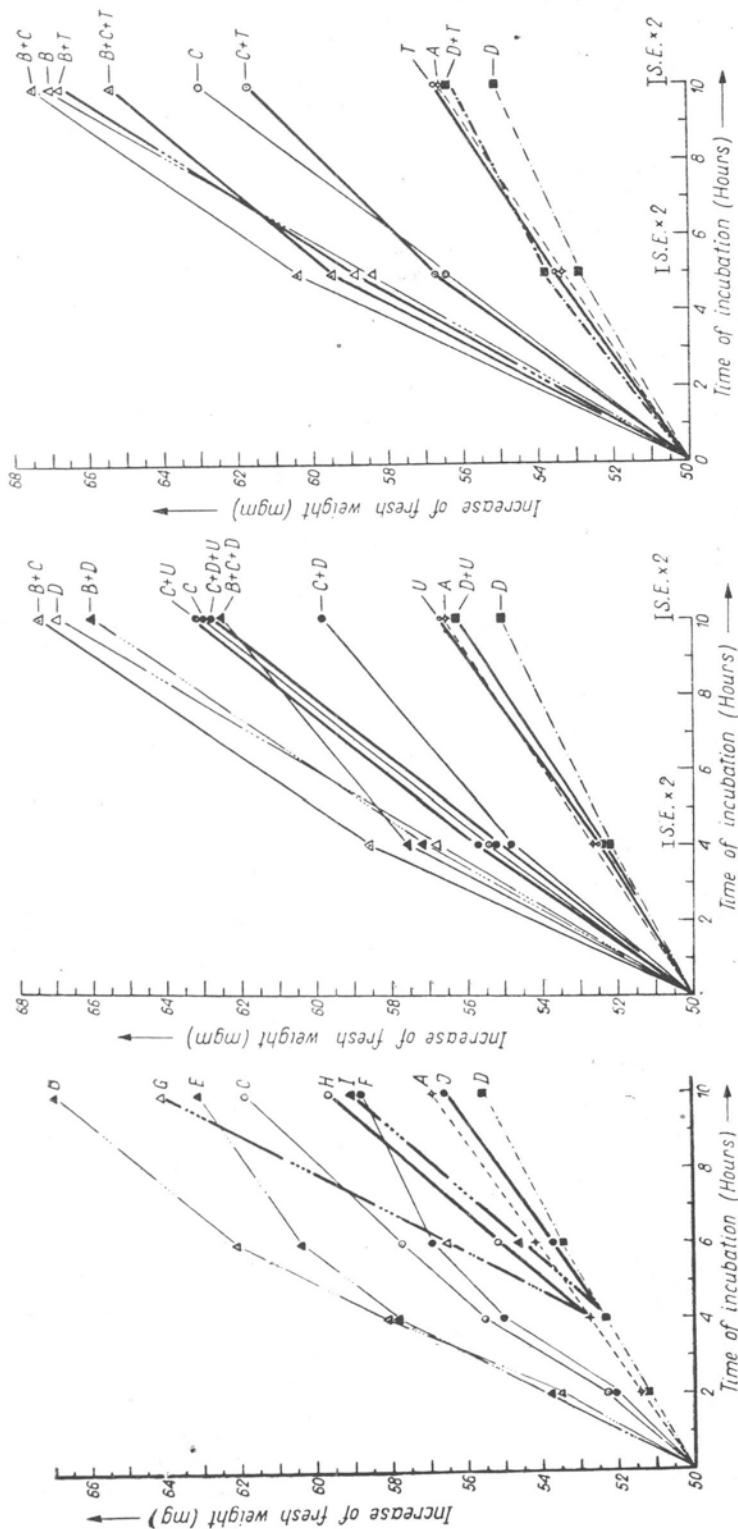


Fig. 5

Fig. 6

Fig. 7

Fig. 5. Effect of diazouracil on the IAA- and coumarin-induced growth of sunflower hypocotyl sections. Description the same as in Fig. 3; instead of PMC read DAU, 40.0  $\mu\text{g/ml}$ .

Fig. 6. Reversion by uracil of the DAU-produced inhibition of the IAA- and coumarin-induced growth of sunflower hypocotyl sections.

A - Sucrose, 2.0% (62.3); B - IAA,  $1.2 \times 10^{-5}$  M (72.4); C - Coumarin, 200  $\mu\text{g/ml}$  (72.4); D - diazouracil, 40.0  $\mu\text{g/ml}$  (58.0); U - Uracil, 150.0  $\mu\text{g/ml}$  (62.4); B+C (76.2); B+D (70.6); C+U (72.2); C+D+U (66.3); B+C+D (66.3); C+D (64.0); D+U (61.4). Concentrations of the compounds tested in mixtures are the same as in the case of single solutions, e.g. B+C = IAA,  $1.2 \times 10^{-5}$  M and coumarin, 200  $\mu\text{g/ml}$ . In parentheses is noted a fresh weight (mg.) of a given sample after 26 hours of incubation in the light.

Fig. 7. Effect of thiouracil on the IAA- and coumarin-induced growth of sunflower hypocotyl sections.

A - thiouracil,  $2.5 \times 10^{-3}$  M; other symbols mean the same as in Fig. 6. Fresh weight (mg.) of samples after 26 hours of incubation in the light: A - 62.3; B - 77.2; C - 72.4; D - 58.7; T - 62.5; B+C - 76.3; B+T - 74.9; B+C+T - 72.5; C+T - 67.8; D+T - 59.3.

27–34 hrs. (see Figs. 1–4). Additionally, CHI in a concentration of  $10^{-3}$  M did not significantly decrease growth rate of the coumarin-treated sample while it markedly affected the auxin-induced growth (unpublished data).

Table 2

Inhibitory effect of puromycin on the IAA- and coumarin-induced growth of sunflower hypocotyl sections

Time of incubation Hours	Series	Increment of fresh weight (mgm.) under the influence of:						S.E.
		O (Control)	IAA	Coumarin	PMC	IAA + PMC	Coumarin + PMC	
5	A	53.4	58.5	56.4	53.1	56.5	55.5	$\pm 0.21$
8	A	55.2	63.1	60.2	54.8	58.2	58.1	$\pm 0.28$
29	A	62.4	74.2	71.1	56.6*	56.4*	59.4*	$\pm 1.10$
4	B	52.7	56.8	54.4	52.4	56.8	54.5	$\pm 0.16$
10	B	56.6	66.0	61.2	55.3	60.9	60.7	$\pm 0.31$
29	B	64.1	75.2	71.6	59.5	64.5	66.4	$\pm 1.10$

Final concentrations: IAA –  $10^{-5}$  M; PMC – 120  $\mu\text{g/ml}$  in a series A and 40.0  $\mu\text{g/ml}$  in a series B.

\* Visible symptoms of toxication: the sections are brown and flexible. Other details as in Table 1.

The results of experiments carried out with application of DAU are very striking. In this case coumarin-induced growth, when compared with the IAA-induced one, was more strongly reduced. After pre-incubation of the sections for 4 hours with diazouracil the growth promoting activity of coumarin manifested only slightly (Fig. 5 J) and the sections did not reach weight of the basal control, i.e. the sucrose alone treated sample (Fig. 5A). The inhibition due to DAU is initially completely, and in the final analysis partially, reversed by uracil applied in 3–4 fold excess (Fig. 6). Moreover, the degree of inhibition is initially decreased in part by thiouracil (Fig. 7). It is very possible, therefore, that DAU competes with uracil in this system and leads probably either to blocking of the synthesis or to the synthesis of defective, i.e. inactive, RNA molecules.

Like DAU, thiouracil used in the concentration slightly affecting the IAA-induced growth ( $2.5 \times 10^{-3}$  M) markedly decreased the coumarin-induced elongation (Fig. 7). Thus, the primary mode of action of coumarin basically differs from that of IAA.

## 2. No effect of the inhibitors of RNA and protein synthesis on the gibberellin induced growth of the first leaf of maize

Since inhibitors used in this study inhibited coumarin- and IAA-induced growth, it was of interest to test whether this specific inhibitors will be active against the gibberellin mediated phenomena engaged in growth.

The experiments were performed with the segments of maize seedlings taken out with the node and a part of the first internode. According to the original data

(Boyarkin and Dmitryeva 1959) growth of the first leaf in this test is either unsensitive to IAA or is inhibited by auxin applied in higher concentrations. As in the case of bean and pea stem sections (Brian et al. 1960), gibberellic acid (GA) inhibits and auxin stimulates rooting and growth of roots of the maize seedling cuttings (cf. Gamburg 1962).

Table 3

Effect of mitomycin C on the GA-stimulated growth of the first leaf of maize (var. *Gibrid Bukovinskiiy*) seedling cuttings

Parameter measured	Time of incubation, Hours	Growth regulators added:			
		O	MMC	GA	MMC + GA
Mean length of the leaf (mm) $\pm$ S.D.	48	8.9 $\pm$ 0.3	10.2 $\pm$ 0.4	24.0 $\pm$ 0.6	32.1 $\pm$ 0.8
	72	12.5 $\pm$ 0.4	14.0 $\pm$ 0.4	29.9 $\pm$ 0.9	28.8 $\pm$ 1.0
Average length of a single root (mm)	48	6.6	4.3	0	0
	72	7.4	7.0	0	0
Average number of roots per cutting	48	1.0	0.8	0	0
	72	1.5	1.5	0	0

Final concentrations: Mitomycin C — 20  $\mu$ g/ml; Gibberellic acid —  $10^{-5}$  M.

Initial length of the first leaf = 0 mm. S. D. = standard deviation.

Table 4

Effect of actinomycin and puromycin on the GA-stimulated growth of the first leaf of maize (var. *Gibrid Bukovinskiiy*) seedling cuttings

Parameter measured*	Growth regulators added**:					
	0	GA	GA + PMC	GA + AMC	PMC	AMC
Mean length of the first leaf (mm) $\pm$ S.D. of the mean	8.8 $\pm$ 0.3	24.4 $\pm$ 0.9	24.0 $\pm$ 0.8	24.0 $\pm$ 0.9	8.0 $\pm$ 0.2	8.7 $\pm$ 0.2
Average length of a single root (mm)	2.3	0	0	0	2.0	2.1
Average number of roots per cutting	1.3	0	0	0	1.1	1.1

\* Measurements were made after 48 hrs. of incubation.

\*\* Final concentrations: GA —  $10^{-5}$  M; AMC — 8.3  $\mu$ g/ml; PMC — 30.0  $\mu$ g/ml.

As it will be seen from the data of Table 3 mitomycin (20  $\mu$ g/ml) did not affect growth of the first leaf of maize, both in presence or in absence of GA. In the contrary, growth of roots was significantly inhibited in comparison with the control not treated with MMC, particularly after 48 hrs. of incubation.

Actinomycin (8.3  $\mu$ g/ml) and puromycin (30.0  $\mu$ g/ml) did not reduce growth

of the GA-stimulated leaves at all (Table 4). Rooting of the segments and the growth of roots were also insignificantly affected. This result is very confusing since there is no doubt that during the growth of roots RNA and proteins are synthesized. Therefore, in subsequent series of analysis the segments were pre-treated for 4 hrs. with AMC (30.0  $\mu\text{g/ml}$ ) or PMC (120.0  $\mu\text{g/ml}$ ) by means of floating on the proper solutions in the dark at 26° C, and subsequently they were planted into glasses containing nutrient media supplemented with GA ( $10^{-5}$  M) and the inhibitors as indicated in Table 5. In this case AMC and PMC reduced the GA-stimulated growth of the first leaf. Nevertheless, the inhibition seemed to be quite unspecific since growth of the proper control was inhibited to the same relative degree. After 3 days of incubation the internode part of the AMC treated cuttings, dipped into the nutrient medium, became brown. It is worth of note that this symptom of an overall toxication in the case of (GA+AMC) treated samples appeared in the second day of cultivation.

Table 5

Effect of actinomycin C<sub>1</sub> and puromycin on the GA-stimulated growth of the first leaf of maize (var. *Gibrid Bukovinskiy*) seedling cuttings

Parameter measured	Time of incubation, Hours	Growth regulators added*:					
		O	GA	GA + AMC	GA + PMC	AMC	PMC
Mean length of the first leaf (mm) ± S.D.	19	4.7±0.1	10.7±0.3	9.6±0.2	8.8±0.2	4.7±0.2	4.3±0.1
	43	9.3±0.3	24.2±0.8	21.6±0.8	20.9±0.7	8.1±0.3	8.4±0.2
	72	13.3±0.5	29.4±0.9	24.3±0.9	26.8±0.9	10.8±0.4	12.6±0.6
Average length of a single root (mm)	19	1.8	0	0	0	1.8	1.8
	43	4.8	0	0**	0	3.4	4.6
	72	5.0	0	0**	0	3.4**	4.6
Average number of roots per cutting	19	0.2	0	0	0	0.2	0.2
	43	0.9	0	0	0	0.9	0.9
	72	1.5	0	0	0	1.5	1.5

\* Cuttings before planting into nutrient media were pre-incubated for 4 hrs. in distilled water (control), AMC (30  $\mu\text{g/ml}$ ) or PMC (120  $\mu\text{g/ml}$ ) and thereafter transferred into nutrient media containing GA or the inhibitors as indicated in the table in the following concentrations: GA -  $10^{-5}$  M; AMC - 8.3  $\mu\text{g/ml}$ ; PMC - 30.0  $\mu\text{g/ml}$ . O and GA samples were pre-incubated in dist. water; AMC and AMC+GA samples were pre-incubated in AMC; PMC and PMC+GA samples were pre-incubated in PMC.

\*\* Visible symptoms of toxication, i.e. browning of the internode part of the cuttings dipped into nutrient media.

As it is seen from the data of Table 6 chloramphenicol ( $5 \times 10^{-3}$  M) and diazouracil (50  $\mu\text{g/ml}$ ) also affected growth of the leaves relatively slightly. In the contrary rooting, and especially the subsequent growth of roots, were markedly inhibited by these antimetabolites. Under their influence the internode part of the treated cuttings, dipped into the nutrient media, became brownish after 48 hrs. Uracil (150  $\mu\text{g/ml}$ ) abolished not only the DAU-dependent toxication but completely reversed also the inhibition of growth of the first leaf and roots, and partially

Table 6

The effect of chloramphenicol and diazouracil on the gibberellic acid stimulated growth of the first leaf, and on the IAA-mediated rooting of maize (var. *W/i-42*) seedling cuttings

Parameter measured*	Growth regulators added**:								
	O	CHI	DAU	IAA	IAA + CHI	IAA + DAU	GA	GA + CHI	GA + DAU
Mean length of the leaf (mm) $\pm$ S.D.	9.3 $\pm$ 0.2	7.3 $\pm$ 0.4	8.0 $\pm$ 0.4	8.9 $\pm$ 0.4	7.3 $\pm$ 0.4	7.6 $\pm$ 0.4	24.6 $\pm$ 1.0	20.0 $\pm$ 1.0	20.1 $\pm$ 1.1
Average length of a single root (mm)	8.6	2.2	2.3	9.1	3.5	3.3	0	0	0****
Average number of roots per cutting***	1.3 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.8 $\pm$ 0.3	1.8 $\pm$ 0.3	1.1 $\pm$ 0.2	0 $\pm$ 0.1	0 $\pm$ 0.3	0 $\pm$ 0.2

\* Measurements were made after 40 hours of incubation.

\*\* Final concentrations: IAA — 10 $\cdot$ 6 M; GA — 10 $\cdot$ 5 M; CHI — 5  $\times$  10 $\cdot$ 3 M; DAU — 50  $\mu$ g/ml.

\*\*\* Roots primordia are noted after „+“.

\*\*\*\* Visible symptoms of toxication (browning, flexibility).

reversed the inhibition of rooting produced by DAU (Table 7). There is no doubt, therefore, that DAU competes with or displaces uracil.

Since inhibitors applied in this study reduced both the IAA- and coumarin-induced longitudinal extension of sunflower hypocotyl sections (Figs. 1—7) and inhibited rooting and growth of the roots of maize seedling cuttings (Tables 3—7), that is affected the auxin-mediated growth phenomena, and decreased the GA-stimulated growth of the first leaf of maize not specifically as seems, it must be concluded that the mode of action of auxins basically differs from the mechanism of action of the gibberellins. This statement does not exclude a possibility that both auxins and gibberellins can act at the same growth promoting centers, and may compete for them (cf. Ng and Audus 1964).

Table 7

Reversion by uracil of the diazouracil-produced inhibition of growth of maize cuttings (*Zea mays* var. *Gibrid Bukovinskiiy*)

Parameter measured	Time of incubation, Hours	Substances added*:			
		O	DAU	U	DAU + U
Mean length of the first leaf (mm) $\pm$ S.D.	48	11.2 $\pm$ 0.4	9.0 $\pm$ 0.3	11.1 $\pm$ 0.3	10.8 $\pm$ 0.2
	92	17.3 $\pm$ 0.6	14.3 $\pm$ 0.6	17.3 $\pm$ 0.6	17.0 $\pm$ 0.4
Average length of a single root (mm)	48	2.7	1.3	2.3	2.4
	92	4.4	2.3	4.5	4.0
Average number of roots per cutting**	48	0.8 $\pm$ 0.4	0.5 $\pm$ 0.3***	1.1 $\pm$ 0.5	0.8 $\pm$ 0.6
	92	1.6 $\pm$ 0.6	0.8 $\pm$ 0.5***	1.6 $\pm$ 0.7	1.2 $\pm$ 0.7

\* Final concentrations: DAU — 50  $\mu$ g/ml; uracil (U) — 150  $\mu$ g/ml.

\*\* Root primordia are noted after „+”.

\*\*\* Visible symptoms of toxication of the internode part of the cuttings.

Recently Neumann (1964) has found that uptake of actinomycin by the shoot of lettuce seedling is very poor in comparison with its uptake by the roots. If it is a case also for etiolated maize seedling cuttings, then tests with AMC and GA should be repeated with use of higher AMC concentrations and direct analysis of the degree of penetration of AMC into the cells must be conducted.

### III. DISCUSSION

The data of Figs. 1—7, as taken generally, lead to the conclusion that IAA and coumarin can realize their biological potency through the induction and/or acceleration of the synthesis of specific protein(s), catalysing processes concerned with growth, and determining the actually observed growth rate. It is quite possible that these growth regulators, as it has been reported for some animal hormones (e.g. Wool and Munro 1963; Korner 1963; Talwar and Segal 1963; Liao

and Williams-Ashman 1962), co-ordinate the synthesis of proteins by means of induction of synthesis of specific messenger ribonucleic acids, either (1) by the direct combination with the genetic apparatus of the cell, (2) by a direct combination with repressors, or (3) by induction of synthesis of specific metabolite(s) which combines with the repressor molecules or with the gene regulators (cf. Jacob and Monod 1961).

A finding that stimulatory activities of the growth regulators examined here were markedly reduced when they were applied 4 hours after AMC, i.e. after a period of time arbitrarily taken as being enough for penetration of the inhibitor into the cell and combination of it with the priming DNA, offers a strong evidence for validity of the assumption that IAA- and coumarin-induced cell elongation is dependent on, and is preceded with, the synthesis of mRNA. If the above formulated assumption is correct, then the enzyme(s) synthesized on the considered mRNA(s) template(s) should be active about 6–8 hours since the rapid inhibition of growth following the application either of AMC or PMC, DAU and CHI occurred after 8 hours (cf. Knypl 1965). It is possible, further, that RNA synthesized owing to the participation of coumarin is rich in uracil since thiouracil and diazouracil, in concentrations slightly active or uneffective against the IAA-induced growth ( $2.5 \times 10^{-3}$  M and  $10^{-4}$  M, respectively), markedly reduced the coumarin-induced growth. The inhibition was reversed by uracil: Such a result indicates that DAU is acting in this system as the antagonist of uracil. Keeping in mind that DAU inhibited the reproduction of tobacco mosaic virus (Schlegel and Rawlins 1954) and stopped the growth of *Datura stramonium* tumours decreasing contents of nucleic acids and proteins in them (Urbanek 1963) one can assume that DAU acts as the specific inhibitor of RNA synthesis.

Specific inhibitors of RNA and protein synthesis reduced a magnitude of response of sunflower hypocotyl sections to IAA and coumarin. Nevertheless, the sections with markedly reduced growth rate as the result of pre-treatment for 4 hrs. with actinomycin (Fig. 1 and Fig. 2) or puromycin (Fig. 3) still continue to respond to these stimulators added later. Such a finding seems to favour a suggestion that protein synthesis is not required for the initiation of the auxin action, though some product(s) of protein synthesis is necessary for the actual process of rapid cell elongation (Cleland 1963). Is, therefore, the formulated assumption about a role of auxin and coumarin as the inducers of mRNA(s) synthesis incorrect? This question cannot be answered until the direct analysis will be carried out with labeled amino-acids and RNA precursors, and with labeled IAA and coumarin. Nevertheless, it is evident that the induced growth is dependent on a protein(s) "X" stable for about 6–8 hours (cf. Fig. 3, and Knypl 1965). RNA preceding the synthesis of protein  $X_1$ , necessary for the coumarin-induced growth, is presumably rich in uracil (cf. Figs. 5–7) whereas the synthesis of protein  $X_2$ , necessary for the IAA-induced growth, is probably directed by RNA rich in cytosine. The latter assumption is drawn from the fact that IAA-induced growth, in difference to the coumarin-induced one, is more sensitive to the inhibitory action of AMC, which specifically binds on to the guanine residues of the priming DNA.

Coumarin in a concentration of 2.0 p.p.m. to 50 per cent inhibited reproduction of tobacco mosaic virus in *Nicotiana tabacum* L. (Knypl and Gubanski 1960), and adenine partially reversed the coumarin (50 p.p.m.) induced inhibition of growth of the first leaf of maize (Knypl 1963). These data indirectly favorise the assumption that coumarin can control the synthesis of RNA and proteins.

As early as 1949 Ber had suggested that auxins could regulate proteins synthesis by influencing RNA metabolism in plants. A few years later Silberger and Skoog (1953) have demonstrated that in the IAA treated tobacco pith tissue growth is preceded by the increment of the level of nucleic acids. Höhn (1954 and 1955) and Skoog and Miller (1957) have maintained that IAA is involved in the metabolism of nucleic acids; other authors have pointed out that auxins are necessary for mitosis and replication of DNA (Das et. al. 1956; Patau et al. 1957). Masuda (1959 and 1960) had found that *Avena* coleoptiles treated with ribonuclease only slightly respond to IAA, and Biswas and Sen (1959) revealed that IAA enhances the rate of turnover of phosphorus in oat and rice coleoptile tissues. On the other hand, there are many evidences that IAA can be bound with protein(s) (Siegel and Galston 1953; Sen Gupta and Sen 1961), nucleotides (Wedding and Black, 1964), or with amino-acids (Südi 1964). It is of interest that incubation of IAA with peroxidase yields a substance of unknown structure (IAA') which forms a complex with purified pea shoot RNA (Kefford et al. 1963). Naphthalene acetic acid-1- $C^{14}$  also binds to the nuclear material of pea internodes (Roychoudhury and Sen 1964): This binding is associated with a release of RNA. IAA in low concentrations stimulated the synthesis of both RNA and DNA in young fruits of coconut and pea internodes, whereas in higher concentrations the auxin stimulated the release of RNA from some its "bound" form (Roychoudhury and Sen 1964). Actinomycin, puromycin and chloramphenicol all inhibited the IAA-induced growth and protein synthesis in artichoke tuber disks, pea stem sections and *Avena* coleoptile sections (Noodén and Thimann 1963 and 1965). Sunflower hypocotyl sections pretreated with these inhibitors still continue to respond to IAA but the magnitude of response is markedly reduced (Knypl 1965). IAA induces enzymatic activity dependent on the synthesis of RNA (Venis 1964). All these data point on the possibility that auxins can act as the inductors of mRNA synthesis. At present it is not possible to build a molecular model of this action. It seems, however, that exogenously applied auxin is, in a first step, enzymatically activated. The activated auxin (e.g. IAA', cf. Kefford et al. 1963, or deuteriauxin, ref. to Bitancourt 1963) in a second step binds on to the genetic apparatus of the cell, complexing either with repressor molecules, which presumably are RNAs or ribonucleoproteins (cf. Jacob and Monod 1961), or complexing directly with the gene regulator. In a result of this binding mRNAs can be synthesized on the cistrons of a given operon. Finally, on the mRNA templates enzymes are synthesized which catalyse the numerous physiological changes following the application of the auxin.

Recently Key (1964) and Key and Shannon (1964) have reported that auxins stimulate synthesis of RNA in soybean hypocotyl tissue. Enhancements of synthesis of RNA and protein by IAA was reported by Datta and Biswas (1965) and by

Hamilton with co-workers (1965). These direct analyses by no means indicate that RNA and protein synthesis are essential for the auxin-induced growth. Nevertheless, it is still unknown whether or not auxins are directly involved in the synthesis of RNA and how, in details, they act on the genetic apparatus of the cell.

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#### SUMMARY

Actinomycin C<sub>1</sub>, puromycin, chloramphenicol, diazouracil and thiouracil all inhibited the IAA- and coumarin-induced growth of sunflower hypocotyl sections, and reduced rooting and subsequent growth of the roots of etiolated maize seedling sections. The inhibitors did not specifically affect the gibberellic acid-stimulated growth of the first leaf of maize cuttings. Thus, auxin mediated growth phenomena are dependent on the protein and RNA synthesis. It is suggested that IAA and coumarin can act as the inductors of the synthesis of messenger RNA(s).

In comparison with each other, the coumarin-induced growth is more sensitive to the inhibitory action of diazouracil and thiouracil, while the IAA-induced growth is more inhibited by actinomycin, puromycin and chloramphenicol. Inhibitions produced by diazouracil are reversed or markedly reduced by uracil.

Mitomycin C was without effect on the IAA- and coumarin-induced growth.

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#### REFERENCES

- Audus L. J., 1959. In *Plant Growth Substances*, Leonard Hill (Books) Ltd., pp. 88—114, London.  
Ber A., 1949, *Experientia* 5: 455.  
Biswas B., and S. P. Sen, 1959, *Nature* 183: 1824.  
Bitancourt A. A., 1963, *Nature* 200: 548—552.  
Boyarkin A. N., and M. I. Dmitryeva, 1959, *Fiziol. Rastenii* (Moscow) 6: 741—747.  
Brian P. W., H. G. Hemming and D. Lowe, 1960, *Ann. Bot.*, N. S., 24: 407—419.  
Brock J. D., 1961, *Bacteriol. Revs.* 25: 32—48.  
Cleland R., 1963, *Nature* 200: 908—909.  
Das N. K., K. Patau and F. Skoog, 1956, *Physiol. Plantarum* 9: 640—651.  
Datta A., and B. B. Biswas, 1965, *Experientia* 21: 633—634.  
Gamborg K. Z., 1962, *Doklady Akademii Nauk S. S. S. R.* 145: 941—944.  
Goldberg I. H., and M. Rabinowitz, 1962, *Science* 136: 315—316.  
Goldberg I. H., M. Rabinowitz and E. Reich, 1962, *Proc. Nat. Acad. Sci. U. S.*, 48: 2094—2101.  
Hamilton L. D., W. Fuller and E. Reich, 1963, *Nature* 198: 538—540.  
Hamilton T. H., R. J. Moore, A. F. Rumsey, A. R. Means and A. R. Schrank, 1965, *Nature* 208: 1180—1183.

- Höhn K., 1954, *Naturwissenschaften* 41: 536.  
 Höhn K., 1955, *Beitr. Biol. Pflanzen* 31: 261.  
 Iyer, V. N. and W. Szybalski, 1963, *Proc. Nat. Acad. Sci. U. S.*, 50: 355—362.  
 Jacob F., and J. Monod, 1961, *J. Mol. Biol.* 3: 318—356.  
 Kefford N. and P. Goldacre, 1961, *Amer. J. Bot.* 48: 643—650.  
 Kefford N. P., R. Kaur-Sawhney and A. W. Galston, 1963, *Acta Chem. Scand.* 17: 313—318.  
 Key J. L., 1964, *Plant Physiol.* 39: 365—370.  
 Key J. L. and J. C. Shannon, 1964, *Plant Physiol.* 39: 360—364.  
 Knypl J. S., 1963, *Proc. 2nd Conf. on Plant Growth Regulation*, Toruń, 4—5 June (in press).  
 Knypl J. S., 1964, *Planta* 61: 352—360.  
 Knypl J. S., 1965, *Nature* 206: 844—846.  
 Knypl J. S. and M. Gubanski, 1960, *Naturwissenschaften* 47: 308—309.  
 Korner A., 1963, *Biochem. Biophys. Res. Commun.* 13: 386—389.  
 Liao S., and H. G. Williams-Ashman, 1962, *Proc. Nat. Acad. Sci. U. S.*, 48: 1956—1964.  
 Masuda Y., 1959, *Physiol. Plantarum* 12: 324—335.  
 Masuda Y., 1960, *J. Inst. Polytechnics, Osaka City Univ.* 11D: 1—23.  
 Matthews R. E. F., 1958, *Pharmacol. Revs.* 10: 359—406.  
 Neuman J., 1964, *Physiol. Plantarum* 17: 363—370.  
 Ng E. K. and L. J. Audus, 1964, *J. Exp. Bot.* 15: 67—95.  
 Noodén L. D. and K. V. Thimann, 1963, *Proc. Nat. Acad. Sci. U. S.*, 50: 194—200.  
 Noodén L. D. and K. V. Thimann, 1965, *Plant Physiol.* 40: 193—201.  
 Van Overbeek J., 1961, In R. M. Klein (ed.) *Plant Growth Regulation*, Iowa State Univ. Press, Ames, Iowa, pp. 449—455.  
 Partanen, C. R., 1965, *Amer. J. Bot.* 52: 204—209.  
 Patau K., N. K. Das and F. Skoog, 1957, *Physiol. Plantarum* 10: 949—966.  
 Roychoudhury R. and S. P. Sen, 1964, *Physiol. Plantarum* 17: 352—362.  
 Schlegel D. E. and T. E. Rawlins, 1954, *J. Bacteriol.* 67: 103.  
 Sen Gupta A. and S. P. Sen, 1961, *Nature* 192: 1290—1291.  
 Siegel S. M. and A. W. Galston, 1953, *Proc. Nat. Acad. Sci. U. S.*, 39: 1111—1118.  
 Silberberger J., and F. Skoog, 1953, *Science* 118: 443—444.  
 Skoog F. and C. O. Miller, 1957, *Symp. Soc. Exp. Biol.* 11: 118—131.  
 Stoner C. D., T. K. Hodges and J. B. Hanson, 1964, *Nature* 203: 258—261.  
 Südi J., 1964, *Nature* 201: 1009—1010.  
 Talwar G. P., and S. J. Segal, 1963, *Proc. Nat. Acad. Sci. U. S.* 50: 226—230.  
 Tuan D. Y. H. and J. Bonner, 1964, *Plant Physiol.* 39: 768—772.  
 Urbanek H., 1963, *Ph. D. Thesis*, Univ. of Lodz (in Polish).  
 Venis M. A., 1964, *Nature* 202: 900—901.  
 Wedding R. T. and M. K. Black, 1964, *Plant Physiol.* 39: 799—806.  
 Wool I. G. and A. J. Munro, 1963, *Proc. Nat. Acad. Sci. U. S.*, 50: 918—923.  
 Yarmolinski M. B. and G. L. de la Haba, 1959, *Proc. Nat. Acad. Sci. U. S.* 45: 1721—1729.

*Hamujące działanie specyficznych inhibitorów syntezy RNA i białka na wzrost wzbudzany przez IAA i kumarynę*

Streszczenie

Aktynomycyna C<sub>1</sub>, puromycyna, chloramfenikol, dwuazouracyl i tiouracyl hamują zarówno wzrost wycinków hypokotyli słonecznika poddanych działaniu IAA i kumaryny, jak i redukują korzenie się i wzrost korzeni wycinków z siewek kukurydzy. Wymienione specyficzne inhibitory nie wpływają wybiórczo na wzbudzany przez kwas giberelinowy wzrost pierwszego liścia wycinków

kukurydzy. Uzyskane wyniki dowodzą, iż procesy wzrostowe wzbudzone przez auksyny zależą od syntezy RNA i białka. Sądzi się, iż kwas indoloocetowy i kumaryna mogą być czynne jako induktory syntezy kwasów ribonukleinowych typu messenger. Przypuszcza się, że mRNA wzbudzany przez kumarynę jest bogaty w uracyl, a mRNA wzbudzany przez IAA zawiera dużo cytozyny. W obu przypadkach odpowiednie białka enzymatyczne, syntetyzowane na odnośnych matrycach mRNA, są czynne przez około 6—8 godzin.

Uracyl odwraca hamujące działanie dwuazouracylu na wzrost wycinków hypocotyli słonecznika i korzenie się wycinków siewek kukurydzy.

Mitomycyna C — specyficzny inhibitor syntezy DNA — nie wpływa na wzrost, wzbudzany przez IAA i kumarynę.

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