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Studies on the Physiological Effect of Gibberellin
VI. Interaction of Gibberellin with Antiauxins[1)]

By

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The author (6, 7) has shown that gibberellin differs from auxin in the physiological effect. He has noted that gibberellin increases stem elongation in additive way even in the presence of optimal concentration of auxin. This fact may suggest that the two groups of growth substance differ from each other in the reaction sequence to be called forth by them.

If it is assumed that antiauxin competes with auxin at their common receptor in the cell, experiments on the interaction of gibberellin with antiauxin may give information concerning whether the receptor for gibberellin is different from that of auxin. Experiments to be reported here admit that the receptors are not common to the two types of growth substance.

Materials and Method

Seedlings of pea (var. Alaska) were grown in the dark room at 25°C for 7 days. Stem sections were cut from the third internode which reached 17–18 mm in length. The initial length of the sections was 5.25 ± 0.11 mm. They were floated in a test solution for 24 hours and the final length was measured with an objective micrometer under a low power binocular microscope.

Cucumber (*Cucumis sativus* L. var. Shogoinfushinari) was used for experiments on the growth of shoot and root of the intact seedling. Seeds were sterilized with 0.1 per cent $HgCl_2$, rinsed, and laid on filter paper moistened with distilled water in a Petri dish. This was put in a dark room at 25°C. After 24 hours, the root was about 2 mm long without the shoot emerged yet. Ten seedlings each were transferred to a Petri dish which was 12 cm in diameter and lined with filter paper moistened with 6 ml of a test solution. The lengths of shoot and root were measured after 48 hours of incubation.

1) Some of this work were presented at the 4th International Conference on Plant Growth Regulation in Boyce Thompson Institute for Plant Research Inc., August 10–13, 1959.

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Each experiment was repeated three times.

Seedlings of the dwarf d-1 mutant of corn, the growth of which depends on applied gibberellin, were grown under field conditions between spring and early fall of 1958 for use as a test material. When the first leaf was not yet fully expanded and was funnel-shaped, the lanolin paste to be tested was smeared in a ring at the base of the leaf. The treated seedlings were kept under field conditions, and the length of the first leaf sheath was measured after 48 hours.

4-Chloro- and 3-chlorophenoxy*iso*butyric acid (4-CIBA and 3-CIBA), 2,4,6-trichlorophenoxyacetic acid (2,4,6-T), 2,4-dichloroanisole (2,4-DCA) and 2-methyl-1,4-dihydronaphthoquinone (K_3) were used as antiauxins, indole-3-acetic acid (IAA) as auxin, and gibberellic acid (GA) as gibberellin. 2,4-DCA and K_3 were purified from commercial material.

The basic medium for the test solutions was 1/30 M phosphate buffer solution of pH 7.0. 4-CIBA, 3-CIBA and 2,4,6-T were dissolved in the form of sodium salts. K_3 was first dissolved in a small quantity of alcohol, which was then dispersed into the basic medium. Hence the test solution contained a little amount of alcohol in this case. The concentrations of K_3, 4-CIBA, 3-CIBA and 2,4,6-T used were 10, 15, 30 and 30 mg/l, respectively.

Results

Experiment on pea stem sections. Interactions of 4-CIBA with the growth effects of IAA and GA are shown in Figure 1. 4-CIBA inhibited the action of IAA at low concentrations of the latter, but not when the auxin concentration was high enough. This result agrees with those of McRAE and BONNER (11). On the other hand, 4-CIBA inhibited the action of GA even to very high concentrations of the latter. No competition was observed between the two growth substances.

The relations of 3-CIBA with IAA and GA were similar to the case of 4-CIBA, as seen in Figure 2.

2,4,6-T (Figure 3) and K_3 (Figure 4) seem also to be competitive with IAA, although, in the concentration used, they did not inhibit the effect of IAA so much as in the above mentioned cases. They, however, inhibited the GA action so well as in those cases. Thus in the experiments using pea stem sections, the four antiauxins used did not seem to be competitive with GA, but inhibited its effect independently of the concentration ratio of the paired substances. The complete inhibition of GA action by the antiauxins may be explained by assuming that GA can not act when the action of natural auxin is inhibited by the antiauxins.

When IAA or indoleacetamide was added to the mixture of GA and 4-CIBA, the GA action was recovered. The inhibition by 4-CIBA, however, was not reversed by any of 2% sucrose, 10^{-3} M pyruvic acid + 10^{-3} M succinate,

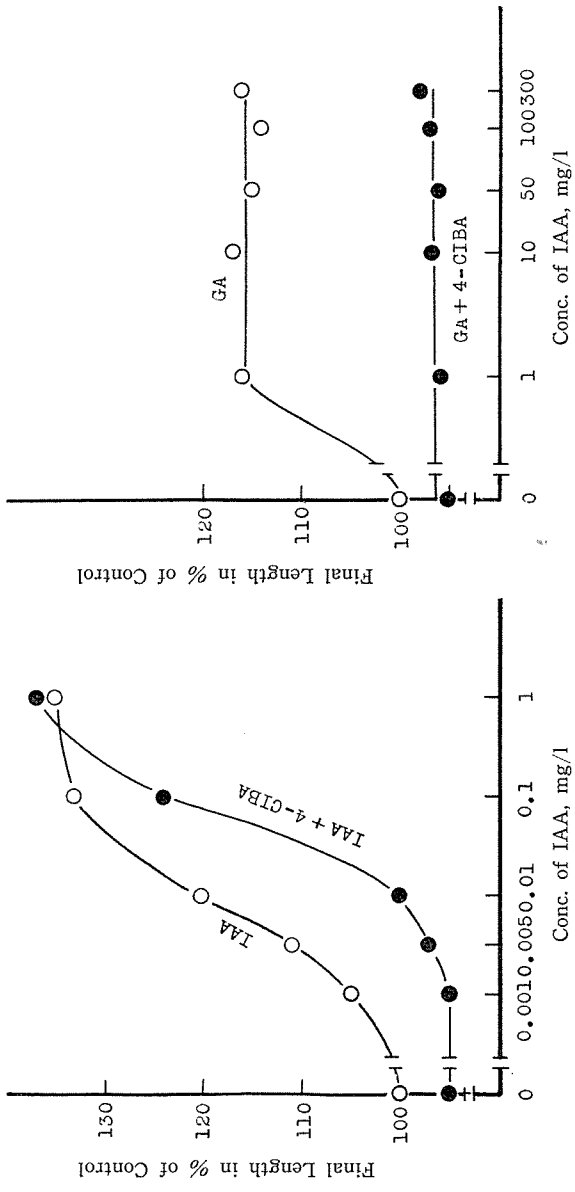

Fig. 1. Interaction of 4-CIBA with IAA and GA in the elongation of pea stem sections.

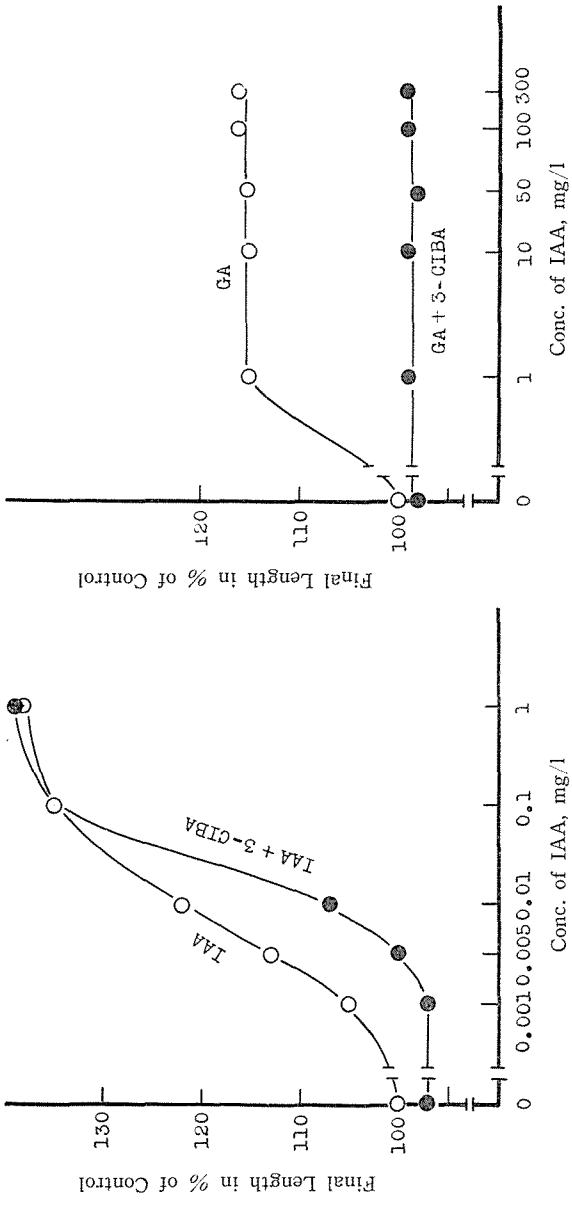

Fig. 2. Interaction of 3-CIBA with IAA and GA in the elongation of pea stem sections.

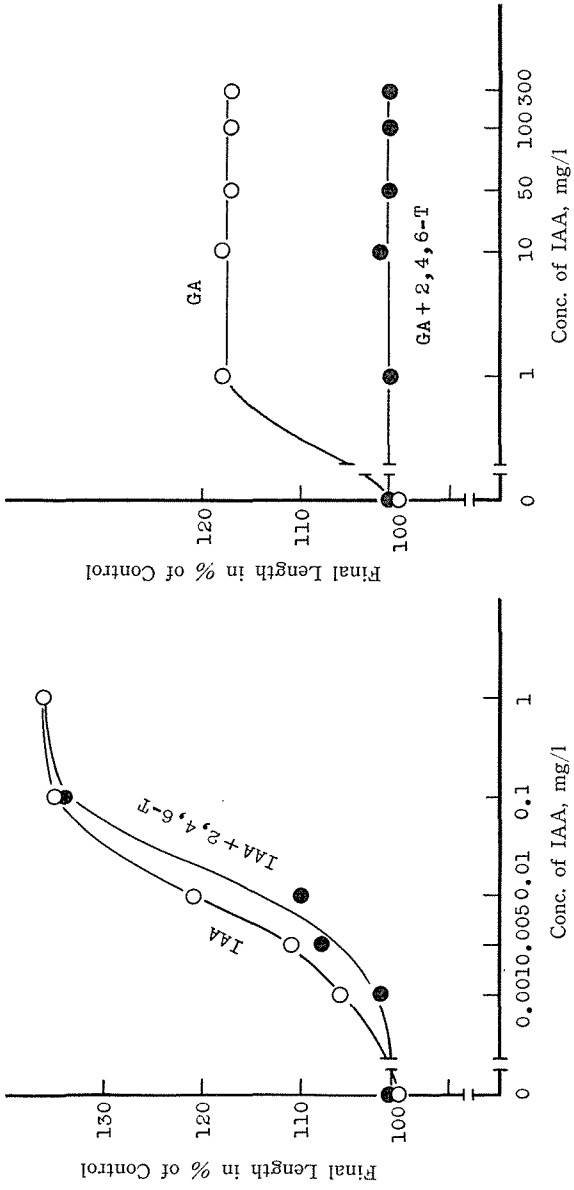

Fig. 3. Interaction of 2,4,6-T with IAA and GA in the elongation of pea stem sections.

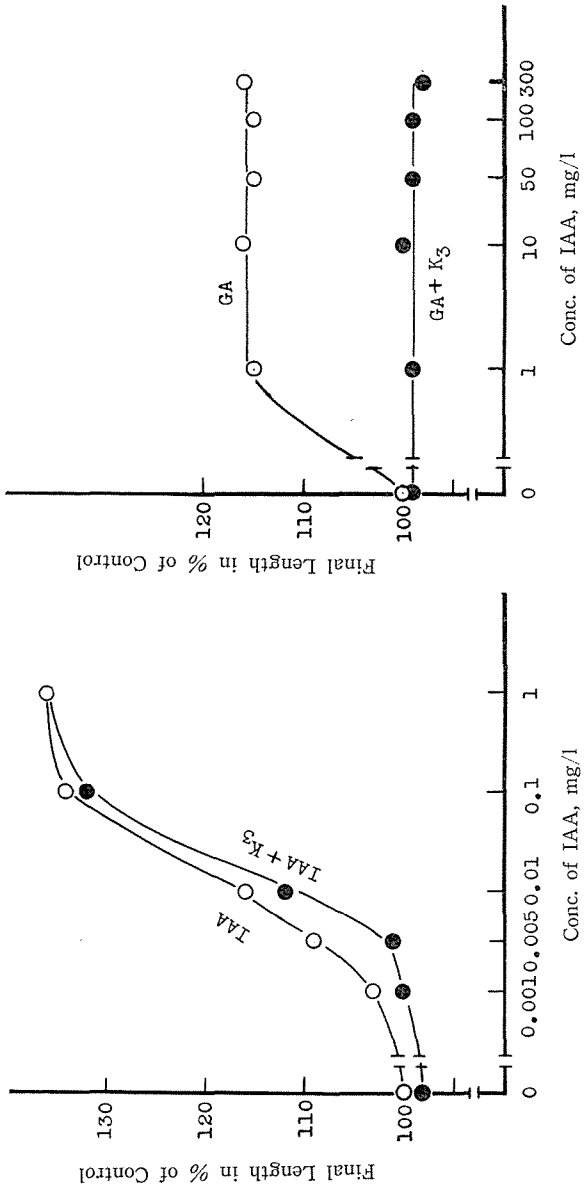

Fig. 4. Interaction of K_3 with IAA and GA in the elongation of pea stem sections.

ATP, adenylic acid, casein hydrolysate, single amino acid (L-leucine, L-histidine, tyrosine, L-phenylalanine, L-methionine, DL-threonine, L-hydroxyproline, DL-*iso*-leucine, aspargic acid, DL-ornithine, L-arginine, L-alanine, L-proline, glycine, L-lysine, DL-valine, or L-glutamic acid), vitamine (thiamine, riboflavin, ascorbic acid, pyridoxine, vitamin E, vitamin K, folic acid, pantothenic acid, nicotinamide, vitamin B_{12} or biotin), yeast extract, diphenylurea, RNA and DNA. Hence it is highly probable that auxin (or the growth process mediated by auxin) is involved in the growth promotion by GA.

Experiment on intact cucumber seedling. Interaction of antiauxins with GA was studied by using intact cucumber seedlings. As seen in Table 1, 4-CIBA promoted the root growth, more at 15 mg/l than at 30 mg/l. On the other hand, GA had no effect on the root growth either given singly (as already reported) or given in combination with 4-CIBA.

As to the shoot growth of the present material, the growth promotion by GA was not interfered with by 4-CIBA, unlike the case of the excised pea stem section.

Table 1. Effect of 4-CIBA on the GA-induced growth of cucumber seedlings. The elongations of root and shoot in absence of both GA and 4-CIBA taken as standards.

Concentration of 4-CIBA, mg/l	Concentration of GA, mg/l							
	0		25		50		100	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	100*	100[1]	98*	124[2]	99*	134[3]	100*	140[4]
15	141**	100[1]	139**	124[2]	141**	133[3]	141**	141[4]
30	116***	100[1]	113***	129[2]	116***	134[3]	114***	139[4]

1, 2, 3, 4, *, ** and ***: Differences among the values in each group are not significant at 1% level.

Table 2. Effect of 2, 4, 6-T on the GA-induced growth of cucumber seedlings. The elongations of root and shoot in absence of both GA and 2, 4, 6-T taken as standards.

Concentration of 2, 4, 6-T, mg/l	Concentration of GA, mg/l							
	0		25		50		100	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
0	100*	100[1]	98*	124[2]	99*	143[3]	101*	146[4]
15	90**	100[1]	89**	124[2]	91**	141[3]	91**	150[4]
30	86***	101[1]	86***	121[2]	87***	141[3]	85***	144[4]

1, 2, 3, 4, *, ** and ***: Differences among in each group are not significant at 1% level.

Table 2 shows that 2,4,6-T was inhibitory on the root growth, but ineffective on the shoot growth. And GA showed no effect on the root growth and increased the shoot growth irrespective of the presence of 2,4,6-T.

Experiment on dwarf d-1 mutant of corn. Lengths of the first leaf sheath measured 48 hours after the smearing of pastes are shown in Tables 3, 4, 5 and 6.

The effect of 0.1% paste of GA was not influenced by the co-existence of various concentrations of 2,4-DCA, 4-CIBA, 3-CIBA or 2,4,6-T in the paste. At the highest concentrations used these antiauxins slightly injured the treated part of the seedling. But even in this case the elongation caused by GA did not differ from the case when GA was given singly.

Table 3. Effect of 2,4-DCA on the GA-induced elongation of first leaf sheath of dwarf d-1 corn. Final length in cm.

Concentration of GA paste, %	Concentration of 2,4-DCA paste, %			
	0	1.4	2.8	4.2
0	1.6* ±0.04	1.7* ±0.05	1.7* ±0.04	1.7* ±0.05
0.1	4.0**±0.08	3.9**±0.12	3.9**±0.05	4.0**±0.13

* and **: Differences among the values belonging to each group are not significant at 1% level.

Table 4. Effect of 4-CIBA on the GA-induced elongation of first leaf sheath of dwarf d-1 corn. Final length in cm.

Concentration of GA paste, %	Concentration of 4-CIBA paste, %			
	0	1	2	3
0	1.7* ±0.03	1.7* ±0.02	1.7* ±0.04	1.7* ±0.04
0.1	3.5**±0.07	3.5**±0.07	3.4**±0.05	3.5**±0.10

* and **: Differences among the values belonging to each group are not significant at 1% level.

Table 5. Effect of 3-CIBA on the GA-induced elongation of first leaf sheath of dwarf d-1 corn. Final length in cm.

Concentration of GA paste, %	Concentration of 3-CIBA paste, %		
	0	1	2
0	1.8* ±0.05	1.8* ±0.02	1.8* ±0.03
0.1	3.9**±0.12	3.9**±0.11	3.8**±0.09

* and **: Differences among the values belonging to each group are not significant at 1% level.

Table 6. Effect of 2, 4,6-T on the GA-induced elongation of first leaf sheath of dwarf d-1 corn. Final length in cm.

Concentration of GA paste, %	Concentration of 2, 4, 6-T paste, %			
	0	1	2	3
0	1.8* ±0.04	1.8* ±0.04	1.7* ±0.04	1.7* ±0.03
0.1	4.3**±0.08	4.4**±0.13	4.3**±0.13	4.2**±0.12

* and **: Differences among the values belonging to each group are not significant at 1% level.

Hence the absence of interference between GA and antiauxins was demonstrated again with dwarf d-1 mutant of corn.

Discussion

In the above-mentioned experiments it has been examined whether IAA and GA have similar relations to various types of antiauxins: that is whether GA has its reaction site in common with IAA in plant tissues.

In pea stem sections growth promotion by low concentrations of IAA was remarkably reduced by the addition of 4-CIBA, 3-CIBA, 2,4,6-T and K_3. This reduction, however, could be restored by high concentrations of IAA. These results agreed with those of McRae and Bonner (11). Although the growth promoting effect of GA was also inhibited by these antiauxins, higher concentrations of GA up to 300 mg/l could not release this inhibition. This fact suggests that the receptive center for GA is not interfered with by antiauxins. Namely, the receptive center for GA probably differs from that for IAA. This suggestion can also be derived from the fact that no interaction on the growth of cucumber seedlings and roots was observed between GA and 4-CIBA or 2,4,6-T. Moreover, the GA-induced elongation of dwarf d-1 mutant of corn seedling was not affected by any one of these antiauxins. Curtis (3) found that, using bean plant (var. Black Valentine), the inhibitory effect of the filtrate from the culture medium of *Aspergillus niger* could be overcome by the application of GA, but not by IAA. Purves and Hillman (13) and Hillman (5) have suggested that the primary actions of gibberellin and auxin are not closely connected. Therefore, the author's conclusion described in the previous paper (7) that the mode of action of gibberellin differs completely from that of IAA, is thus reassured.

Brian and Hemming (2) and Kuse (9), using dwarf pea and the petiole of young sweet potato stem, respectively, demonstrated the necessity of IAA for the growth effect of GA. But, at least in some other cases, gibberellin seems to require for its operation some factor(s) different from IAA. Vlitos and Meudt (15), using etiolated pea cuttings, have shown that a certain factor(s)

present in their shoot apex is required for the operation of gibberellin. PURVES and KATO (14) also observed the presence of such a factor(s) in the cotyledons of darkgrown cucumber seedlings. BRIAN and HEMMING (2), however, proposed the presence of third factor required for GA-IAA interaction and GALSTON and WARBURG (4) regarded that the third factor operated through an auxin-sparing system.

APPLEGATE (1) reported that auxin was not involved directly in the GA-induced elongation of *Zinnea elegans*. MALTZHAN and MACQUARRIE (10) reported that GA was effective in the growth of protonemata of *Splachnum ampullaceum* (L.) HEDW. but IAA, amino acids and vitamins were not. The dominant dwarf corn does not respond to gibberellic acid either with or without concomitant presence of IAA both under short day and long day conditions at various temperatures (8). No synergism between gibberellin and exogenously supplied auxin was observed by NEELY and PHINNEY (12). Thus IAA itself is not always necessary for the GA-effect, but seems to be replaceable by another factor(s).

Summary

Gibberellic acid differed from indole-3-acetic acid in the growth effect when they were applied together with a variety of antiauxins. The reaction site of GA in plant tissues seems to be indifferent of that of IAA.

An antiauxin activity of 2-methyl-1,4-dihydronaphthoquinone was demonstrated.

In conclusion, the author wishes to thank Professor Joji ASHIDA for his guidance and Professor R.L. WAIN, University of London, and Professor H. BURSTRÖM, University of Lund, for giving the author antiauxins.

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