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Studies on the Physiological Effect of Gibberellin

III. Effects of pH and Enzyme Inhibitors on GB action

By

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Introduction

In the previous papers[4,5] the author has reported that gibberellin is a kind of growth substance different from auxin. Recently PHINNEY *et al.*[6] have succeeded to extract gibberellin-like substances from various kinds of higher plants and indicated the possibility that the growth of higher plants is controlled by those substances as well as by auxin.

As an approach to the action mechanism of gibberellin, effect of some enzyme inhibitors on the gibberellin-induced stem elongation was studied. This paper reports those experiments, together with studies on the effects of pH on the gibberellin-induced elongation and water uptake.

The abbreviations, GB and IAA, respectively, stand for gibberellin A* and indole-3-acetic acid in this report.

Material and Methods

Pea stem sections were used as the material. Pea seedling (*Pisum sativus* L., variety Alaska) was grown in a darkroom at 25°C. Sections were excised from the third internode, 15–20 mm. in length, of 7 days old seedlings, and were floated in 1/30 M phosphate buffer both with and without addition of GB for the duration of experiment.

The length of stem sections was measured by an object micrometer under a low power binocular microscope. The average initial length of sections was 5.26 ± 0.15 mm.

Fresh stem sections were weighed on a torsion balance, and the water uptake was determined by the gain over the initial weight.

Respiration was measured by the cmm. of oxygen taken up by the sections in Warburg respirometer, shaken at 80 r.p.m. in a water bath at 30°C. in darkness. Each flask contained 10 sections (100–120 mg. fresh weight), bathed in 2 ml. of 1/30 M phosphate buffer solution with and without addition of GB. Experiments ran

*) Gibberellin A was provided through the courtesy of Prof. Y. SUMIKI.

for 4.5 hours, preceeded by a half-hour equilibration period. Manometers were read every half hour.

Since 10 mg./l. was found in preliminary experiments to be the optimum concentration of GB for the elongation of pea stem section, this concentration was used in the following experiments.

Results

pH and the elongation.—Stem sections were floated in 1/30 M phosphate buffer mixtures for 24 hours. As shown in Table 1, the more acidic the reaction, the more was the elongation, in the presence of GB as well as in control. The stimulation induced by GB, however, was the largest at pH 7.0.

No diametrical growth of stem sections was observed in the 24 hour period, either in the presence or absence of GB.

Table 1. Effect of GB (10 mg./l.) and pH on the elongation, in 24 hours, of pea stem sections. Each value represents the average of 10 sections.

pH	Increase in length of section in 0.1 mm.		
	Control	GB	% Increase over control
5.3	11.1	17.1	54
6.0	10.2	16.3	60
7.0	7.4	13.0	76
8.0	6.6	9.7	47

pH and the water uptake.—Water uptake was determined by the fresh weight increase of the sections during the 24 hours' floating. It is evident from Figure 1 that the water uptake of pea stem sections were increased by GB (10 mg./l.) at any pH tested. The water uptake in the presence of GB was the highest at 5.3, followed by 6.0, 7.0 and 8.0 in that order. However, since the value of control was the lowest at 7.0, the effect of GB over control was the largest at this pH.

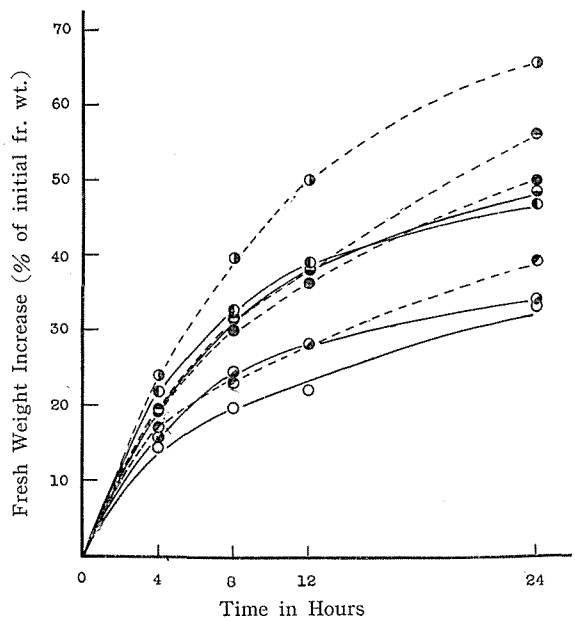

Figure 1. Effect of GB (10 mg./l.) on the water uptake of pea stem sections at various pH.
Control at pH 5.3,
" " " 6.0,
" " " 7.0,
" " " 8.0,
GB-treatment at pH 5.3,
" " " " 6.0,
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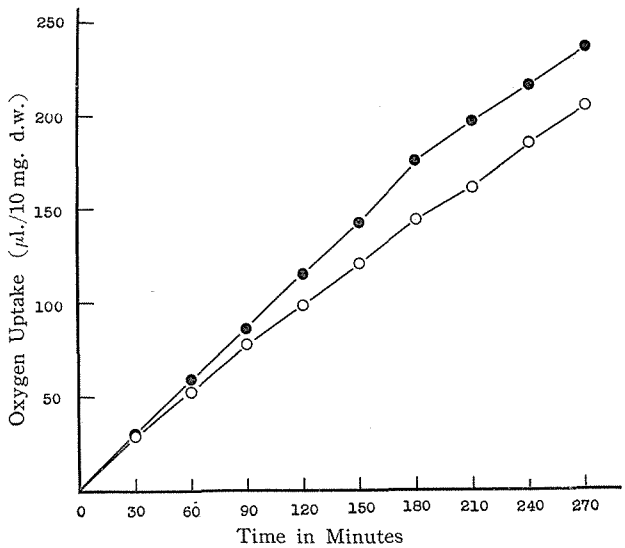

Figure 2. Effect of GB (10 mg./l.) on the oxygen uptake by pea stem sections.

○—○ Control,
●—● in the presence of GB

Effect of GB on the respiration.— Figure 2 shows the time course of oxygen uptake by stem section at pH 7.0 in the GB and control solutions. The oxygen uptake was stimulated by GB about 15%. In the same experimental period, IAA stimulated it about 28%.

Stem age and the GB effect.— In order to see difference in the effect of GB according to the age, elongation, water uptake and respiration were measured using sections from the first, the second and the third internodes of the 7 days old seedling. The third internodes was the most actively growing portion of the whole stem, while the other two showed no measurable elongation. The results obtained are summarized in Table 2. GB exerted practically no effect on any of the measured activities of the sections from the two internodes which has passed the growing age.

Effect of enzyme inhibitors on the GB-induced elongation.— Stem sections

Table 2. Effect of GB (10 mg./l.) on the elongation, the water uptake and the respiration of pea stem sections at the three different ages at pH 7.0.

Internode No.	Increase in length of section in 0.1 mm		
	Control	GB	% Increase over control
1	1.2	1.2	0
2	1.4	1.4	0
3	7.4	13.0	+76
	Water uptake, % of initial fresh wt.		
	Control	GB	% Increase over control
1	10	10	0
2	12	12	0
3	25	40	+60
	Oxygen uptake, μlit/10mg d.w./hr.		
	Control	GB	% Increase over control
1	25.8	27.5	+6
2	31.3	29.5	−6
3	51.3	59.5	+16

were floated in the buffer solution both with and without addition of GB. To these, cyanide, arsenite and *p*-chloromercuribenzoate were added after 4 hours. Growth changes followed are illustrated in Figure 3, 4 and 5, each representing one of three similar sets of experiments. Each of the enzyme inhibitors used inhibited the growth both in the presence and in the absence of GB. Since the GB sections elongated more than water control before the inhibitors revealed their full effect, the former always exceeded the latter in the final length.

Elongation under the effect of GB is inhibited almost completely at the inhibitor

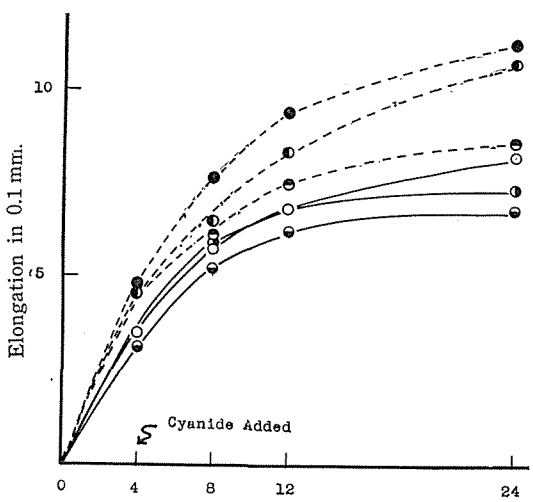

Fig. 3

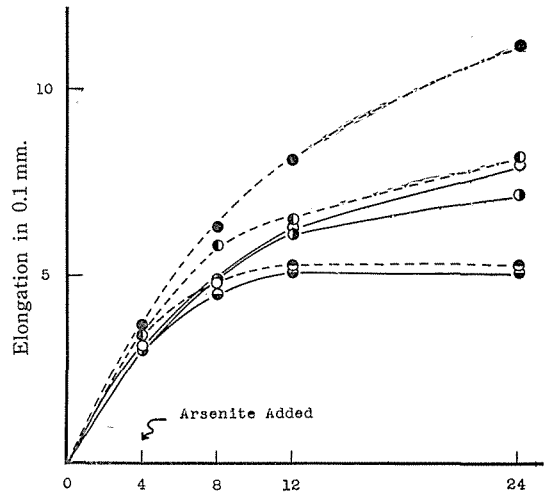

Fig. 4

Figure 3. Effect of cyanide on the elongation of pea stem sections in the presence and absence of GB (10 mg./l.).
○——○ Control, ◑——◑ 10^{-4}M NaCN,
◒——◒ 2×10^{-4}M NaCN,
◐- - -◐ GB+10^{-4}M NaCN, ●- - -● GB,
◓- - -◓ GB+2×10^{-4}M NaCN.

Figure 4. Effect of arsenite on the elongation of pea stem sections in the presence and absence of GB (10 mg./l.).
○——○ Control, ◒——◒ 10^{-4}M Arsenite,
◑——◑ 2×10^{-5}M Arsenite,
◐- - -◐ GB+2×10^{-5}M Arsenite,
◓- - -◓ GB+10^{-4}M Arsenite, ●- - -● GB.

Figure 5. Effect of *p*-chloromercuribenzoate (PCMB) on the elongation of pea stem sections in the presence and absence of GB (10 mg./l.).
○——○ Control, ◑——◑ 4×10^{-4}M PCMB,
●- - -● GB, ◐- - -◐ GB+4×10^{-4}M PCMB.

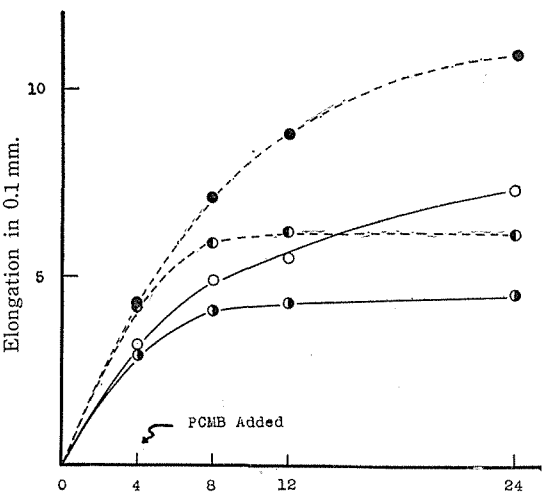

Fig. 5

concentrations which also inhibited the elongation in the absence of GB. Thus cyanide, *p*-chloromercuribenzoate and arsenite, which are known to inhibit the growth induced by auxin[3,7,8], inhibit the GB-induced growth as well.

On the other hand, sodium diethyldithiocarbamate did not inhibit in the concentrations used the elongation in the presence as well as in the absence of GB (Table 3). Hence the copper enzyme does not seem to play an important role both in the normal and in the GB-induced growth.

Table 3. Effect of sodium diethyldithiocarbamate on the elongation of pea stem sections in the presence of GB (10 mg./l.)

Concn. of inhibitor (Mol.)	Final length in percentage of control	
	Control	GB
0	100*	109**
10^{-3}	99*	107**
10^{-4}	99*	109**

*) **) Differences in each of the series are not significant at 1% level.

Discussion

Although the GB action seems to involve effects on some step of metabolism which is not involved in the main sequence of reactions caused by auxin[4,5], the final effects, such as the stimulation of elongation, water uptake and respiration, are common to both of the growth regulators. As to auxin, many investigators have tried to find a relation between the effect of auxin on the respiration and that on the growth. COMMONER and THIMANN[1], finding that iodoacetate inhibits only about 10% of the total respiration at the concentration which inhibits growth completely, have concluded that only a part of respiration participates in the growth process. FRENCH and BEEVER[2] have reported that the optimal concentrations of auxin promoting growth and respiration coincide with each other, although no increase in respiration is observable at concentrations which induce a small growth response. HACKETT and THIMANN[3] have established that water uptake induced by auxin is dependent upon oxidative metabolism in which cytochrome oxidase is the terminal oxidase system. Thus, accumulating data point to the importance of respiration in the auxin-induced growth. It has also been shown by the use of sulfhydryl inhibitors as arsenite and *p*-chloromercuribenzoate that sulfhydryl enzymes are included in the growth process[3,7,8].

In the experiments reported in this paper cyanide, arsenite and *p*-chloromercuribenzoate inhibited completely the growth in the presence of GB at respective concentrations which also inhibited the growth without GB. And at the concentrations thus far used, sodium diethyldithiocarbamate and α, α'-dipyridyl (unpublished) did not inhibit the growth either in the presence and in the absence of GB. The growth measured in the absence of GB may have been caused by the natural auxin which the stem sections contained. Hence so far as the experiments of the author concerns, the GB-induced growth seems to involve the same oxdoreductive reactions as the growth occurring without external application of GB.

Summary

Gibberellin stimulated elongation, water uptake and respiration of pea stem sections at various pH values, and most strongly at 7.0. Effects by gibberellin were observed only in the young, growing part of stem.

The gibberellin-induced elongation was inhibited by cyanide, arsenite and *p*-chloromercuribenzoate, but not by diethyldithiocarbamate, just as the elongation without gibberellin. It is thus suggested that enzymes containing heavy metal and sulfhydryl group do, and those containing copper do not, participitate in the gibberellin-induced growth, just as in the normal growth.

In conclusion, the author wishes to thank Professor Joji ASHIDA for his cordial guidance.

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