EFFECT OF GIBBERELLIN ON THE INHIBITOR-β FORMATION IN THE EXCISED HYPOCOTYL SECTIONS OF ETIOLATED *PHASEOLUS* SEEDLINGS

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Introduction

It has already been suggested by HEMBERG[1,2] that the high content of inhibitor-β in their tissues may bring about the dormancies in potato tubers and in terminal buds of *Fraxinus*. BOO[3] has also observed the decrease in inhibitor-β content in connection with the breaking of the dormancy of potato tubers by treatment with gibberellic acid (GA). GA stimulates the growth of plant tissues in various species and possible mechanisms of action of GA are proposed in several ways, (ref. PHINNEY and WEST[4]). One of which is that GA acts as an antagonist to a natural growth inhibitor in a plant tissue. Inhibitor-β is contained not only in the resting tissues of potato tubers and of *Fraxinus* buds, but also in the growing stems and roots of various plants (KEFFORD[5]).

Evidence has accumulated to show that natural gibberellin and growth inhibitor may be considered as growth regulators for the normal growth of plants and the level of their content in organs and tissues is variable. Accordingly the further understanding of their statuses in the growing tissues could yield new information about the role of this active material on the plant growth.

The writers have also found this inhibitor-β in apical stems and root apexes where the cell division and cell elongation are occurring actively (unpublished).

The present investigation was undertaken to see the changes in the activities of inhibitor-β and of GA infiltrated into the sections which were excised from the apical zone of hypocotyls of etiolated *Phaseolus* seedlings, in the hope of finding further information of the effect of inhibitor on cell division and cell elongation of plants.

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Material and Methods

Phaseolus vulgaris were selected as material, since they yield uniform seedlings within several days and adequate experimental materials may obtained from the growing tissue of the hook zone of a hypocotyl. Seeds of *Phaseolus vulgaris* var. "Kintoki" were germinated in a moist sawdust and grown in a dark room at 25°C for 4 to 6 days. Sections were obtained from the hypocotyl ranging from tip to 5 *mm* under the hook zone, where the cell division and elongation are active. GA solution (0.5 or 1 *mg* per liter) was introduced into sections of about 2 to 4 *g* in fresh weight by means of a vacuum infiltration under reduced pressure. Then the sections were washed with distilled water, blotted with filterpaper, and the fresh weight was measured again. The increase in fresh weight after the infiltration was considered to correspond to the amount of GA solution infiltrated into the tissues. By this treatment the increase in fresh weight was about 6 to 10 per cent of the initial weight. Thus it was possible to estimate a rough amount of GA solution absorbed. As a control the sections having the same fresh weight were treated similarly with distilled water.

Each lot of treated sections was divided into two equal parts by weight, and one of them was extracted immediately with ethanol, while the other was extracted after overnight incubation at 25°C in the dark moist room. Each alcoholic extract was evaporated under reduced pressure at 40°C, then fractionated into neutral and acidic fractions with ether. It should be noted here that in the present plant materials, no appreciable activities of GA or inhibitor were detected in neutral fraction, therefore only acidic fraction was used for the experiment.

The acidic fraction was separated on paper chromatograms using the isopropanol-ammonia-water (10:1:1) mixture as developer, the developed chromatograms were divided into ten equal transverse strips. Each of the strips was placed in a 6 *cm* petri dish with 1 *ml* of distilled water containing IAA 2 *mg* per liter. Activities of GA and inhibitor were assayed using the etiolated wheat leaf section test. Other experimental procedures used in the present investigation are similar to those described in the previous paper (YOSHIMURA and TAGAWA[6]), so full descriptions are omitted here.

Results

Experiment 1. Formation of inhibitor-β and GA consumption

The experimental sections of 2 *g* in fresh weight were excised from the

apical parts of hypocotyls cultured 5 days in the dark, then GA-solution (1 mg/l) was infiltrated into them under negative pressure. Excess solution adsorbed on the outer surface of the sections was washed out with distilled water, and then filter papers were applied for removing excess moisture. The difference in fresh weight before and after the infiltration was regarded as the amount of GA-solution absorbed in the sections. By this treatment an increase of about 7 per cent of the original weight, due to the infiltration, was recognized. As the control the sections of 2 g in fresh weight were treated with distilled water.

These sections of GA-treatment and of control were divided into two groups respectively. One of them was used for extraction with alcohol, immediately after the treatment, and the other was extracted with alcohol, after allowing to stand overnight in the dark moist room at 25°C. Ether soluble neutral and acidic fractions were obtained from alcohol extracts, and these fractions were developed by the paper chromatographic procedure to measure the activities of GA and of inhibitor by means of bioassay. The results obtained with acidic fraction are illustrated in Fig. 1.

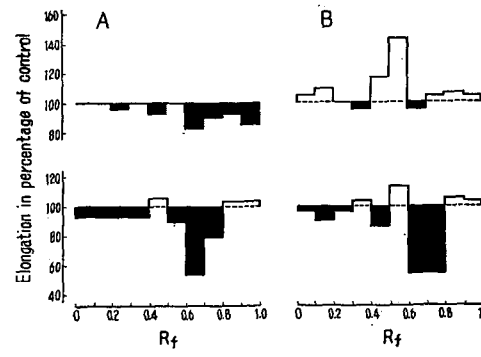

Fig. 1. Biological assay of chromatograms of acidic fraction of ethanol extract, which were obtained from apical sections of hypocotyls of etiolated *Phaseolus* seedlings, and treated with GA solution. Each lot of sections used for extraction was 1 g in fresh weight, and amount of GA solution infiltrated into the sections was about 0.07 *cc*. A is control and B represents GA (1 *mg/l*) treatment. Upper and lower histograms show before and after incubation respectively, and those that follow are the same.

The activity of inhibitor in the control and in sections treated with GA was not clear in the samples extracted immediately after infiltration, but after overnight incubation significant activities were recognized in both lots. In this case the inhibitor activity in the tissue treated with GA was higher than that of the control. From this it would appear that the formation of inhibitor was enhanced by the GA-treatment.

So far as measured by means of bioassay, the whole activity of GA can be recovered when the extraction is made immediately after the GA-treatment. If the extraction is made after overnight incubation, however, GA activity could not be recovered. Although the data at hand do not answer the question

as to the fate of infiltrated GA during the incubation, for the sake of convenience of description, this phenomenon was referred to as "consumption of GA" hereafter.

In any cases of incubation with neutral fractions, no significant effects on the acceleration or retardation of wheat leaf growth was observed. In further experiments, accordingly, only the acidic fractions were assayed.

Experiment 2. Effect of GA concentration on inhibitor formation

Preliminary experiments have made it obvious that GA accelerated the formation of inhibitor in the hypocotyl sections of *Phaseolus*. In experiment 2, the effect of concentration of GA on the inhibitor formation was studied. Experimental materials and methods were the same as those in Experiment 1. The fresh weight of each lot of the sections was 2.6 *g* and concentrations of GA were used at 0.2, 1.0 and 5.0 *mg* per liter. The results are shown in Fig. 2.

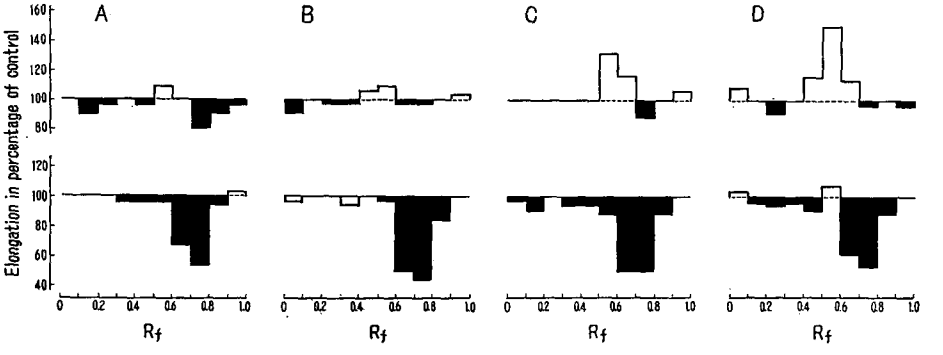

Fig. 2. Apical sections of hypocotyls were treated with various concentrations of GA solution. Each lot of sections used for extraction was 1.3 *g* in fresh weight and amounts of solution infiltrated were about 0.06 *cc*; other conditions were the same as described in figure 1. Concentrations of GA solutions of B, C and D were 0.2, 1.0 and 5.0 *mg/l* respectively and A is a control. Upper and lower figures show the results obtained before and after the incubation.

By treatment with GA solution at a concentration of 0.2 *mg/l*, as well as at that of 1 *mg/l*, the formation of inhibitor was enhanced. When the concentration was lowered to 0.05 *mg/l* in another experiment, the formation of inhibitor was still accelerated. Although at concentration of 0.2 and 0.05 *mg/l*, the activity of GA could not detected in the extract even when this GA was extracted immediately after the treatment, the enhancement of the inhibitor formation was recognized at the low GA concentrations such as 0.2 or 0.05 *mg/l*. This highly suggests that the native gibberellin are supposed to be con-

cerned with the inhibitor formation in the control plant*. GA infiltrated into the tissues was completely consumed during the incubation even at the high concentration of 5 *mg/l*

Experiment 3. Differences in the inhibitor formation between various hypocotyl zones

In Exp. 3 the difference in the inhibitor formation in the sections obtained from various zones of hypocotyls was measured. Three lots of sections were excised from hypocotyls as indicated in Fig. 3: *a* and *b* are active growing zones of hypocotyls and *c* is the zone where the elongation growth has already finished. The experimental results are shown in Fig. 4.

As seen from Fig. 4, in *a* and *b* sections obtained from growing zone where the cell division and elongation are occurring actively, the inhibitor production and GA consumption are as similarly significant as those recognized in the previous experiment. While in *c* section, obtained from the zone where the elongation growth has already completed, neither inhibitor formation nor GA consumption were observed.

5-day-old seedlings on which hypocotyls 4 lines were marked at intervals of 5 *mm*, starting from just beneath the hook zone to the proximal zone, were cultured at 25°C in the dark. The rate of elongation of each zone from upper

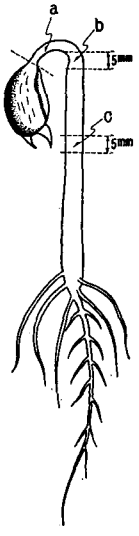

Fig. 3. Various zones of the hypocotyl of etiolated *Phaseolus* seedling where lots of experimental sections were excised.
a : apical zone including meristematic hook zone.
b : a part of active elongating zone.
c : middle zone where the elongation has terminated already.

* In the ordinary experiments when sections of 1-2 *g* in fresh weight were used for extraction, the activity of endogenous GA could not detected, but when hypocotyl sections of 5-8 *g* in fresh weight obtained from growing zone were used for extraction and bioassay, GA activity was clearly indicated.

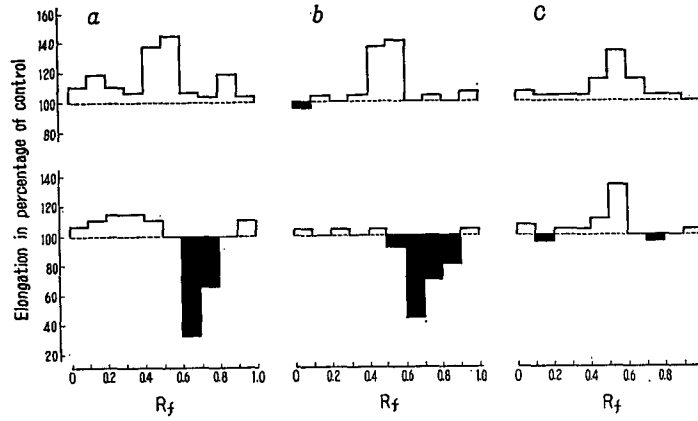

Fig. 4. Sections were excised from various zones of hypocotyls as indicating in fig. 3 and treated with GA (1 *mg/l*) solution; fresh weight of sections used for extraction was 2 *g*, and amount of introduced GA solution in *a*, *b* and *c* were 0.15, 0.18 and 0.10 *cc* respectively.

to lower, after 18 hours, were 6.5, 5.4, 2.3 and 1.2 *mm* respectively. On the other hand, the inhibitor formation and GA consumption were measured with other materialls of 4 *g* in fresh weight corresponding to these 4 zones. The results are shown in Fig. 5

The formation of inhibitor was significant in the uppermost apical section, but it was insignificant in the proximal sections. In the upper 3 zones, infiltrated GA was completely consumed during the incubation, while in the lowest

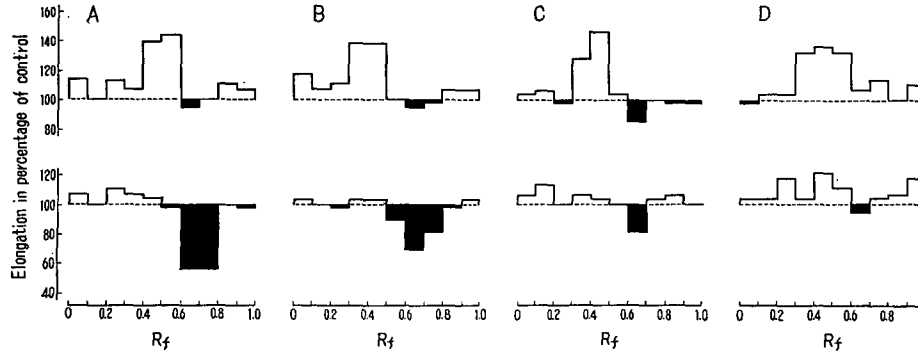

Fig. 5. Sections were taken from various parts of hypotyls and incubated overnight after the treatment with GA (1 *mg/l*) solution. The fresh weight of the sections for extraction was 2 *g*. A, B, C and D were lots of sections, each 5 *mm* in length, which were excised from the hypocotyl in succession starting from hook zone toward the base.

zone a part of infiltrated GA was recovered. From these facts it would appear that the activities of inhibitor production and GA consumption are restricted within narrow zones where the growth is actively taking place.

Experiment 4. Studies with etiolated leaves.

The primary leaves of *Phaseolus* can grow tolerably well in the dark, although developmental status is far behind the green one. Experiment 4 was planned to see whether the conclusion obtained with hypocotyl could be extended to the leaf. The leaf blades of 2 *g* each in fresh weight were excised from the etiolated seedling, and cut into several pieces to be treated with GA solution. The increase in fresh weight of the leaf sections was about 30 per cent over the original weight after infiltration with GA solution. The rate of inhibitor formation and GA activity were measured immediately after the infiltration and after the overnight incubation. The experimental data are shown in Fig. 6.

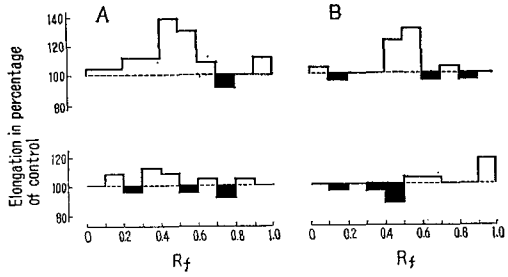

Fig. 6. Extraction of leaves of etiolated *Phaseolus* seedlings treated with GA (0.5 *mg/l*) solution. The fresh weight of leaves used for extraction was 2 *g*, and an amount of GA solution infiltrated into A and B sections were 0.3 and 0.28 *cc* respectively. A and B section were obtained from leaves of 6 day- and 8 day-old seedlings respectively.

As seen from Fig. 6, no appreciable formation of inhibitor was observed, but GA was consumed completely during the overnight incubation.

Experiment 5. Basal application of GA to excised hypocotyl.

In the preceding four experiments GA or distilled water were introduced into the sections by means of infiltration, so the intercellular spaces were filled with liquid giving a transparent appearance to the tissues, accordingly there leaves a little doubt as to whether the inhibitor formation and GA consumption in the infiltrated sections have occurred under such abnormal conditions. In the present experiment, the basal parts of the excised hypocotyls were dipped into GA solution (1 *mg/l*) for 20 minutes to absorb GA solution (GALSTON and WARBURG[7]). The control hypocotyls were similarly dipped into water. Immediately after such treatment the apical sections were excised from both materials and each lot of sections was divided into two equal parts, and one of them was extracted immediately, while the other was extracted after incubation overnight in a moist chamber, then the sections were assyed in the

usual manner. The experimental results obtained with sections excised immediately after the treatment, have made it obvious that GA which was absorbed from the base of the hypocotyls during the 20 minutes treatment, was translocated to the apical zones of the hypocotyls. In the sections which were incubated overnight after GA treatment, the inhibitor formation and GA consumption were ascertained to be similar to the sections infiltrated with GA.

Experiment 6. Effect of water deficiency

When the sections were incubated in a moist chamber, as was shown in the previous experiments, the inhibitor formation was the result, while the elongation of the sections was not so remarkable. If adequate water was supplied to the sections, a significant elongation of these sections resulted, without showing any inhibitor formation. Such differences in responses would seem to be attributed to a shortage of water in the sections, so in the present experiment the effects of water deficiencies on these responses were explored.

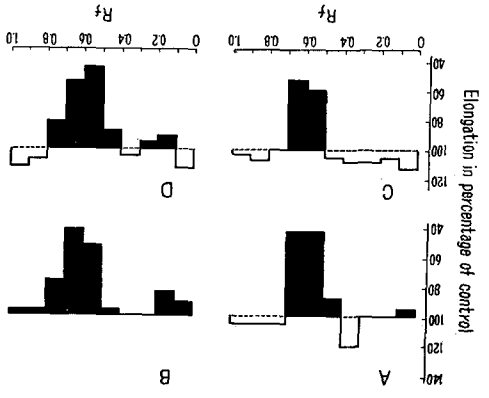

Fig. 7. The rate of inhibitor formation by *Phaseolus* hypocotyls which were allowed to stand in a dried chamber for 2 days prior to extraction, in order to restrict the water supply. The fresh weight of each lot of section was 4 *g*.

A : sections were obtained ranging from the apical part to 5 *mm* under the hook zone of the hypocotyls.

B : sections were obtained just following A, ranging from 5 to 25 *mm* under the hook zone.

C : sections were taken from the leaving proximal part of the hypocotyls.

D : young primary leaves were obtained from the same seedlings.

5-day-old seedlings grown in moist sawdust were picked out and let stand in a dry dark room of about 40 per cent relative humidity for 2 days at 25°C. They continued to elongate for a short period of time, but after two days they became distinctly flaccid, yet having the capacity to recover normal growth if adequate water is supplied at this stage. The results of bioassay obtained with hypocotyls and leaves are shown in Fig. 7.

By the hypocotyls of *Phaseolus* seedlings grown in a moist dark room the location of inhibitor formation is usually restricted in the apical zones. While by the hypocotyls cultured in a dry dark room, inhibi-

tor accumulated abundantly throughout the whole parts of the hypocotyls and leaves.

Experiment 7. Effects of temperature and oxygen during the incubation

In the previous experiments, the sections were incubated at 25°C after GA treatment. In order to see the effect of temperature during the incubation on inhibitor formation, the sections were incubated at room temperature ranging from 15 to 20°C and at 25°C. The results are shown in Fig. 8.

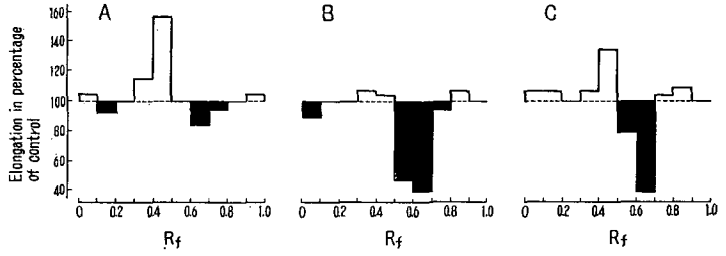

Fig. 8. Influence of incubation temperature after GA (1 *mg/l*) treatment. The fresh weight of each lot of sections for extract was 2 *g*. A : Before incubation; B and C : After incubation at 25°C and at room temperature respectively.

In the sections incubated at room temperature, the inhibitor formation was less than that at 25°C. And the GA consumption at room temperature was not so significant as that at 25°C and a part of the GA infiltrated could recovered by extraction after the incubation.

Next, in order to see the effect of oxygen supply during the incubation on the inhibitor formation, the sections were divided into two groups after the GA treatment. The first one was incubated overnight in a moist chamber, and the other was incubated overnight in a moist chamber filled with nitrogen gas. As is seen in Fig. 9, neither GA consumption nor inhibitor formation were observed in the absence of oxygen.

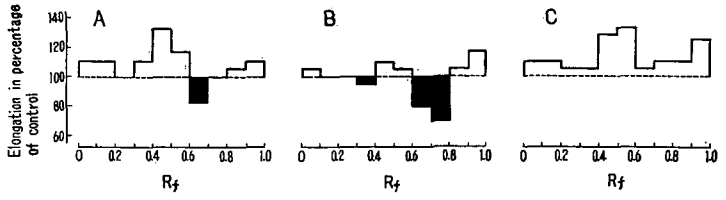

Fig. 9. Influence of oxygen during incubation after GA (1 *mg/l*) treatment.
A : Before incubation; B and C : After incubation in a usual moist chamber and under nitrogen gas.

Experiment 8. Observation with green seedlings

The experiments stated above were made with etiolated *Phaseolus* seedlings grown in the dark. The point to be considered next is whether these evidences are applicable to other material plants. In the present experiment the *Phaseolus* seedlings were raised in pots filled with soil, under natural day light in a green house. The sections were obtained from the stems and leaves two days after the unfolding of the primary leaves and the bioassay was made by the usual method previously stated, and the results are shown in Fig 10.

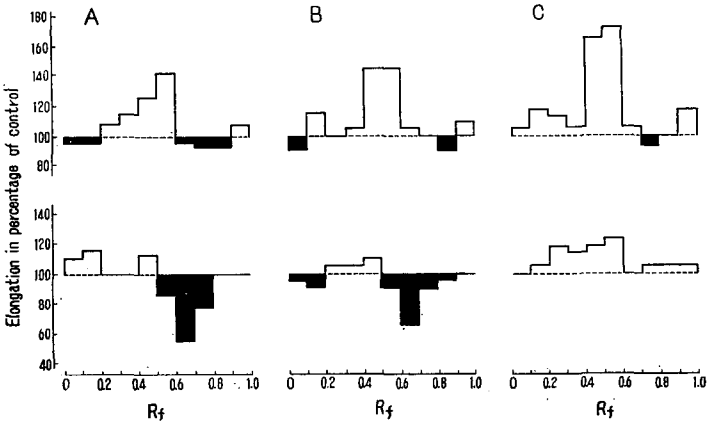

Fig. 10. Extraction of various parts of green seedlings of *Phaseolus* which were treated previously with GA (1 *mg/l*) solution. A is 1 *cm* section obtained from under part of the primary leaves; B is 1 *cm* section obtained from under part of the cotyledones; and C is primary leaf. The fresh weight of sections for extraction was 2 *g*, and amounts of GA solution infiltrated into A, B and C sections were 0.13, 0.2 and 0.7 *cc* respectively.

Both in the stem sections which were obtained from the under parts of the primary leaves where the active elongation is going on, and in the hypocotyl sections which were obtained from the under parts of the cotyledons where the elongation has already terminated, a significant formation of inhibitor was recognized, and GA infiltrated was found to be completely consumed. The experimental data obtained with the sections in which elongation growth has already finished, do not agreed with that obtained with etiolated materials in Exp. 3. Although no satisfactory explanation can be offered here, it might be attributed to the differences in moisture content and cell constituents between these two different materials. In the leaf sections, only the GA consumption was ascertained to occur in the same manner as with etiolated leaves.

Experiment 9. Inhibitor formation in intact seedlings

All the preceding experiments were made with excised hypocotyl sections. So a question may arise whether similar result might be obtained with an intact seedling. To make sure of this point, *Phaseolus* seeds were germinated in sawdust moistened with GA (1–2 *mg/l*) solutions or with water as control for 5 days, in the dark, at 25°C. Mean lengths of the hypocotyls of the control and of GA treated were 6.3 *cm* and 10.2 *cm* respectively, and these data show clearly the acceleration of hypocotyl elongation due to GA treatment.

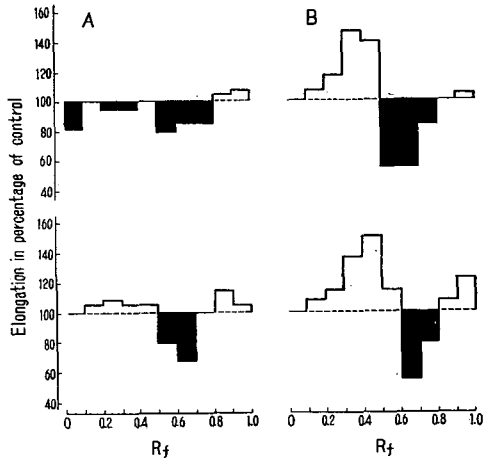

Fig. 11. *Phaseolus* seeds were germinated in sawdust moistened with water as control (A) and with GA (1 *mg/l*) solution (B). One lot of sections of about 10 *mm* in length was excised from hook zone of hypocotyls (lower), and the other from the adjoining distal zone (upper).

Sections of about 10 *mm* in length were excised from the hook zone of the hypocotyl and from the adjoining distal part of the same hypocotyl. Experimental materials were extracted from each experimental lot of 5 *g* in fresh weight and bioassayed by the usual method.

As seen from Fig. 11, the inhibitor activity of the hook zone of the hypocotyls increased markedly due to the GA treatment. These data are suggestive of some correlation between the acceleration of elongation growth and the increase of inhibitor-β content.

Experiment 10. Acceleration of germination by inhibitor-β obtained from potato tuber

In natural growth processes inhibitor-β is contained abundantly in the hook zone of *Phaseolus* hypocotyl, where the cell division and cell elongation are going on actively, and inhibitor activity in the same zone is increased by GA treatment. These facts provide the support for the concept that any relation might exist between the stimulation of hypocotyl elongation and the increase in inhibitor-β activity caused by GA treatment.

BONDE[8] extracted neutral inhibitor from leaves of cocklebur and assayed by *Avena* coleoptile curvature test. He found that this inhibitor suppressed the coleoptile curvature reaction due to IAA treatment when the neutral in-

hibitor was applied at a high concentration, while it accelerated clearly at low concentration, and it was also ascertained that such acceleration was independent to the IAA concentrations applied. VARGA and KÖVES[9] examined the function of inhibitor-β obtained from bean plants by means of coleoptile section test and proved similar facts.

The present authors have attempted to see whether such growth acceleration may occur at certain dilutions of inhibitor-β which were extracted from potato tubers and partially purified paper chromatographically.

Using the basal solution of inhibitor-β obtained from potato tubers, it was studied to determine whether some acceleration of growth may be recognized at certain weaker concentrations than that of the basal inhibitor solution. The stimulating effect of inhibitor-β for growth could not recognized utilizing the elongation test with *Avena* hypocotyl section or wheat leaf section at various rates of dilution of the basal inhibitor solution, while, by the seed germinating test of several crop species, a significant accelerating effect on germination was ascertained at the appropriate rate of dilution.

a) Germination of summer wheat (var. 'Norin' No. 75).

4 sheets of filter papers were laid on the bottom of each beaker of 100 *cc* capacity and moistened with 8 *cc* of diluted inhibitor-β solution. As the control, distilled water was used instead of the inhibitor solution. Fifteen wheat grains were placed on the filter papers in each beaker, setting the embryo upward, and beakers were put together in a large container and covered with a glass plate in order to avoid evaporation of the culture solution during the incubation. After incubation at 25°C in the dark for 4 days, coleoptile lengths were measured. The data illustrated in Table 1 were indicative of several replicate measurements. Concentrations of inhibitor were expressed in gram(s) of fresh weight of potato tissues which were used to obtain 1 *cc* of the inhilitor solutions, At the inhibitor concentration of $\times 0.5$, the length of coleoptile was about 24 per cent over the control.

TABLE 1. *Effect of inhibitor-β obtained from potato tuber on the germination of wheat seeds*

Concentration of inhibitor	Control (no addition)	×0.1	×0.5	×1.0
Length of coleoptile (mm)	36.9	39.7	45.9	41.3
Percentage	100	108	124	112

b) Germination of dwarf pea

In order to keep suitable conditions of water and air supply for germination, the germination tests were made in moist sawdust. The Sawdust was repeatedly boiled with water to remove all water soluble materials, and finally dried. Six *g* of dried sawdust and 30 *cc* of the test solution were put into 100 *cc* conical beakers. One of the test lots contained inhibitor solution only and the other was the one to which GA solution was added. Ten seeds were embeded in each beaker and allowed to germinate at 25°C in the dark for 3 days. Then the lengths of the roots and epicotyls were measured and the average was calculated. The results are shown in Table 2.

TABLE 2. Effect of inhibitor-β obtained from potato tuber on the germination of dwarf pea seeds

Addition of gibberellin (1 mg/l)	−			+		
Concentration of inhibitor	0	×2	×4	0	×2	×4
Length of root (mm)	53.8	48.1	47.5	53.4	53.8	51.4
Percentage	100	89	88	100	101	96
Length of epicotyl (mm)	10.1	10.3	9.8	13.4	15.5	16.6
Percentage	100	102	97	100	116	124

The influence of inhibitor-β on the root elongation was not clearly established in both cases, alone and in collaboration with GA. As to the elongation of epicotyls, inhibitor-β gave no clear effect when it was supplied independently, while in the presence of GA a significant increase of elongation growth was observed. Inhibitor-β showed also a growth stimulation indicated by the germination of *Phaseolus* seeds or by the culture of excised root tips of *Phaseolus* seedlings.

Discussion

It has already been shown that the apical hook part of the hypocotyls of etiolated *Phaseolus* seedlings is a zone of cell division and the adjoining proximal part of it is a zone of active cell elongation, while in the further lower part, the cell division and the cell elongation are already finished. When GA is applied to such young seedlings exogenously, the elongation of the hypocotyls stimulated markedly. However, the region of hypocotyls which is sensitive to the exogenous GA, is restricted to the young growing part of them,

while the region where the elongation growth is already terminated, no growth was caused even by the exogenous supply of GA. In order to obtain further information on these problems, the writers excised the sections from various parts of hypocotyls of young *Phaseolus* seedlings and the sections were infiltrated with GA solution. After the treatment the sections were divided into two groups, one of them was used for GA extraction immediately after the GA treatment and the other was used for GA extraction after being allowed to stand overnight, and the changes of GA activity in the tissue due to such treatment were measured by means of bioassay.

According to the results, GA activity in the sections obtained from hypocotyl zones where the cell division or the cell elongation is occurring actively, disappeared almost completely after overnight incubation, while infiltrated GA could be recovered almost completely from the sections which were excised from the hypocotyl zone where the cell elongation has almost terminated.

It was also ascertained in the sections excised from zone of cell division that the content of the inhibitor-β increased significantly according to the decrease in GA activity.

These data lead to the assumption that GA introduced into such sections exogenously may suffer any changes. In intact hypocotyls of *Phaseolus* seedlings, inhibitor-β is contained only in the hook zone, and when excised sections of them are treated with GA solution inhibitor-β is formed in the same zone. These results highly suggest that the formation of the inhibitor-β in the sections may be influenced by GA introduced exogenously. It is also interesting to see whether a similar pattern may occur in the intact seedlings.

Phaseolus seeds were germinated in sawdust moistened with GA solution or with water as control, in the dark. The elongation of hypocotyl treated with GA was more significant than that of the control, and the activity of inhibitor-β in the hook zone of the hypocotyls treated with GA was much intensified, compared with those of the control. The above-mentioned results lead to the assumption that native GA may play an important role in the normal growth of plants and the growth acceleration caused by GA is causally related to the formation of inhibitor-β in the growing zone.

Inhibitor-β is contained abundantly in dormant potato tubers and dormant *Fraxinus* buds, so it is considered that this substance may control the dormancy of these plants (ref. BENTLEY[10]). On the other hand, the same substance is also contained richly in actively growing parts of plants (YOSHIMURA and TAGAWA[6]; VARGA and KÖVES[9]; BOICHUK[11]; DÖRFFLING[12]). These facts highly suggest that inhibitor-β may participate in the active growth process (BENTLEY[10]; BOICHUK[11]) or may be responsible for the cell division[6].

The present authors have examined the influence of inhibitor-β obtained from potato tuber on the seed germination of several species of crops and ascertained that inhibitor-β stimulated the elongation of wheat coleoptile and of dwarf pea epicotyl at proper diluted concentrations. These effects on growth were observed only at the early stage of germination and became less distinctive with the developmental march of a seedling. The reason for this is not yet clear, but it is supposed that a seedling would become soon able to produce this substance so much that it no longer appeared to be a limiting factor of the growth. Further explanation of this is deserving of further study.

The formation of inhibitor-β was accelerated by an irradiation of light (SIMPSON and WAIN[13]). Such formation was also detected by the authors over wide regions covering not only the hook zone but also all the elongating zones and further proximal zone of the hypocotyl of green *Phaseolus* seedlings. While in the etiolated one inhibitor-β is localized only in the hook zone of the hypocotyl. The wider distribution of inhibitor-β in the hypocotyls of seedlings grown in light makes it highly probable that its physiological role may be a suppression of cell elongation in light.

MOHR and PETERS[14] have found that light inhibited the elongation of hypocotyl of *Sinapis alba*, while accelerated the cell division of them. Taking into account their experimental data, the writers wish to express a suggestion that the physiological role of inhibitor-β may be considered under two categories; one is the influence on cell division and the other is the regulation of cell elongation.

It has long been known that GA stimulate not only the cell elongation but also the cell division (GREULACH and HAESLOOP[15]; SACHS and LANG[16]; FEUCHT and WATSON[17]). The difference in the reaction due to GA treatment may be attributed to the difference in the developmental stages of tissues. According to our experimental results, it was ascertained that GA treatment increased inhibitor-β content in the meristematic regions, so it seems very reasonable to assume that some relations might exist between the stimulation of cell division and increased formation of inhibitor-β; both caused by GA treatment.

After the consideration of huge data on the physiology of growth regulator, the writers wish to express their general suggestion that the normal growth status of plants might be attributed to a balance among the natural growth factors. And inhibitor-β would be one of these factors and this may play an important role in regulating cell division and cell elongation of plants under close co-operation with GA.

Summary

1) Sections were excised from the apical part of hypocotyls of etiolated *Phaseolus* seedlings. Distilled water or gibberellin (GA) solution were infiltrated into the sections, then they were divided into two groups. One of them was used for extraction with alcohol immediately, and the other was used for extraction after being allowed to stand overnight incubation, in the dark, at 25°C. The changes in activities of inhibitor-β and of GA were measured by the wheat leaf elongation test.

2) The contents of GA and inhibitor-β in sections were not so significant immediately after the sections were cut, but after the sections were incubated overnight in a moist chamber, the activity of the inhibitor increased appreciably. When GA solution was introduced into the sections, prior to the incubation, the inhibitor production increased far more than it did in the former experiment. GA introduced into the sections could be recovered readily if an extraction was made immediately after the treatment, but after overnight incubation no activity was detected.

3) Using with the sections excised from various parts of the hypocotyls, the ability of inhibitor-β formation and changes in the activivity of introduced GA were examined. Both the formation of inhibitor and GA consumption were found to be localized only in the hook zone of the hypocototyls where cell division is going on actively, while in the proximal part of this hook zone of the hypocotyl where cell elongation is occurring actively GA consumption was recognized, however, no formation of inhibitor was observed. In the further proximal part of the hypocotyl where the growth is terminated, neither GA consumption nor inhibitor formation were ascertained.

4) In the leaves of etiolated *Phaseolus* seedlings, GA consumption was shown, but no inhibitor formation was detected.

5) When the apical sections were placed in a moist chamber overnight inhibitor-β was formed, but the elongation was not appreciable, while if distilled water was supplied to the sections the elongation was significant and in this case no inhibitor-β was indicated in their extract. These data suggest that by the formation of inhibitor-β in excised sections, a limited supply of water might cause suppressions of growth and of inhibitor-β production.

6) When intact seedlings were placed in a water deficiency condition for 2 or 3 days, the elongation of hypocotyls was restricted appreciably and inhibitor-β content increased excessively in all regions.

7) Both the inhibitor-β formation and GA consumption in the sections did not occurred in the absence of oxygen.

8) In green seedlings of *Phaseolus* the rates of inhibitor formation and of GA consumption were similar to those in the etiolated ones.

9) Using the inhibitor-β preparation which was extracted from potato tubers and separated by means of paper chromatography, the effect of this preparation on the germination of seeds was studied, and it was ascertained that this accelerated the growth of wheat coleoptiles and epicotyls of dwarf pea.

10) The writers wish to express their general suggestion that inhibitor-β would be one of the natural plant growth factors and this may play an important role in regulating cell division and cell elongation in plants under close co-operation with gibberellin.

Literature

1) HEMBERG, H. 1958. The occurrence of acid inhibitors in resting terminal buds of *Fraxinus*. Physiol. Plant., **11**, 610-614.
2) HEMBERG, H. 1958. The significance of the inhibitor-β complex in the rest period of the potato tuber. Ibid., 615-626.
3) BOO, L. 1961. The effect of gibberellic acid on the inhibitor-β complex in resting potato. Physiol. Plant., **14**, 676-681.
4) PHINNEY, B. O. and WEST, C. A. 1960. Gibberellins as native plant growth regulators. Ann. Rev. Plant Physiol., **11**, 411-436.
5) KEFFORD, N. P. 1955. The growth substances separated from plant extracts by chromatography. II. J. Exp. Bot., **6**, 245-255.
6) YOSHIMURA, F. and TAGAWA, T. 1961. Growth regulator in germinating rice seed. J. Fac. Agr., Hokkaido Univ., **51**, 559-573.
7) GALSTON, A. W. and WARBURG, H. 1959. An analysis of auxin-gibberellin interaction in pea stem tissue. Plant Physiol., **34**, 16-22.
8) BONDE, E. K. 1953. Growth inhibitors and auxin in leaves of cocklebur. Physiol. Plant., **6**, 234-239.
9) VARGA, M. and KÖVES, E. 1959. Distribution and quantitative changes of the β-inhibitor in the various organs of the bean plant during ontogeny. Acta Biol. Acad. Sci. Hungaricae, **9**, 369-378. (Biol. Abst., **35**, (1960), 1925).
10) BENTLEY, J. A. 1958. The naturally-occurring auxins and inhibitors. Ann. Rev. Plant Physiol., **9**, 47-80.
11) BOICHUK, O. B. 1962. The distribution and dynamics of growth substances in young tomato plants. Ukrain Bot. Zhur., **19** (3), 34-45. (Biol. Abst., **40** (1962), 1248).
12) DÖRFFLING, K. 1963. Die Bedeutung von Inhibitor β für die korrelative Hemmung und für die Winterruhe der Knospen von *Acer pseudoplatanus*. Planta, **59**, 346-350.
13) SIMPSON, G. M. and WAIN, R. L. 1961. A relationship between gibberellic acid and light in the control of internode extention of dwarf peas (*Pisum sativum*). J. Exp. Bot., **12**, 207-216.

14) MOHR, H. und PETERS, E. 1960. Der Phototropismus und das lichtabhängige Längenwachstum des Hypocotyls von *Sinapis alba* L. Planta, **55**, 637–646.
15) GREULACH V. A. and HAESLOOP, J. G. 1958. The influence of gibberellic acid on cell division and cell elongation in *Phaseolus vulgaris*. Amer. J. Bot., **45**, 566–570.
16) SACHS, R. M. and LANG, A. 1957. Effect of gibberellin on cell division in *Hyoscyamus*. Science, **125**, 1144–1145.
17) FEUCHT, J. R. and WATSON, D. P. 1958. The effect of gibberellins on internodal tissues of *Phaseolus vulgaris* L. Amer. J. Bot. **45**, 520–522.