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SUMMARY

Measurements were made of the effects of indoleacetic acid (IAA), gibberellic acid (GA), benzyladenine (BA) and tryptophan (TP) on the growth of segments taken from the apex of decapitated, etiolated seedlings of *Pisum sativum* L. var. Alaska.

In terms of long-term growth responses, low levels of IAA caused elongation, whereas high levels caused expansion. At low levels, GA had no effect, at high levels it caused elongation. Tryptophan caused responses similar to those caused by GA, but these 2 substances did not act additively. BA had little promotory or inhibitory effect on growth.

Two hypothesis were concluded that either; a) two growth systems exist and the one governed by GA is suppressed in the presence of IAA, or b) only one growth system exists and IAA is dominant in its control (i.e. expansion and elongation are manifestations of the same growth system operating at different magnitude).

The main purpose of this study is to find evidence to support either of these hypothesis.

INTRODUCTION

The complexity of the control mechanisms of hormonal action on growth in higher organisms is only partially understood. The basic goal of the study of plant hormones is to determine the actions of the hormones both separately and through interaction with other hormones on growth or other processes involved. However, there are many difficulties in trying to achieve this goal.

This study was done under the supervision of Prof. E. DAVIES at the University of NEBRASKA, U.S.A. (Doctoral dissertation).

It has been reported that high concentrations of indoleacetic acid (IAA) in lanolin cause lateral expansion in the sub-apical region of decapitated, etiolated pea epicotyls (FAN and MACLACHLAN, 1966; 1967; MAC QUARRIE, 1965; RIDGE and OSBORNE, 1969; DAVIES, 1974).

Similar concentrations of gibberellic acid (GA), however, cause marked elongation (DAVIES and MACLACHLAN, 1968; DAVIES, 1974). Since decapitation removes almost the all source of IAA (SCOTT and BRIGGS, 1963) but not of GA (RADLEY, 1958), the "abnormal growth" caused by IAA and the continuance of "normal growth" caused by GA seem unexpected.

The differing effects of IAA and GA could be due to either; a) each hormone affecting a different growth system, or b) both hormones affecting the same growth system, but at different magnitudes, so that at low magnitudes elongation ensues, but at high magnitudes, expansion ensues.

The studies described in this paper were conducted to find evidence to support either hypothesis. Expansion is accompanied by increases in activity of the enzyme, cellulase (β -1, 4 glucan 4-gluconohydrolase, EC 3.2.1.4), increases in polyribosome content and increases in protein synthesis.

But this part of growth studies is not given in this paper because of limited space. They will be given in another paper.

MATERIAL AND METHODS

Pea seeds (*Pisum sativum* L., var. Alaska) were obtained from Earyl May Seed and Nursery Co., Shenandoah, Iowa, 51601, U.S.A. They were washed for 20 minutes in 0.5 % sodium hypochloride, rinsed thoroughly and allowed to imbibe overnight in tap water. The imbibed seeds were sown in moist artificial soil (vermiculite) and grown at 23 to 25 °C in darkness until ready for further treatment. Under dimgreen light, these epicotyls with third internode 2 to 5 cm long were decapitated, i.e. the plumule and hook were detached (FAN, 1967). This removed meristematic tissue which, in the intact plant, is the major source of, natural

auxin to the internode below (SCOTT and BRIGGS, 1963). The internode was marked with ink 10 mm from the cut apex to delineate a "segment" of tissue. This region contained virtually all of the elongation cells in the epicotyl (WENT and THIMANN, 1937; YOUNG, 1962), and was comparable to that used in many previous growth studies.

In most experiments, the decapitated epicotyls were each supplied at the apex with about 2 to 2.5 mg lanolin paste (hydrous) in which IAA or other hormones were suspended at varying concentrations. Methods for preparing the suspensions are described in detail elsewhere (FAN, 1967; DATKO, 1968). The segments were allowed to grow while still attached to the plant for various periods of time when they were removed for analysis, lanolin paste was wiped off from the segments and fresh weight and length were measured rapidly to the nearest 0.1 mg and 0.5 mm respectively. Segments were rinsed briefly in dilute hypochloride and distilled water, then wiped dry and chilled to 2 °C prior to fractionation.

RESULTS AND DISCUSSION

A. Enhancement of Elongation and Expansion in Response to Various Levels of Indoleacetic acid (IAA).

Indoleacetic acid (IAA) applied at high concentrations (0.5 % in lanolin) to decapitated, etiolated pea stem causes massive expansion (FAN and MACLACHLAN, 1966, 1967; MAC QUARRIE, 1965; RIDGE and OSBORNE, 1969) whereas at the same concentrations GA causes elongation (DAVIES, 1974).

In an effort to determine whether these differences in growth were due to different growth systems or alternatively, whether expansion and elongation are manifestations of the same growth system operating at different magnitudes, varying concentrations of IAA and GA were applied separately or together. It was found that in the absence of IAA, increasing amounts of GA (0.005 to 0.5 %, i.e. 0.1 – 10.0 microgram/plant) caused increased elongation but had no effect on expansion.

However, all IAA levels (0.005 to 0.5 %) caused similar inhibition at elongation and enhancement of expansion or swelling

(weight/length) whether GA was present or not. The effects of IAA were completely dominant even when GA was supplied at 100 times the concentration of IAA. It was concluded that either; a) two growth systems exist and the one governed by GA is suppressed in the presence of IAA, or b) only one growth system exists and IAA is dominant in its control.

1. Effects of Low Levels of IAA

Although most researchers have emphasized the expansion caused by high levels of IAA, relatively little has been published on the effects of low IAA levels on the growth of decapitated, etiolated pea epicotyls.

If GA acts prior to IAA in the same growth system and this system can result in elongation, an automatic implication is that some (low) auxin level should stimulate elongation rather than expansion. Accordingly, low amounts (0.5 – 10 ng/plant) of IAA were applied to decapitated peas and the resulting growth is recorded in Fig. 1. At 0.5 ng/plant (i.e. 0.000025 %, 1/20,000 the amount normally applied – FAN and MACLACHLAN, 1966), IAA caused a measurable increase in elongation. Elongation increased with IAA level up to 4 ng per plant, but above this IAA level, elongation was increasingly inhibited (Fig. 1). Total growth (expressed as fresh weight) also increased with auxin concentration up to about 4 ng per plant, but was maintained (rather than reduced) at higher IAA levels. This caused expansion (weight/length) to increase above 4 ng IAA per plant. In various similar experiments, the optimal amount of IAA for elongation has varied between 4 and 10 ng per plant, but noticeable expansion only occurred above this optimal amount. This means that the type of growth taking place at low auxin levels is a continuance of "normal" elongation, but that at higher auxin levels elongation is inhibited and "abnormal" expansion occurs. Hence, IAA can govern both elongation and expansion in the same epicotyl and the relative amount of each type of growth is dependent on the amount of IAA applied.

If IAA can cause elongation or expansion through the same system then the elongation brought about by GA may also be mediated through this system and should be inhibited by an

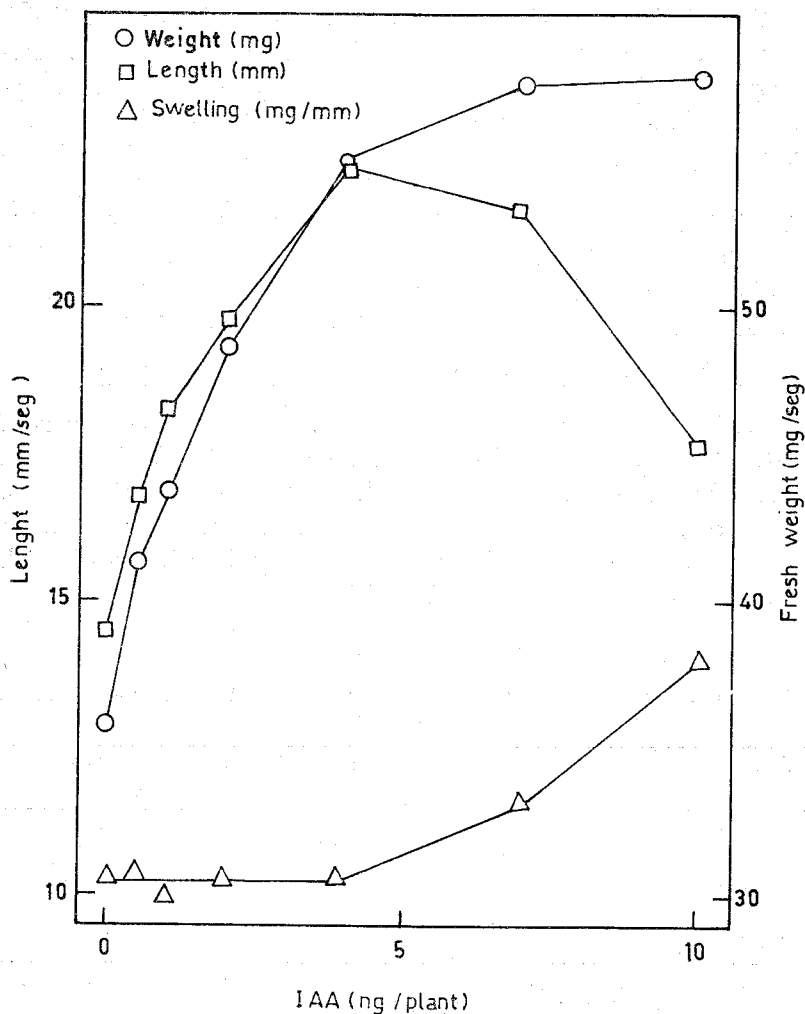


FIGURE 1. Effect of Low Levels of IAA on Elongation and Expansion of Pea Epicotyls.

Peas were grown 7-8 days in the dark at 23-25° C until third internode was 2-5 cm long. The plumule and hook were excised, a mark made 10 mm below the cut apex to delineate a segment of tissue. The cut apex was treated with about 2 mg lanolin plus IAA in the amounts indicated. Measurements were made of length and fresh weight of the segment of tissue which developed from the 100 mm of epicotyl after 2 days growth. Length was measured to the nearest 0.1 mm and fresh weight to the nearest mg.

Expansion (swelling) is expressed as fresh weight/length (mg/mm).

anti-auxin if the effect of GA is prior to IAA. However, if GA acts through a separate system, or if it acts after IAA in the same system its effects should not be modified by an anti-auxin. Application of increasing amounts (0.5 – 5.0 $\mu\text{g/plant}$) of TIBA, a rather specific inhibitor of basipetal auxin transport (NIEDER-GANG – KAMIEN, E. and F. SKOOG, 1956), caused increasing inhibition of elongation in all instances (DAVIES, 1974). This means that GA-induced growth is modified to the same extent as IAA-induced growth and it would appear that GA must be acting through a system controlled primarily by IAA.

2. Effects of Various Concentration of IAA and GA

If GA is acting on a separate growth system, then levels of GA which cause elongation should have an additive effect when applied together with levels of IAA which also cause elongation. However, the data in Table 1 shows that GA had very little influence on elongation brought about by low levels of IAA (nor on the expansion caused by high levels of IAA).

TABLE 1

Effects of IAA and GA, separately and together on Elongation and Expansion of Pea Epicotyls.

Treatment	Length (mm/seg)	Fresh Weight (mg/seg)	Swelling (mg/mm)
LANOLIN (Control)	16.0	40.1	2.5
IAA (Low) ^a	23.8	76.2	3.2
IAA + GA (Low)	24.6	76.3	3.1
IAA (High) ^b	14.5	85.5	5.9
GA (High)	25.8	72.2	2.8
IAA + GA (High)	15.8	88.5	5.6

a Low level (0.0005 %) equals 10 ng/plant.

b High level (0.5 %) equals 10 $\mu\text{g/plant}$.

Lanolin \mp IAA, GA and TP at 0.0005 and 0.5 % w/w, i.e. 0.01 and 10 μg per plant, respectively, were applied to the decapitated epicotyl. Length and fresh weight were recorded after 48 hrs growth. At least 20 seedlings were used for each hormone treatment. Values denote average of 2 experiments.

Nevertheless, evidence presented elsewhere (DAVIES, 1974) showed that after 4 days growth GA did cause a noticeable inc-

rease in elongation of tissue treated with low levels of IAA. This support the suggestion that GA is acting to maintain the level of IAA in the growing region. Maintenance of the IAA level by GA could be accomplished through one of two ways. GA could either prevent break down of IAA already there (HALEVY, 1963; RUSSEL and GALSTON, 1969) or cause synthesis of new IAA (WIGHTMAN and COHEN, 1968; BLACK and HAMILTON, 1971; GIBSON and WHIGHTMAN, 1972; TRUELSON, 1972). Since tryptophan is considered the major precursor of IAA in higher plants (WILDMAN and BONNER, 1948; BLACK and HAMILTON, 1971; GIBSON and WHIGHTMAN, 1972) its effects on growth were studied first.

3. Effects of Tryptophane on Growth

The data in Fig. 2 shows that increasing amounts of Tryptophane (TP) above 4 μg /plant cause increase in length and fresh weight up to 400 μg /plant. Concentration of TP higher than 400 μg /plant could not used as suspension because it is more concentrated which then could not be made.

In this experiment the growth of apical segments of 7-8 days dark grown decapitated pea seedlings induced by TP (see Table 11 and Fig. 2), but did not considerably enhanced in the presence of GA. If GA acts to conversion of TP to IAA then TP in the presence of GA should give much better growth than GA alone. The data in Table 11 show that TP does not enhance growth in the presence of GA, this would suggest that GA is not involved in the synthesis of IAA through conversion of TP to IAA.

The other possibility is that TP is probably being used as a nutrient. Many other amino acids were tested some of them gave some growth, but none of them gave as much growth as TP (data not shown). So most likely the role of TP is not as a nutrient.

The final possibility is that TP is presumably contaminated by IAA in purchased bottle. This is hard to show. During the experiment when 400 μg /plant TP was added to decapitated seedlings some expansion, 3.5 mg/mm (see Fig. 2), resulted. This

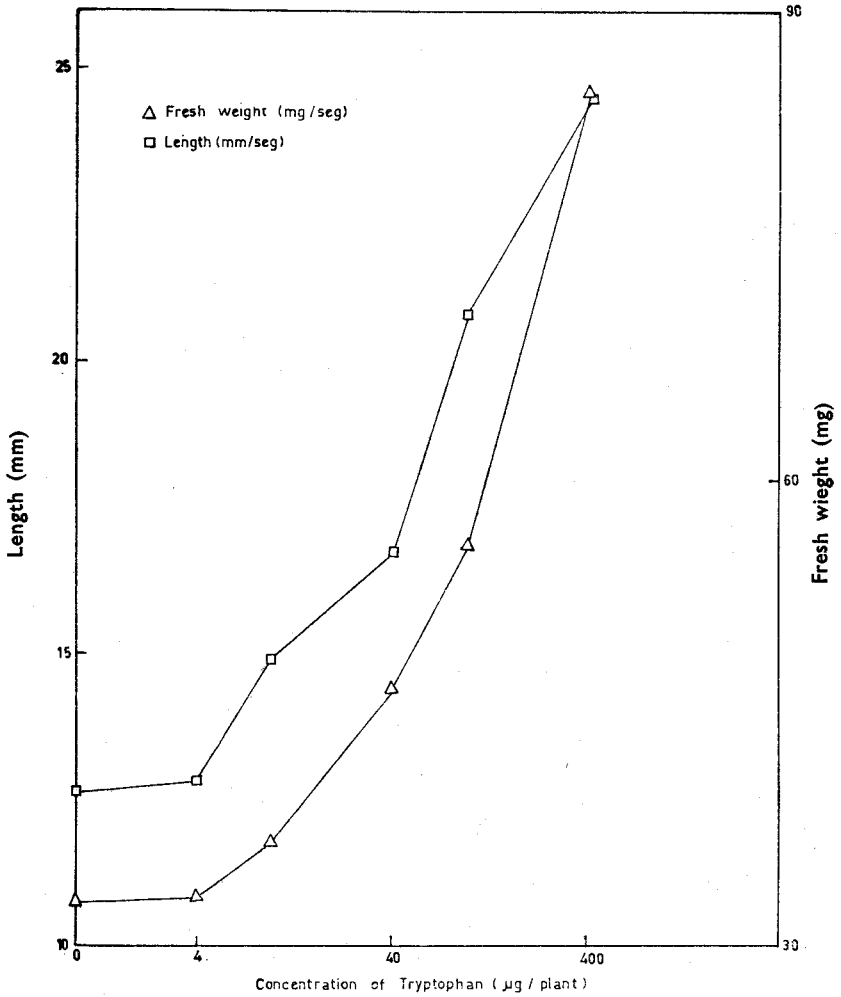


FIGURE 2. Effect of Tryptophane on Growth.

Methods were the same as in Fig. 1, except tryptophane, at 0, 4, 10, 40, 100 and 400 $\mu\text{g/plant}$ was applied to the decapitated seedlings.

TABLE 11
Effects of GA and TP, separately and together, on Growth

Tryptophan ($\mu\text{g/plant}$)	Length (mm/seg)	-GA		+ GA ^a	
		Fresh weight (mg/seg)	Length (mm/seg)	Fresh weight (mg/seg)	
0	16.8	40.7	28.4	84.2	
2	17.3	42.7	27.9	74.6	
5	18.5	51.2	29.3	79.9	

^aGA applied at 10 μg per plant (0.5 %).

See legend to Table 1. Cut apices were treated with 2 mg lanolin paste containing GA and TP at the amounts indicated. much expansion looks like less than that caused 100 ng IAA. So this gives $100 \text{ ng}/400 \mu\text{g} = 1/4000$ contaminant. That means it cannot be measured, as it is so small.

4. Enhancement by 6-Benzyladenine – 6-Benzylaminopurine – (BA) of Auxin-Promoted Growth in Decapitated Pea Seedlings

In studies on auxin-cytokinin interaction in growth, some workers have shown an inhibition of auxin promoted elongation by cytokinin (De ROPP, 1956; HASHIMOTO, 1961; KATSUMI, 1963; SOMMER, 1961), whereas others have shown an enhancement (SHRANK, 1958; HEMBERG and LARSSON, 1972; HEMBERG, 1972; JORDAN and SKOOG, 1971; DENİZCİ, 1966).

The effects of BA, a synthetic cytokinin, on endogenous and low and high levels of IAA-induced growth of decapitated, etiolated pea seedlings are presented in Table 111. Low levels of BA caused a slight increase in growth of elongating tissue. High levels of BA caused a greater increase in fresh weight than in elongation. So that the tissues expanded more. In no case did BA cause a marked increase in growth and in no case did it inhibit growth.

This data suggests that BA, rather than generally inhibiting growth, is affecting a cellular activity (e.g. cell division) which is associated specifically with auxin activity and during the interaction of BA and IAA, IAA is dominant.

HEMBERG and LARSSON (1972), proposed that cytokinin promoted elongation by increasing endogenous auxin concent-

TABLE III

Effects of Benzyladenine (BA) Alone and in Conjunction with IAA on Growth

Treatments	Length (mm/seg)	Fresh Weight (mg/seg)	Swelling (mg/mm)
Lanolin (Control)	14.5	32.2	2.2
BA (High) ^a	16.9	42.8	2.5
BA (Low) ^b	15.4	35.7	2.3
IAA (High)	14.2	78.2	5.5
IAA (Low)	20.5	50.0	2.4
BA + IAA (High)	13.2	81.0	6.1
BA + IAA (Low)	21.7	53.4	2.5
BA (High) + IAA (Low)	21.9	64.6	3.0
BA (Low) + IAA (High)	14.0	76.7	5.5

^a High level equals 10 $\mu\text{g/plant}$ (i.e., 0.5 % w/w)^b Low level equals 0.01 $\mu\text{g/plant}$ (i.e., 0.0005 % w/w)

See legend to Table 1. After 2 days growth, at least 20 segments were measured for each treatment. Values denote average of 2 experiments.

ration, thereby enhancing auxin-promoted elongation at low auxin concentrations and inhibiting auxin-promoted elongation at high auxin concentrations by inducing supraoptimal auxin levels in the cell.

This hypothesis is supported by growth experiment with *Avena* (SHRANK, 1958; HEMBERG and LARSSON, 1972; DENİZCI, 1966) and measurement of cellular and diffusable auxin concentrations in the presence and absence of exogenous cytokinin (HEMBERG, 1972; JORDAN and SKOOG, 1971). The interaction of cytokinin and auxin in this work with *Pisum sativum* shows similar results to HEMBERG's that the inhibitory effects of BA on elongation of tissue treated with high levels of IAA which was accompanied by a promotion of expansion.

CONCLUSIONS

In this study, the most striking results are as follows:

1. High levels of indoleacetic acid (IAA) cause expansion, similar levels of gibberellic acid (GA) cause elongation. When both hormones are applied together, the effects of IAA are domi-

nant and expansion ensues. Very low levels of IAA, however, cause elongation. At low levels GA had no effect.

2. The increasing amounts of tryptophan cause increase in elongation, but when both tryptophan and GA are applied together, GA does not considerably enhance the elongation.
3. Benzyladenine had little promotory or inhibitory effect on growth.
4. Two hypothesis were concluded that either; (a) two growth systems exist and one governed by GA is suppressed in the presence of IAA, or (b) only one growth system exists and IAA is dominant in its control.

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ÖZET

Bu çalışmada, karanlıkta büyütülmüş, epikotilin ucundaki plumula kısmı koparılmış bezelye (*Pisum sativum* L.var. Alaska) fidelerinin uç kısımlarından alınan parçaların büyümesine indolasetik asit (IAA), giberellik asit (GA), benziladenin (BA) ve triptofan (TP) ın yaptığı etkileri göstermek için gerekli ölçmeler yapılmıştır.

Hormon tatbikiyle uzun-büyüme devresinde (0-72 saat), IAA'nın düşük konsantrasyonları dokularda uzamaya, buna karşılık yüksek konsantrasyonları şişmeye neden olmuştur. Giberellik asitin düşük konsantrasyonları etki etmediği halde, yüksek konsantrasyonları dokularda uzamayı sağlamıştır. Triptofanın büyümeye etkisi GA'ninkine benzediği halde, herikisi birlikte tatbik edildiğinde, ayrı ayrı tatbik edildiklerinden farklı olarak, büyümede fazla bir artış kaydedilmemiştir. Benziladeninin ise büyümeyi artırmakta veya engellemekteki etkisinin çok az olduğu belirlenmiştir.

Burada iki varsayım düşünülmüştür. Bunlardan birisi; iki büyüme sistemi vardır ve sistemlerden birisi GA tarafından idare edilmektedir. Bu sistemde GA'nın etkisi, IAA'nın varlığıyla bastırılmaktadır. Ötekisi; sadece bir büyüme sistemi vardır ve IAA'nın büyümeyi kontroldeki etkisi, dominanttır (bu demektir ki şişme ve uzama, farklı büyüklükte oluşan aynı büyüme sisteminin sonuçlarıdır).

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