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# Effects of Light, Abscisic Acid, and $^6$N-Benzyladenine on the Metabolism of [$^3$H]Gibberellin $A_4$ in Seeds and Seedlings of Lettuce, cv. Grand Rapids[1]

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RICHARD C. DURLEY,[2] J. DEREK BEWLEY,[3] IAN D. RAILTON,[4] AND RICHARD P. PHARIS
*Department of Biology, University of Calgary, Calgary, Alberta T2N IN4, Canada*

## ABSTRACT

**Gibberellin $A_4$ ($GA_4$) can substitute for light in the germination of Grand Rapids lettuce seeds. Seeds imbibed in [$^3$H]$GA_4$ do not convert this to other GAs prior to, or immediately following, visible germination: thus $GA_4$ alone can promote radicle expansion. Abscisic acid inhibited [$^3$H]$GA_4$-induced germination, but did not significantly affect [$^3$H]$GA_4$ uptake or metabolism during germination. $^6$N-benzyladenine overcame the inhibitory effect of abscisic acid and increased [$^3$H]$GA_4$ uptake, although radicle emergence was delayed somewhat.**

**During hypocotyl extension there was a large conversion of [$^3$H]$GA_4$ to [$^3$H]$GA_1$ in light or darkness, the major conversion site being the growing root. Hypocotyls of dark-grown seedlings contained more [$^3$H]$GA_1$ than those of light-grown seedlings. The apparent inability of exogenous $GA_1$ to promote greater hypocotyl extension than $GA_4$ is related to its poorer uptake. Abacisic acid markedly inhibited hypocotyl expansion, root growth, and the conversion of [$^3$H]$GA_4$ to [$^3$H]$GA_1$.**

The physiological action of gibberellins on lettuce seed germination has been widely studied. Not all GAs are equally effective in promoting germination of seeds of the lettuce cultivars Arctic King or Grand Rapids, but among the most potent are $GA_4$ and $GA_7$ (4). Subsequent hypocotyl growth is effectively promoted by $GA_7$ in cultivars Arctic (8) and Grand Rapids (reported herein), although $GA_4$ is very promotive in Grand Rapids (reported herein, and 18) relative to Arctic (8). ABA is known to prevent $GA_3$-induced germination, and BA will overcome this inhibitory effect (2).

Herein we report on the metabolism of [$^3$H]$GA_4$ during seed germination and subsequent hypocotyl extension of Grand Rapids lettuce both in the presence and absence of ABA and in the presence of ABA + BA.

## MATERIALS AND METHODS

Seeds of *Lactuca sativa* L. (cv. Grand Rapids) were obtained from Ferry-Morse Inc., California and stored at 2 C until required. For specific experimental conditions see "Results and Discussion."

**Synthesis of [$^3$H]$GA_4$.** Using hydrogen enriched with tritium, $GA_7$ was hydrogenated to 1, 2-[$^3$H]$GA_4$, (by Amersham Searle Corp.) according to Cross *et al.* (6) and subsequently purified (9) to a constant specific radioactivity of 1.87 Ci/mmol ($12.6 \times 10^6$ dpm/$\mu$g). The product was stored at −20 C in 95% ethanol purified immediately prior to use on a silica gel partition column (10).

**Extraction and Chromatography.** After incubation, seeds or seedlings were plunged into methanol at −22 C and stored at −22 C until extraction in a mortar with acid-washed sand at 0 C in 80% methanol. Methanol was removed *in vacuo* and an equal volume of 0.5 M phosphate buffer (pH 8) was added to the residual aqueous phase. After adjusting to pH 9 with 3 N KOH the extract was partitioned (4X) against equal volumes of diethyl ether, adjusted to pH 3, partitioned (6X) against ethyl acetate and then (4X) against l-butanol. Thus, for each harvest four fractions were obtained: neutral, ether extract; acidic, ethyl acetate extract; acidic, butanol extract; residual buffer solution. Only the acidic, ethyl acetate extract, which contained almost all of the extractable radioactivity (Table III), was examined further.

The acidic, ethyl acetate-soluble fractions were dissolved in a minimum volume of 0.5 M phosphate buffer (pH 8) and purified on a PVP column eluted with 0.1 M phosphate buffer (pH 8) (14). After extraction at pH 3 into ethyl acetate and evaporation, the residues were chromatographed on a silica-gel partition column (10). Twenty-seven fractions were collected and combined according to counts obtained from direct liquid scintillation using an external standard. Typical combinations are shown in Table I. The combined fractions were converted to trimethylsilyl ethers of the methyl ester derivatives as per Cavell *et al.* (5) and examined on three column liquid phases (2% AFl, 2% SE30, and 1% XE60) by gas-liquid radiochromatography as per Durley *et al.* (12). The radioactive peaks were compared to those of standards and results of a typical extract are summarized in Table I. Fractions 4 to 8 contained $GA_4$, fractions 13 and 14 contained $GA_1$, fractions 15 and 16 contained an unknown compound A, and fractions 17 to 19 contained $GA_2$. When [$^3$H]$GA_4$ (not fed to seeds) was taken through the work-up procedure outlined above it was found that compound A and $GA_2$ were produced in appreciable quantities.

**Uptake and Metabolism of Gibberellins in Roots, Hypocotyls, and Cotyledons.** Conversion of [$^3$H]$GA_4$ to other GAs in the root, hypocotyl, and cotyledons was determined in seedlings germinated and grown under continuous white fluorescent light, or in darkness, on 3 ml of 1.5 $\mu$g/ml [$^3$H]$GA_4$ ($1.5 \times 10^7$ dpm/ml) for 5 days. After this time 40 seedlings from two replicate dishes were dissected into the appropriate parts; root and hypocotyl lengths were measured. The parts were extracted separately in cold 80% methanol, the extract was transferred to Whatman GF/A paper discs and eluted on a silica gel column (10). Consecutive fractions were pooled, taken to dryness, and

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[2] Present address: Department of Crop Science, University of Saskatchewan, Saskatoon, Saskatchewan, S7N OWO, Canada.

[3] To whom requests for reprints should be addressed.

[4] Present address: Botany Department, Rhodes University, Grahamstown, P.O. Box 94, South Africa.

Table I. *Gas-Liquid Radiochromatography Retention Times of Trimethylsilyl Ethers of Methyl Ester Derivatives of Silica Gel Partition Column Fractions of a Typical Extract, with Comparative Standards*

Comparison was made between the observed radioactive peak and the mass peak of an appropriate co-injected standard GA.

| | Retention Time on 3 Columns | | | Probable Identity |
|---|---|---|---|---|
| | 2% QF1 (203 C) | 2% SE30 (203 C) | 1% XE60 (209 C) | |
| | *min* | | | |
| Column fractions | | | | |
| 4–8 | 10.4 | 9.1 | 11.7 | $GA_4$ |
| 13–14 | 14.4 | 15.3 | 15.2 | $GA_1$ |
| 15–16 | 16.0 | 15.3 | 15.5 | A (artifact?) |
| 17–19 | 20.4 | 19.4 | 20.6 | $GA_2$ (artifact) |
| Standard GAs | | | | |
| $GA_1$ | 14.4 | 15.3 | 15.3 | |
| $GA_2$ | 20.4 | 19.6 | 20.5 | |
| $GA_3$ | 16.5 | 16.9 | 18.5 | |
| $GA_4$ | 10.4 | 9.1 | 11.7 | |
| $GA_8$ | 17.3 | 25.0 | 18.6 | |
| $GA_{34}$ | 12.6 | 15.2 | 13.4 | |

radioactivity was determined by standard liquid scintillation counting techniques.

Uptake of [$^3$H]$GA_1$ and [$^3$H]$GA_4$ was followed into the roots, hypocotyls, and cotyledons of growing seedlings by placing 70 seeds under continuous white fluorescent light on 2 ml of [$^3$H]$GA_1$ (3.27 $\mu$g, 4.58 $\times$ $10^6$ dpm) or [$^3$H]$GA_4$ (3 $\mu$g, 3.55 $\times$ $10^6$ dpm) for 3 days. Three replicates of 20 seeds were then taken and dissected into the three parts (hypocotyl and root lengths were measured) and each part was subjected to tritium oxidation using a Packard Tricarb Sample Oxidiser. Total dpm in each seedling part was determined and for comparative purposes the values for [$^3$H]$GA_1$ uptake were adjusted to account for the lower specific activity of [$^3$H]$GA_4$.

## RESULTS AND DISCUSSION

**Relative Activity of $GA_3$, $GA_4$, and $GA_7$ on Hypocotyl Elongation.** $GA_4$ and $GA_7$ are very effective in promoting lettuce seed germination (1, 4) relative to the commonly used $GA_3$. $GA_4$ is also more effective than $GA_3$ in promoting subsequent hypocotyl extension (18), although $GA_7$ was not tested. Using the same technique as Paleg and coworkers (18) we found that light-grown seedlings kept for 3 days on 0.1 $\mu$g/ml and 1 $\mu$g/ml $GA_3$ showed hypocotyl extension of 5.85 $\pm$ 0.28 mm and 8.04 $\pm$ 0.35 mm, respectively; on 0.1 $\mu$g/ml and 1 $\mu$g/ml $GA_4$, 6.94 $\pm$ 0.35 mm and 9.65 $\pm$ 0.12 mm respectively; and on 0.1 $\mu$g/ml and 1 $\mu$g/ml $GA_7$ an extension to 7.4 $\pm$ 0.19 mm and 11.0 $\pm$ 1.35 mm, respectively. Seedlings kept on water for the same period under the same conditions only exhibited hypocotyl elongation to 4.8 $\pm$ 0.16 mm. Thus, the potency in Grand Rapids appears to be $GA_7 > GA_4 > GA_3$.

**Promoter and Inhibitor Action.** The time courses of germination of seeds imbibed in 5-cm Petri dishes in darkness at 25 C in a total volume of 1.5 ml of 21 $\mu$M $GA_4$, or 21 $\mu$M $GA_4$ + 20 $\mu$M ABA + 60 $\mu$M BA, are shown in Figure 1. Seeds imbibed in 21 $\mu$M $GA_4$ + 20 $\mu$M ABA did not germinate and after 40 hr only 16% of those seeds imbibed on water in darkness had germinated. While BA overcame the inhibitory effect of ABA on $GA_4$-induced germination, there was a marked increase in the time required for radicle protrusion to occur. These observations are similar to those made for $GA_3$, ABA, and BA interactions (2, 13).

Seeds imbibed for 18 hr in darkness on 21 $\mu$M $GA_4$ and then transferred to a solution containing 21 $\mu$M $GA_4$ + 20 $\mu$M ABA for a further 18 hr in darkness exhibited no hypocotyl elongation, but seeds transferred to an equivalent $GA_4$ solution and kept under the same conditions produced hypocotyls 2 to 5 mm in length over the same time period.

**[$^3$H]$GA_4$ Metabolism in Light- and Dark-imbibed Seeds and Seedlings.** Five hundred milligrams of seeds were imbibed at 25 C in 3 ml [$^3$H]$GA_4$ (21 $\mu$M, 15 $\times$ $10^7$ dpm) in the outer rim of a covered Conway unit for 1 or 4 days in continuous white fluorescent light, or in darkness. Seeds in the light and dark showed 98% germination after 24 hr, with radicles protruding 1 to 2 mm. After 4 days in the dark hypocotyls were 9 to 12 mm long and the cotyledons were expanded and green, indicating that 21 $\mu$M $GA_4$ was insufficient to overcome completely the inhibitory effects of light on hypocotyl extension. Hypocotyl elongation of seedlings in Conway units was less than those planted in Petri dishes for the hypocotyl elongation studies.

[$^3$H]$GA_4$ was readily taken up by germinating seeds and seedlings (Table II). $GA_1$ is the major metabolite of $GA_4$; unknown compound A and $GA_2$ must be considered artifacts of the work-up procedure until further experiments determine unequivocally whether they are produced by the seed or seedling (Table II). There was little or no metabolism of [$^3$H]$GA_4$ up to 24 hr, the time at which germination is obvious and the radicles have emerged and begun to grow. In the seedling stage, while the hypocotyl was elongating, there was massive conversion to $GA_1$. There was slightly more conversion of $GA_4$ to $GA_1$ in dark-grown seedlings (44%) than in the light (37%), and the hypocotyls of the former were up to twice the length of the latter. The difference in rate of metabolism of $GA_4$ in light- and dark-grown seedlings noted here is much lower than the difference in the conversion of $GA_5$ to a $GA_1$-like compound between dark- and

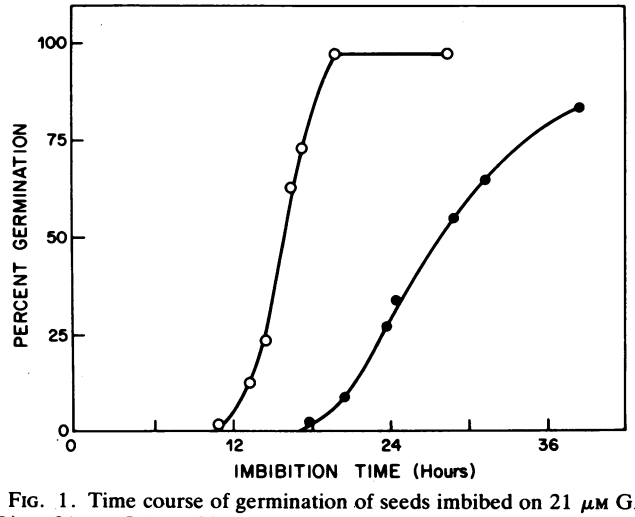


FIG. 1. Time course of germination of seeds imbibed on 21 $\mu$M $GA_4$ (○) or 21 $\mu$M $GA_4$ + 20$\mu$M ABA + 60 $\mu$M BA (●).

Table II. *Level of Ethyl Acetate-soluble Metabolites in Seeds and Seedlings*

The tissues imbibed [$^3$H]$GA_4$ in continuous light or darkness for 1 day (seed) or 4 days (seedlings).

| Metabolite | Radioactivity | | | |
|---|---|---|---|---|
| | Seed | | Seedling | |
| | Dark | Light | Dark | Light |
| | *dpm* $\times$ $10^{-6}$ | | | |
| $GA_4$ | 69.6 | 79.4 | 76.3 | 93.6 |
| $GA_1$ | 2.0 | 2.2 | 59.5 | 53.9 |
| A | 1.2 | 1.2 | 0.9 | 0.7 |
| $GA_2$ | 3.5 | 5.5 | 3.6 | 2.7 |
| $GA_8$? | 0 | 0 | 0.4 | 0.3 |

light-grown 5-day-old pea seedlings (15). Conversion of $GA_4$ to $GA_1$ during lettuce hypocotyl elongation involves C-13 hydroxylation. Perhaps this hydroxylation is an activation step for the hypocotyl elongation process. Similar C-13 hydroxylations of gibberellins $A_4$, $A_9$, and $A_{14}$ have been observed in experiments involving shoot elongation of dwarf rice and dwarf pea (11, and references cited therein).

A small peak, tentatively identified by gas-liquid radiochromatography as $GA_8$, was observed in the seedlings, but not consistently in the seeds. It has been demonstrated that $GA_8$ is a metabolite of $GA_1$ (16, 17, 19).

It appears that $GA_4$ itself promotes seed germination but that $GA_1$ could be responsible for the stimulation of hypocotyl elongation. In cv. Grand Rapids, exogenous $GA_4$ is a very potent germination promoter and $GA_1$ is relatively ineffective (1, 3). Surprisingly, $GA_4$ is slightly more promotive than $GA_1$ in the Grand Rapids lettuce hypocotyl extension test (18), although such differences could possibly be explained on the basis of differential uptake.

**Uptake of [$^3$H]$GA_1$ and [$^3$H]$GA_4$ by Seedlings.** Seeds were germinated and grown in continuous light for 3 days on [$^3$H]$GA_4$ or [$^3$H]$GA_1$ and then dissected into roots, hypocotyls, and cotyledons. Uptake of radioactive label into each seedling part after this 3-day period is shown in Table III. Exogenously applied $GA_4$ was slightly more promotive for hypocotyl elongation than was $GA_1$ (confirming the observation in [17]) but root growth was (un)affected equally by these GAs, thus eliminating any possibility that differential uptake was due to variabilities in root surface area. That $GA_4$ was taken up more effectively by the seedlings than was $GA_1$ is clearly shown: in fact, 250% more label from $GA_4$ was absorbed into the whole seedling and 310% more into the hypocotyls. While conversion of $GA_4$ to $GA_1$ appears to be an event associated with hypocotyl elongation, any potentially greater promotion by exogenously applied $GA_1$ is restricted by impaired uptake.

**Metabolism of [$^3$H]$GA_4$ by Roots, Hypocotyls, and Cotyledons of Light- and Dark-grown Seedlings.** From the histograms showing the distribution of radioactive gibberellins in the dissected parts of 5-day-old light- and dark-grown seedlings (Fig. 2) total uptake into the seedling and seedling parts was calculated (Table IV). Hypocotyls and roots were significantly longer in dark-grown seedlings. The greatest increase in labeled gibberellin was in the dark-grown roots (Table IV), where 62% of the label had been converted to $GA_1$ and only 18.2% remained as $GA_4$ (Fig. 2). In light-grown seedlings the same amount of gibberellin was present as $GA_1$ (62.5%) and only 11.2% was unconverted $GA_4$ (Fig. 2). The growing roots appear to be an important site for the conversion of $GA_4$ to $GA_1$, with light apparently inhibiting the uptake of $GA_4$ into this organ. The expanded radicles of 1-day-old seeds were incapable of the $GA_4$ to $GA_1$ conversion (Table II). It has been indicated that conversion from one gibberellin to another can occur within the roots of light-grown *Phaseolus coccineus:* one of the products of this conversion is $GA_1$ (7).

In dark-grown hypocotyls 60.7% of the [$^3$H]GA was in the form of $GA_1$ and 12% as $GA_4$, whereas in the shorter light-grown hypocotyls (Table IV) only 43.3% was present as $GA_1$ and 9.25% as $GA_4$ (Fig. 2). Total radioactivity in the hypocotyls from light- and dark-grown seedlings was the same (Table IV). At present we do not know whether the $GA_1$ in the hypocotyls was formed from $GA_4$ *in situ* or transported there as $GA_1$ from the growing roots. If $GA_4$ was transported from the roots for conversion in large amounts in the aerial parts, then that $GA_4$ would at some stage have to avoid the very effective $GA_4$ to $GA_1$ conversion mechanism present in the roots. Whatever the source of $GA_1$, it is obvious that there is a restriction imposed by white light on its synthesis in, or transport to, the hypocotyl. Furthermore, there is a good correlation between endogenous $GA_1$ levels and length of the hypocotyls.

Table III. *Uptake of [$^3$H]$GA_4$ and [$^3$H]$GA_4$ into Light-grown Lettuce Seedling Hypocotyls, Roots, and Cotyledons*

| | $GA_1$ | | $GA_4$ | |
|---|---|---|---|---|
| | Length ± SE | dpm/seedling part | Length ± SE | dpm/seedling part |
| | *mm* | | *mm* | |
| Hypocotyl | 6.53 ± 0.22 | 489 ± 44 | 8.1 ± 0.27 | 1,550 ± 37 |
| Root | 19.13 ± 0.62 | 4,609 ± 557 | 18.93 ± 0.68 | 10,309 ± 834 |
| Cotyledons | | 796 ± 48 | | 3,007 ± 380 |
| Total uptake | | 5,894 | | 14,866 |

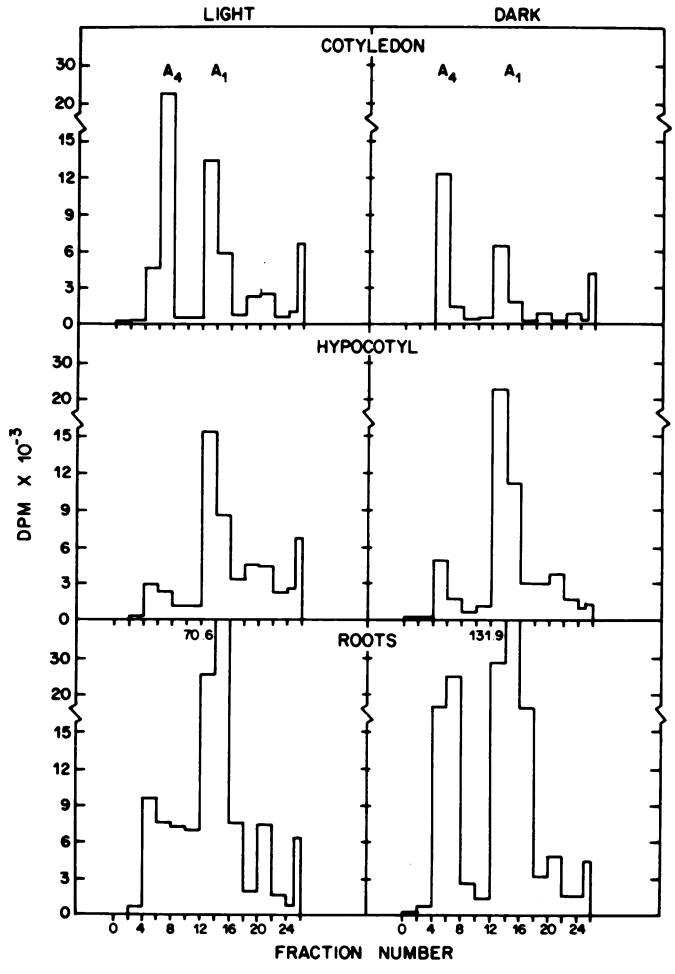


FIG. 2. Conversion of [$^3$H]$GA_4$ to other gibberellins by roots, hypocotyls and cotyledons of light and dark-grown seedlings. For details on uptake into, and growth of, seedling parts see Table IV. Column fractions 5 to 8: $GA_4$; 13 to 16: $GA_1$; 17 and 18: $GA_2$; 21 and 22: $GA_8$ (?).

Table IV. *Uptake of [$^3$H]$GA_4$ into Light- and Dark-grown Lettuce Seedling Hypocotyls, Roots, and Cotyledons*

See Fig. 2 for distribution of radioactivity in various gibberellins.

| | Light-grown | | Dark-grown | |
|---|---|---|---|---|
| | Length ± SE | dpm/20 seedling parts × $10^{-3}$ | Length ± SE | dpm/20 seedling parts × $10^{-3}$ |
| | *mm* | | *mm* | |
| Hypocotyl | 7.0 ± 0.36 | 55.3 | 11.20 ± 0.52 | 55.8 |
| Roots | 13.55 ± 0.62 | 153.0 | 17.73 ± 1.35 | 241.3 |
| Cotyledons | | 60.3 | | 29.8 |

In light-grown cotyledons 45% of the gibberellin present was maintained as $GA_4$ and 31.2% converted to $GA_1$. In dark-grown cotyledons, which contained less than 50% of the radioactivity found in the light-grown ones (Table IV), approximately the same level of $GA_4$ was noted (47%) and conversion to $GA_1$ (27.4%) was similar (Fig. 2). Since light-grown cotyledons appear more expanded than dark-grown ones, and green, they may act as a better sink for transported gibberellins. It is also possible that conversion of $GA_4$ to $GA_1$ could take place in the cotyledons themselves from $[^3H]GA_4$ absorbed directly from the imbibition solution, since these organs would take up gibberellin during germination and early growth, until lifted by the expanding hypocotyl. No significant conversion occurred in the cotyledons during the 1st day of imbibition, however (Table II).

It is clear from these results that the growing roots, and not the emerging radicles, are a major site of conversion from $GA_4$ to $GA_1$. It is also interesting that while the conversion of $GA_4$ to $GA_1$ in the whole dark-grown seedlings is only slightly higher than in the whole light-grown seedlings (7%, Table II; 1%, Fig. 2), it is in the hypocotyl, whose expansion is known to be affected by gibberellins, that the only increase (17%) in $GA_1$ levels is observed. We have yet to determine the time during seedling growth at which this conversion commences.

**Effects of ABA and BA on $[^3H]GA_4$ Metabolism.** Three hundred milligrams of seeds were imbibed under the same conditions as in the experiment presented in Figure 1, except that $[^3H]GA_4$ was used. Seeds were either imbibed in 21 $\mu$M $[^3H]GA_4$ ($15 \times 10^7$ dpm) for 10 hr (a time prior to visible germination), for 16 hr in $[^3H]GA_4$ + 20 $\mu$M ABA, or for 16 hr (a time prior to visible germination) in 60 $\mu$M $[^3H]GA_4$ + ABA + BA. To inhibit hypocotyl elongation, seeds were imbibed on 21 $\mu$M $[^3H]GA_4$ for 18 hr and then transferred to a fresh solution of 21 $\mu$M labeled $GA_4$ with ABA (a parallel treatment without ABA was also performed). Transfer was effected under a dim green safelight. Seedlings were harvested after a further 18 hr; hypocotyls had not emerged from those seeds imbibed upon ABA.

When the seedlings began to grow there was a large increase in $[^3H]GA_1$ production (Table IV [seedlings]), and this was markedly suppressed in the presence of ABA over the 36-hr incubation period (Table V [seedlings]). Thus $GA_4$ conversion to $GA_1$ is not merely an event associated with the length of time that seeds have been exposed to $GA_4$, but is indeed related to the growth of the seedling. The levels of $GA_1$ are probably reduced in all organs since hypocotyl extension, radicle elongation, and cotyledon expansion are all inhibited by ABA.

The low level of $[^3H]GA_1$ in the ABA-treated seeds is apparently not due to enhanced conversion of this compound to $GA_8$ and $GA_8$-glucoside (as occurs in barley aleurone layers [17]), since no detectable levels of $GA_8$ were found nor were BuOH soluble dpm increased (Table VI). Thus, ABA may inhibit $GA_4$-induced growth in Grand Rapids lettuce by blocking directly or indirectly, conversion of $GA_4$ to $GA_1$, thus preventing hypocotyl expansion. We cannot determine from experiments using this inhibitor whether $GA_1$ formation is essential for hypocotyl expansion since growth of the roots, a major site of $GA_1$ production, is also prevented.

On the basis of the results presented here we can only speculate that the conversion of $GA_4$ to $GA_1$ is necessary for enhanced hypocotyl elongation. Whether the 17% increase in $GA_1$ could account for the 60% increase (Table IV) in length of dark- over light-grown hypocotyls is not known, although it is interesting that it is only in the hypocotyl of dark-grown seedlings that $GA_1$ levels increase.

In this study we have monitored the conversion of $GA_4$ to $GA_1$ only after 5 days. We cannot ignore the possibility that there was a consistently higher synthesis of $GA_1$ throughout the several days of hypocotyl expansion, or that the conversion was initially higher when the rate of hypocotyl growth was higher. Studies of the kinetics of hypocotyl growth and $GA_1$ synthesis are planned. Also, we do not known whether $GA_1$ causes hypocotyl elongation, or if it is present as a consequence of increased growth. We tend to favor the first possibility, for if increased growth resulted in increased $GA_1$ production, then dark-grown seedlings should produce substantially more than the smaller light-grown seedlings. The relationship between hypocotyl extension and $GA_1$ production is complex because it is not easy to apply the converse argument, namely that increased growth is a consequence of increased $GA_1$ production: other factors may well be involved.

Table V. *Levels of Ethyl Acetate-soluble Metabolites in Seeds treated with $GA_4$, ABA, and BA*

The seeds were imbibed in darkness with $[^3H]GA_4$ (10 hr seeds and 36 hr seedlings), $[^3H]GA_4$ + ABA (16 hr seeds and 36 hr seedlings) and $[^3H]GA_4$ + ABA + BA (16 hr seeds only).

| Treatment | Counts | | | |
|---|---|---|---|---|
| | $GA_4$ | $GA_1$ | A (artifact) | $A_2$ |
| | *dpm* $\times 10^{-6}$ | | | |
| $[^3H]GA_4$ (seeds) | 52.6 | 0 | 0.94 | 2.3 |
| $[^3H]GA_4$ + ABA (seeds) | 48.7 | 0.13 | 0.56 | 0.83 |
| $[^3H]GA_4$ + ABA + BA (seeds) | 55.6 | 0.78 | 1.1 | 2.4 |
| $[^3H]GA_4$ (seedlings) | 76.2 | 35.1 | 0.20 | 0.37 |
| $[^3H]GA_4$ + ABA (seedlings) | 78.1 | 4.1 | 0.14 | 4.8 |

Table VI. *Distribution of Radioactivity in Total Methanolic Extract and Four Fractions from this Extract from Seeds Imbibed in Darkness*

The seeds were imbibed in darkness with $[^3H]GA_4$ (10 hr seeds and 36 hr seedlings), $[^3H]GA_4$ + ABA (16 hr seeds and 36 hr seedlings) and $[^3H]GA_4$ + ABA + GA (16 hr seeds only).

| Treatment | Counts | | | | |
|---|---|---|---|---|---|
| | Methyl alcohol | Ether | Ethyl acetate | Butyl alcohol | Buffer |
| | *dpm* $\times 10^{-6}$ | | | | |
| $[^3H]GA_4$ (seeds) | 53.4 | 0.48 | 52.7 | 0.57 | 0.24 |
| $[^3H]GA_4$ + ABA (seeds) | 49.8 | 0.27 | 49.2 | 0.66 | 0.16 |
| $[^3H]GA_4$ + ABA + BA (seeds) | 61.2 | 0.18 | 59.8 | 0.67 | 0.15 |
| $[^3H]GA_4$ (seedlings) | 121.5 | 0.27 | 116.0 | 4.11 | 0.70 |
| $[^3H]GA_4$ + ABA (seedlings) | 85.3 | 0.28 | 86.6 | 1.50 | 0.36 |

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