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# Metabolism of Tritiated Gibberellins in *d-5* Dwarf Maize

## I. IN EXCISED TISSUES AND INTACT DWARF AND NORMAL PLANTS[1]

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### ABSTRACT

**Metabolism of [$^3$H]gibberellin $A_1$ ([$^3$H]$GA_1$) was followed in intact seedlings and excised apices and leaf tissue of both dwarf and normal (tall) plants of *d-5* maize (*Zea mays* L.). The three metabolites produced were tentatively identified as [$^3$H]$GA_8$, [$^3$H]$GA_8$-glucoside ([$^3$H]$GA_8$-glu), and [$^3$H]$GA_1$-X, an unknown.**

**In 3-hour, pulse-labeling experiments with tissues of incubated, expanding leaves, more than 70% of the [$^3$H]$GA_1$ taken up was metabolized to the three products within 12 to 15 hours. [$^3$H]$GA_1$ fed to the roots of 7-day-old seedlings was readily translocated to the leaves, and all three metabolites were found in both roots and leaves. [$^3$H]$GA_1$-X was the major metabolite in roots, whereas in leaves the major metabolite was [$^3$H]$GA_8$-glu. There were no consistent differences in [$^3$H]$GA_1$ metabolism between dwarf and normal plants, indicating that dwarfism in *d-5* maize is not associated with modified $GA_1$ metabolism.**

**In excised, mature leaf tissue, [$^3$H]$GA_1$ metabolism was slower than in excised, young leaf tissue. Mature leaf tissues produced [$^3$H]$GA_8$-glu as by far the major metabolite, with [$^3$H]$GA_8$ and [$^3$H]$GA_1$-X as minor metabolites. In contrast, in young leaves the three metabolites appeared sequentially in significant proportions: [$^3$H]$GA_8$ first, followed by [$^3$H]$GA_8$-glu and, finally, [$^3$H]$GA_1$-X.**


Single gene dwarf mutants are potentially the simplest systems available for studying the genetic and physiological control of gibberellin levels in vascular plants. There are more than 20 such mutants of *Zea mays*, five of which are of special interest because they are readily responsive to applied GA and have low levels of endogenous GA (13). Because a single gene may control the biogenesis of a substance, Phinney (13) postulated that each dwarfing gene in *Zea mays* controls a different step in the biosynthetic pathway leading to a GA(s) essential for normal growth. Similar genetic control of GA level has been proposed by Moh and Alan (7) for single gene dwarf mutants of bean (*Phaseolus vulgaris*) and by Murakami (8) for rice (*Oryza sativa*).

In view of the increasing interest in the role of GA metabolism in controlling GA levels in plants (2, 6, 9, 12, 14, 17), we have considered the possibility that GA metabolism, as distinct from GA biosynthesis, might be involved in the phenomenon of dwarfism in maize. Plants have the ability to convert biologically active gibberellins to more polar derivatives. The metabolites of [$^3$H]$GA_1$ in barley aleurone layers have been tentatively identified as [$^3$H]$GA_8$, [$^3$H]$GA_8$-glucoside ([$^3$H]$GA_8$-glu), and [$GA_1$]-glu (9, 12). The first two of these metabolites have very low biological activity (3). Gibberellin metabolism could act to regulate the supply of GA and may explain the origin of GA-glucosides in plants (16, 18). GA metabolism has been correlated with plant growth phenomena including seed maturation and germination (2, 15), dwarfism (6), and flowering (14, 17) with varying success. For instance, there was an increase in the metabolism of [$^3$H]$GA_5$ in *Silene armeria* under inductive daylengths (17), however in *Solanum andigena* the metabolism of [$^3$H]$GA_1$ was not influenced by daylength (14).

In this study, we investigated the relationship between metabolism of [$^3$H]$GA_1$ and the growth habit of *d-5* maize, a single gene, GA-responsive mutant.

### MATERIALS AND METHODS

**Radioactive $GA_1$.** We used tritiated $GA_1$ because this hormone is widespread in plants and there is tentative evidence for the presence of $GA_1$, or the chemically and biologically similar $GA_3$, in maize (5, 12). [1,2-$^3$H]Gibberellin $A_1$ ([$^3$H]$GA_1$), prepared and purified as described previously (9, 10), was used in all experiments. The two specific activities used were 1 Ci/mmole ($2.3 \times 10^6$ cpm/$\mu$g) and 43 Ci/mmole ($1 \times 10^8$ cpm/$\mu$g).

**Plant Material.** Seeds of *d-5* dwarf maize (*Zea mays* L.) were immersed in aerated, deionized $H_2O$ overnight, then placed in glass-covered plastic trays between layers of paper towels moistened with deionized $H_2O$. After 48 hr at 23 C, those seedlings with strong root and coleoptile growth were selected for use.

In experiments involving root application of [$^3$H]$GA_1$, seedlings were transferred singly to vials containing 3 ml of quarter-strength Hoagland's solution. Each vial was covered with aluminum foil to exclude light, and air was bubbled through the medium in each vial. The seedlings were grown for 4 days under a bank of mixed fluorescent and incandescent lamps (1500 ft-c) at 16-hr day length and 26 C, before treatment.

Leaf tissues were obtained from plants grown in a greenhouse for 4 to 5 weeks in a mixture of equal parts of soil, peat, and sand.

**Treatment of Intact Seedlings with [$^3$H]$GA_1$.** For root application, 2.5 ml of quarter-strength Hoagland's solution containing [$^3$H]$GA_1$ was added to vials containing 6-day-old seedlings growing under nonsterile conditions. Plants were grown for 24 hr under a light bank and then separated into (*a*) roots and mesocotyl, (*b*) first leaf and coleoptile, and (*c*) second and

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younger leaves and shoot apex. Roots were washed twice in deionized water to remove surface radioactive substances, and all plant parts were weighed and analyzed for [$^3$H]$GA_1$ and its metabolites.

For leaf application, 12 normal and 12 dwarf seedlings were grown in the greenhouse for 10 days. Then a 30-$\mu$l drop of a solution was carefully inserted into the cone formed by the leaf sheaths of the two older leaves and the lamina of the emerging youngest leaf. Plants were treated at midday and harvested 24 hr later. At harvest, the tops were severed from the roots, and the third leaf was carefully withdrawn from the enclosing leaf sheaths. The lower 2 cm of the third leaf of normal plants, or 1 cm of dwarf plants, was then cut off. These leaf sections were quickly washed in two changes of deionized $H_2O$ to remove surface radioactivity, and the sections immediately were placed in 80% ethanol.

**Incubation of Excised Leaf Tissues in [$^3$H]$GA_1$.** Apical regions of 4- or 5-week-old plants, or leaf discs from the sixth or seventh mature leaf, were incubated in solutions of [$^3$H]$GA_1$. A typical plant had one or two stem internodes undergoing extension and 12 or 13 identifiable leaves. The apical region consisted of the shoot apex and the two youngest leaves coiled around it. In normal plants this region was 20 to 40 cm in length, and in dwarf plants 10 to 20 mm.

The discs were punched from surface-sterilized leaf laminae, using a 9-mm diameter sterilized cork borer. The laminae, previously moistened with 0.1% Tween solution, were sterilized by immersion in 0.5% sodium hypochlorite for 4 min. Shoot apical regions were surface sterilized by immersion in 0.2% sodium hypochlorite for 2 min.

Leaf tissues were floated on 1 ml of incubation medium in 25-ml Erlenmeyer flasks and incubated at 26 C for various lengths of time on a reciprocating shaker. The 150 ft-c of light came from mixed incandescent and fluorescent sources. Ten leaf discs (approx 80 mg fresh weight of normal leaf tissue; 95 mg fresh weight of dwarf leaf tissue) or 8 to 10 shoot apical regions (approx 400 to 500 mg fresh weight) were used per flask. The incubation medium consisted of 0.05 M potassium phosphate buffer (pH 6.2) containing 2% sucrose in addition to [$^3$H]$GA_1$. All glassware and media were autoclaved before addition of plant material under aseptic conditions.

At the end of an incubation period, the medium was decanted, and the tissues were washed three times, 1 min each time, in potassium phosphate buffer to remove surface radioactivity. In pulse-labeling experiments, leaf tissues were initially floated for 2 or 3 hr on a medium containing [$^3$H]$GA_1$. They were then washed in three changes of buffer containing cold $GA_1$ at the same concentration as the [$^3$H]$GA_1$ supplied initially (Figs. 2 and 3). The leaf tissues were then incubated in the same cold $GA_1$ buffer medium for various lengths of time before extractions of [$^3$H]$GA_1$ and its conversion products.

**Extraction of Radioactive Compounds.** The washed plant tissue was boiled 1 min in 5 ml of 80% ethanol. It was then homogenized in a glass tissue grinder with an additional 5 ml of 80% (v/v) ethanol. The resulting material was centrifuged, and the supernatant liquid was decanted. The pelleted residue was twice extracted by shaking with 80% ethanol and recentrifuging. The resultant ethanolic extracts were assayed for radioactivity by liquid scintillation counting and were reduced in volume to near dryness under reduced pressure for direct application to ChromAR TLC sheets.

**Thin Layer Chromatography.** The metabolism of [$^3$H]$GA_1$ was followed by TLC, using 5 × 20 cm or 20 × 20 cm strips or sheets of ChromAR (Mallinckrodt Chemical Works). The solvent systems were: A, isopropanol-3 N $NH_4OH$ (5:1, v/v); B, benzene-acetic acid (4:1, v/v); and C, ethyl acetate-chloroform (3:1, v/v). Solvent system A was run for 12 or 15 cm and solvent C for 15 cm. The ethanolic extracts were streaked on ChromAR strips which were developed first in solvent A to a distance of 12 cm, then developed for 70 min in solvent B, with the solvent being permitted to run to the top of the strip and evaporate. Radioactive compounds on the TLC strips were located with a radiochromatogram scanner. The radioactive compounds were eluted with 80% ethanol. Portions of the eluate were assayed for radioactivity by scintillation counting, using Beckman's cocktail D as the scintillation fluid.

For identification, the radioactive compounds were further purified in solvents A or B. Authentic GA standards and the radioactive compounds were co-chromatographed on ChromAR and visualized under UV light after being sprayed with 5% $H_2SO_4$ in ethanol and heated at 100 C for 10 min. The radioactive products of [$^3$H]$GA_1$ metabolism were eluted, then methylated with diazomethane in ether (9) and further purified by TLC. Free gibberellin methyl esters were run in solvent C and gibberellin $A_1$ and $A_8$-glu methyl esters in solvent A, along with authentic GA methyl ester standards. Free GA methyl esters were eluted with methanol-ethyl acetate (1:1) and GA-glu methyl esters with absolute methanol. The compounds were dried and then derivatized to produce methyl ester-trimethylsilyl ethers, using Sil Prep (hexamethyldisilazane-trimethylchlorosilane-pyridine, 3:1:9).

**Gas-Liquid Chromatography.** Metabolic products of [$^3$H]$GA_1$ were tentatively identified by comparing them with authentic standards on a gas chromatograph equipped with a flame ionization detector and an effluent splitter. Columns were of stainless steel (3.5 mm × 180 cm) packed with 3% SE-30 or 2% QF-1 on Gas-Chrom Q (Applied Science Laboratories, State College, Pa.). Column temperatures and carrier gas flow rates were as described by Nadeau and Rappaport (9). Radioactive peaks were located by collecting column effluent samples at 0.5-min intervals and checking for radioactivity by liquid scintillation counting. Retention time of methyl ester-trimethylsilyl ester derivatives and metabolites of [$^3$H]$GA_1$ were compared with those of mass peaks from co-injected authentic standards (9).

## RESULTS

**Fate of [$^3$H]$GA_1$ in Intact Dwarf and Normal Plants.** The distribution of [$^3$H]$GA_1$ and its conversion products is shown in Table I. The roots of normal plants took up and translocated more radioactivity to the leaves than did roots of dwarf plants, probably because normal leaves were considerably larger than those on dwarf plants. There was little difference in root weight between the two types of plants at this age.

When the crude ethanolic extracts from both dwarf and normal plants were chromatographed, four zones containing radioactive compounds were detected on the TLC strips. The $R_F$ values of these compounds were: I, 0.03 (0.0–0.06); II, 0.36 (0.32–0.40); III, 0.62 (0.57–0.67); and IV, 0.81 (0.75–0.86). The radioactive compounds were eluted and tentatively identified on TLC by comparison with authentic GA standards. On radiochromatograms developed in solvent B for 90 min, compounds III and IV migrated to $R_F$ values 0.30 and 0.69, corresponding, respectively, to those of authentic $GA_8$ and $GA_1$. Compound II, chromatographed in solvent A, had an $R_F$ of 0.42, corresponding to that of a $GA_8$-glu standard.

Compounds II, III, and IV were derivatized as their methyl esters and run on TLC. In solvent C, the methyl esters of III and IV had $R_F$ values of 0.65 and 0.82, which corresponded, respectively, to the $R_F$ values of authentic methyl esters of $GA_8$ and $GA_1$ ($GA_1$ and $GA_8$ are immobile in solvent C). The methyl ester of II had an $R_F$ of 0.72 in solvent A, corresponding to that of authentic $GA_8$-glu-methyl ester. On the basis of TLC,

Table I. *Distribution of Radioactivity in Organs of d-5 Maize Seedlings 24 Hr after Root Application of [$^3$H]$GA_1$*

Six-day-old seedlings growing in vials were supplied with [$^3$H]-$GA_1$ (0.16 $\mu$g/ml, 2.3 $\times$ $10^6$ cpm/$\mu$g) in 2.5 ml of quarter-strength Hoagland's solution. After 24 hr, the seedlings were divided into three parts which were weighed, and the radioactive substances were extracted with 80% ethanol and assayed. Data are averages of two replicates of four plants each.

| Plant Part | Dwarf Plants | | | Normal Plants | | |
|---|---|---|---|---|---|---|
| | Amount of total recovered radioactivity | | Fresh wt | Amount of total recovered radioactivity | | Fresh wt |
| | *cpm* | % | *mg* | *cpm* | % | *mg* |
| First leaf and coleoptile | 12,890 | 11.6 | 135 (±17)[1] | 29,980 | 16.3 | 205 (±20) |
| Second and younger leaves | 11,420 | 9.9 | 95 (±12)[1] | 34,040 | 18.5 | 145 (±14) |
| Roots | 87,420 | 78.5 | 450 (±19)[1] | 120,250 | 65.3 | 455 (±22)[1] |
| All | 111,360[2] | | 680[2] (±25)[1] | 184,270[2] | | 805[2] (±30)[1] |
| Uptake as % of cpm supplied to roots | 12.0 | | | 19.9 | | |

[1] Numbers in parentheses are SE.
[2] Total cpm or fresh weight.

Table II. *Distribution of Radioactivity in Extracts of d-5 Maize Seedlings after Treatment with [$^3$H]$GA_1$ for 24 Hr*

Ethanolic extracts from the plants treated with [$^3$H]$GA_1$ (Table I) were subjected to TLC on strips of ChromAR 500 (silica gel-glass fiber sheet). Strips were developed first in isopropanol-3 N $NH_4OH$ (5:1, v/v) and then in benzene-acetic acid (4:1, v/v). Radioactive compounds on the strips were first located by radioscanning. They were then eluted for scintillation counting and identified by GLC.

| Plant Part | Compound | Dwarf Plants | | Normal Plants | |
|---|---|---|---|---|---|
| | | Amount of total recovered radioactivity | | Amount of total recovered radioactivity | |
| | | *cpm* | % | *cpm* | % |
| First leaf and coleoptile | [$^3$H]$GA_1$ | 2,790 | 30.2 | 7,040 | 28.9 |
| | [$^3$H]$GA_8$ | 510 | 4.9 | 2,160 | 9.5 |
| | [$^3$H]$GA_8$-glucoside | 4,960 | 53.6 | 13,670 | 55.1 |
| | [$^3$H]$GA_1$-X | 1,240 | 11.9 | 1,840 | 6.6 |
| Second and younger leaves | [$^3$H]$GA_1$ | 2,920 | 29.8 | 7,470 | 29.3 |
| | [$^3$H]$GA_8$ | 810 | 8.1 | 2,480 | 10.2 |
| | [$^3$H]$GA_8$-glucoside | 4,420 | 46.2 | 11,930 | 48.2 |
| | [$^3$H]$GA_1$-X | 1,790 | 15.8 | 2,692 | 12.0 |
| Roots | [$^3$H]$GA_1$ | 45,640 | 77.0 | 64,810 | 83.4 |
| | [$^3$H]$GA_8$ | 3,690 | 6.2 | 2,950 | 3.8 |
| | [$^3$H]$GA_8$-glucoside | 4,460 | 7.5 | 4,770 | 6.2 |
| | [$^3$H]$GA_1$-X | 5,600 | 9.4 | 5,100 | 6.5 |

compounds II and III were tentatively identified as the metabolites [$^3$H]$GA_8$-glu and [$^3$H]$GA_8$, respectively, and IV as unreacted [$^3$H]$GA_1$. The identity of I is unknown, and this metabolite, reminiscent of one reported by Nadeau *et al.* (11), is hereafter called [$^3$H]$GA_1$-X. It does not move in solvents A or B, but it moves to $R_F$ 0.41 in the organic phase of 1-butanol-acetic acid-water (4:1:4, v/v/v). Gas-liquid chromatography substantiated the results with TLC. Retention times for methyl ester-trimethylsilyl ethers of the other metabolites were very similar to those obtained by Nadeau and Rappaport (9) for metabolites of [$^3$H]$GA_1$ in bean seeds.

All parts of the seedlings supplied with [$^3$H]$GA_1$ contained the same metabolites (Table II). In leaves, the metabolite present in largest amount was [$^3$H]$GA_8$-glu. Roots, however, contained relatively more [$^3$H]$GA_1$-X than did leaves and larger amounts of unmodified [$^3$H]$GA_1$. Although there was little difference between the amounts of metabolites extracted from the first and second leaves, there was an obvious difference between roots and shoots in the proportions of the metabolites present. When dwarf and normal plants were compared, there was little apparent difference between the metabolic patterns in any plant part (Table II).

[$^3$H]Gibberellin $A_1$ was also applied to the lamina of the emerging third leaf of 10-day-old seedlings, and 24 hr later sections were cut from the rapidly growing base of these leaves for extraction. Unmodified [$^3$H]$GA_1$ accounted for about half the radioactivity extracted. Of the three metabolites [$^3$H]$GA_1$-X was present in the largest amount. There was little difference in the pattern of [$^3$H]$GA_1$ metabolism between young dwarf and normal leaf tissue.

**Metabolism of [$^3$H]$GA_1$ in Excised Leaf Tissues of Different Physiological Ages.** Leaf discs were cut from mature leaves of 4- or 5-week-old dwarf and normal plants and incubated in duplicate 25-ml Erlenmeyer flasks containing [$^3$H]$GA_1$ in 1 ml of incubation medium. Shoot apical regions were also excised from the same plants and incubated. [$^3$H]$GA_1$ at 210,400 and 685,000 cpm/ml were supplied, respectively, to leaf discs and shoot apical regions. In leaf discs, [$^3$H]$GA_8$-glu was the major metabolic product and dwarf and normal tissues produced it in equal amounts (Table III). [$^3$H]$GA_8$ and [$^3$H]$GA_1$-X were very minor components, collectively accounting for less than 5% of the total radioactivity recovered. [$^3$H]$GA_8$-glu and [$^3$H]$GA_1$-X were the main metabolites in the apical sections, but [$^3$H]$GA_8$ accounted for at least 9% of the total radioactivity recovered. Dwarf shoot apical regions produced [$^3$H]$GA_8$-glu and [$^3$H]$GA_1$-X in almost equal amounts, whereas normal shoot apical regions produced considerably more [$^3$H]$GA_1$-X than [$^3$H]$GA_8$-glu.

Shoot apical regions had a considerably higher metabolic rate, with some 75% of the [$^3$H]$GA_1$ taken up being converted to metabolites in 22 hr; the comparable value for leaf discs was 55%. Differences in total metabolism between dwarf and normal tissues appeared to be minor, regardless of age of tissue.

**Pulse Labeling of Tissues with [$^3$H]$GA_1$.** In a time-course experiment, apical tissue was incubated in a medium containing

Table III. *Distribution of Radioactivity among [$^3$H]$GA_1$ and Its Metabolites in Excised Leaf Tissues of d-5 Maize after 22 Hr of Incubation in [$^3$H]$GA_1$*

Leaf discs or shoot apical regions contained in duplicate 25-ml Erlenmeyer flasks were incubated in 1 ml of buffer (0.05 M potassium phosphate, pH 6.2, 2% sucrose) with [$^3$H]$GA_1$ for 22 hr under dim light at 26 C. Leaf discs (10 discs/flask; fresh weight 80 mg for normal leaf discs, 95 mg for dwarf leaf discs) were punched from mature leaves of 5-week-old plants. Shoot apical regions (350 mg/flask) were from the same plants. [$^3$H]$GA_1$ (2.3 $\times$ $10^6$ cpm/$\mu$g) concentrations were 0.093 $\mu$g/ml for discs, 0.30 $\mu$g/ml for shoot apical regions.

| Radioactive Compound | Leaf Discs | | Shoot Apical Region | |
|---|---|---|---|---|
| | Dwarf | Normal | Dwarf | Normal |
| | *cpm/10 leaf discs* | | *cpm/10 shoot apical regions* | |
| [$^3$H]$GA_1$ | 11,605 | 11,940 | 76,340 | 65,600 |
| [$^3$H]$GA_8$ | 580 | 495 | 22,730 | 35,590 |
| [$^3$H]$GA_8$-glucoside | 12,390 | 11,420 | 82,150 | 45,880 |
| [$^3$H]$GA_1$-X | 520 | 620 | 78,600 | 105,160 |
| Total cpm recovered | 25,095 | 24,576 | 259,820 | 252,230 |

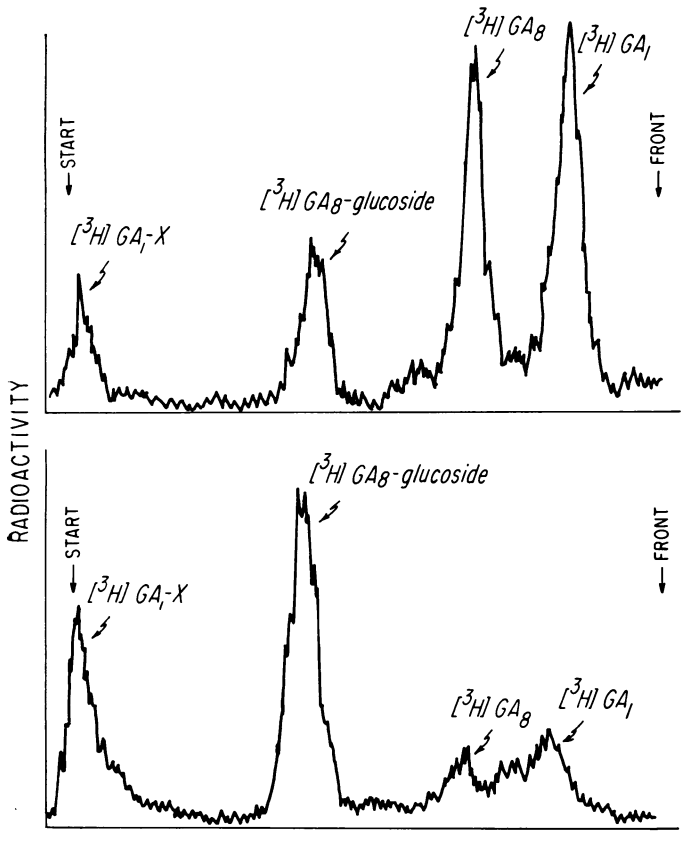


FIG. 1. Radiochromatogram tracings of metabolites obtained by incubation of normal maize shoot apical regions pulse-labeled with [$^3$H]GA$_1$. TLC on ChromAR strips developed (1) with isopropanol-3 N $NH_4OH$ (5:1, v/v) to 12 cm, then (2) with benzene-acetic acid (4:1, v/v) for 70 min. See legend to Fig. 2 for details of incubation procedure. Upper: extract from tissues incubated 6 hr; lower: extract from tissues incubated 13 hr.

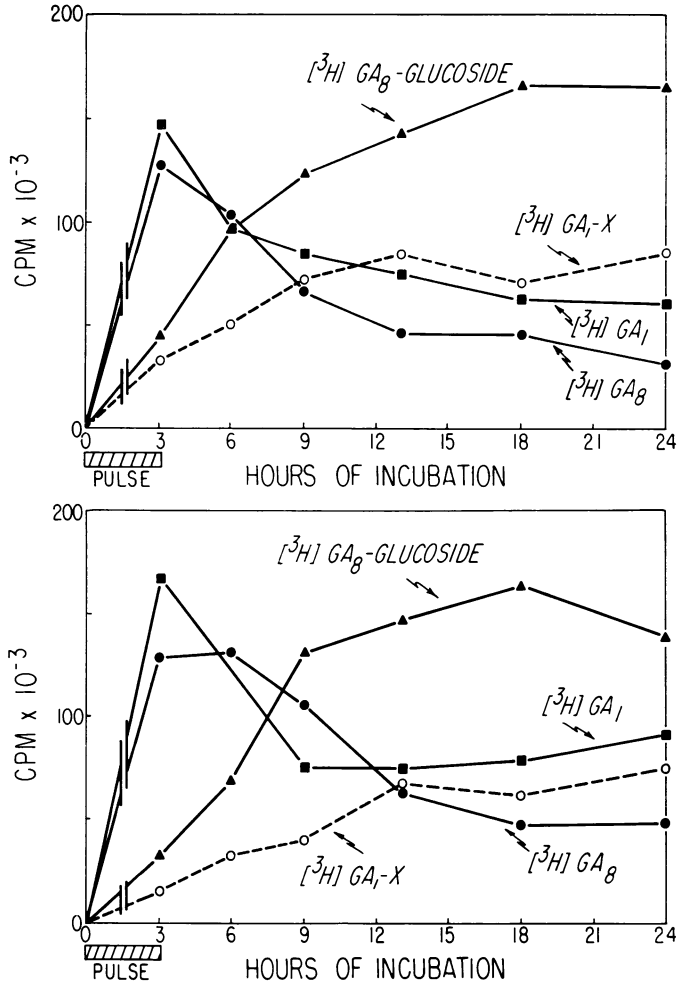


FIG. 2. Time course of [$^3$H]GA$_1$ metabolism in incubated shoot apical regions from dwarf and normal *d-5* maize plants. A 3-hr uptake period in [$^3$H]GA$_1$ (0.05 $\mu$g/ml, 43 Ci/mmole) was followed by incubation in GA$_1$ at the same concentration. Plant materials, incubation conditions, extraction, and chromatography were as described in Table III. Upper: normal shoot apical regions; lower: dwarf shoot apical regions.

[$^3$H]GA$_1$ for 2 or 3 hr, and then was transferred to nonradioactive GA$_1$ medium of the same concentration. Samples of tissue were analyzed periodically during the incubation period to determine the extent of metabolism of the pulse of [$^3$H]GA$_1$. This brief uptake experiment was designed to reveal the sequence of appearance of metabolites and to minimize the effects of any possible long term uptake difference between dwarf and normal tissues.

Shoot apical regions were incubated in [$^3$H]GA$_1$ (43 Ci/mmole; $5.25 \times 10^6$ cpm/ml; 0.05 $\mu$g/ml) for 3 hr, after which they were transferred to an incubation medium containing GA$_1$ (0.05 $\mu$g/ml) for periods up to 24 hr. At each sampling, normal leaf tissue and dwarf leaf tissue were extracted for chromatography. Extracts of the shoot apical regions yielded very clean separation of metabolites on TLC (Fig. 1).

In both dwarf and normal leaf tissues, more than 30% of the radioactivity recovered at the end of the 3-hr uptake period was already in the form of the metabolite [$^3$H]GA$_8$ (Fig. 2). The amount of radioactive [$^3$H]GA$_8$ peaked between 3 and 6 hr, then declined to a minimum after 12 hr. Only small amounts of [$^3$H]GA$_8$-gly were detected at the end of the 3-hr uptake period, but the compound accounted for about 40% of recovered radioactivity after 12 to 15 hr. [$^3$H]GA$_1$-X appeared later and accumulated more slowly than any other metabolite, leveling off at 20% of the total radioactivity after 12 hr. It is apparent that the [$^3$H]GA$_1$ taken up in the first 3 hr was rapidly metabolized in the following 9 hr, after which time less than 20% remained unmetabolized. Metabolic reactions appeared to cease after a total of 12 to 15 hr. Metabolism in dwarf and normal shoot apices was virtually identical (Fig. 2).

Leaf discs from mature leaves were also used in a time course experiment. This time low specific activity [$^3$H]GA$_1$ (1 Ci/mmole) was used. Also, the uptake period was shortened to 2 hr, and the discs were incubated for periods up to 40 hr. [$^3$H]GA$_1$ was supplied at 2.5 $\mu$g/ml ($5.6 \times 10^6$ cpm/ml.). As no differences between dwarf and normal leaf discs in pattern of [$^3$H]GA$_1$ metabolism were observed, the data from the two types of tissue were combined and the means plotted in Figure 3. Metabolism of [$^3$H]GA$_1$ in leaf discs was considerably slower than that of high specific activity [$^3$H]GA$_1$ in shoot apical regions (Fig. 2). After 30 hr, metabolism in leaf discs was still continuing (Fig. 3). [$^3$H]GA$_8$ was the first metabolic product detected in the leaf discs (Fig. 3) but, unlike the situation in shoot apical regions, it did not accumulate initially. Instead, it was rapidly converted to [$^3$H]GA$_8$-glu, which appeared to be the final product. [$^3$H]GA$_1$-X was a very minor metabolite in the leaf discs from mature leaves. At 40 hr, when less than 30% of the [$^3$H]GA$_1$ taken up remained unmetabolized, [$^3$H]GA$_1$-X accounted for no more than 7% of the total radioactivity (Fig. 3).

Generally, the qualitative and quantitative differences in

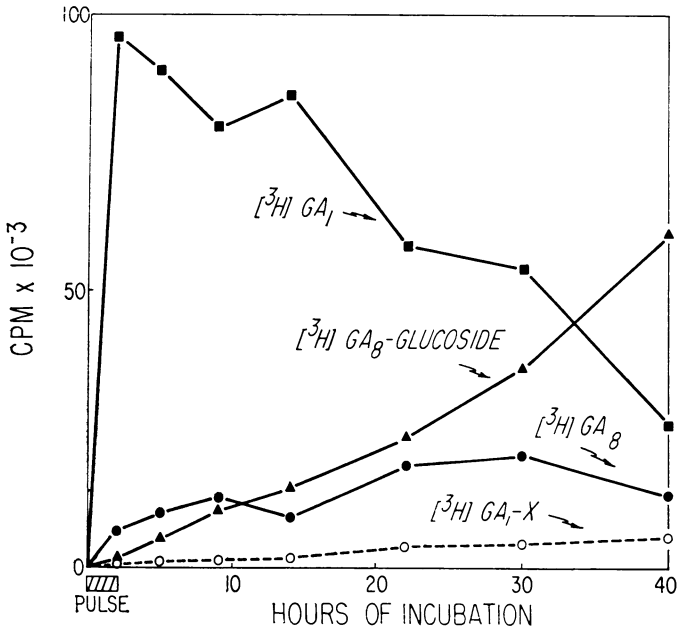


FIG. 3. Time course of [$^3$H]$GA_1$ metabolism in incubated leaf discs from *d-5* maize plants. A 2-hr uptake period was provided in [$^3$H]$GA_1$ (2.5 $\mu$g/ml) of low specific activity (1 Ci/mmole, 2.28 $\times$ $10^6$ cpm/$\mu$g). Experimental details as described in Fig. 2. Data from dwarf and normal leaf discs were combined and averaged.

[$^3$H]$GA_1$ metabolism in young and mature leaves (Fig. 2 *versus* Fig. 3), were similar to the differences observed in the incubation experiment outlined in Table II.

## DISCUSSION

The principal question in this study was: Is dwarfism in *d-5* maize related to differences between dwarf and normal plants in metabolism of a gibberellin? To investigate this possibility, it was convenient to use a single gene maize mutant, *d-5*, in which dwarfism appears to be regulated by some gibberellin-related phenomenon (13).

Tissues of *d-5* maize are capable of converting [$^3$H]$GA_1$ to three metabolites: [$^3$H]$GA_8$, [$^3$H]$GA_8$-glu, and an unknown compound, [$^3$H]$GA_1$-X (Fig. 1). To further identify these metabolites, they have been subjected to acid hydrolysis (4), and both [$^3$H]$GA_8$ and [$^3$H]$GA_8$-glu, as expected (9), yielded acid-rearranged derivatives having the same retention time on GLC as authentic acid-rearranged $GA_8$. The [$^3$H]$GA_1$-X yielded an acid-rearranged derivative of [$^3$H]$GA_1$ on prolonged acid hydrolysis, indicating that the conjugate was formed directly from $GA_1$. Both [$^3$H]$GA_8$ and [$^3$H]$GA_8$-glu were products of [$^3$H]$GA_1$ metabolism in germinating bean seeds (9) and barley aleurone layers (12). The unknown metabolite [$^3$H]$GA_1$-X was also detected as a [$^3$H]$GA_1$ metabolite in barley aleurone layers (12) and has been characterized as an amphoteric conjugate of [$^3$H]$GA_1$ (11). On no occasion, however, was [$^3$H]$GA_1$-glu produced in maize, although this glucoside is a product of [$^3$H]$GA_1$ metabolism in barley aleurones (12).

One of the problems involved in treating intact whole seedlings of the dwarf and normal types is that they differ considerably in shoot size and morphology. The same amount of [$^3$H]$GA_1$ fed to morphologically different shoot systems may be taken up, translocated, and converted at different rates, not necessarily because of an inherent genetic character, but because of the differences in growth behavior of the two types. The problem is clearly seen in Table I in which normal maize seedlings, root-fed with [$^3$H]$GA_1$, took up 70% more radioactivity than did dwarf plants. Furthermore, the roots of normal plants exported nearly three times as much radioactivity to the leaves as did those of dwarf plants. This result is probably linked to the much larger size and area of leaves on normal plants, which afforded greater uptake of [$^3$H]$GA_1$ via the transpiration stream (Table I). To minimize these problems in tissue sampling, similar fresh weights of excised dwarf and normal leaf tissues were used in some experiments.

In intact seedlings root-fed [$^3$H]$GA_1$, the roots contained large amounts of unmetabolized [$^3$H]$GA_1$ and smaller proportion of metabolites compared with the leaves (Table II). Much of the [$^3$H]$GA_1$ in roots had probably been drawn into the cell walls and vascular system by the transpiration stream, and was thus unavailable for metabolism. The roots contained large amounts of [$^3$H]$GA_1$-X relative to [$^3$H]$GA_8$ and [$^3$H]$GA_8$-glu, whereas in leaves [$^3$H]$GA_1$-X was a relatively minor metabolite. This raises the possibility that [$^3$H]$GA_8$ and [$^3$H]$GA_8$-glu may be translocated to the shoots, whereas [$^3$H]$GA_1$-X is not, and that the unknown compound accumulated at the site of its production in the roots.

A factor bearing on the amount of [$^3$H]$GA_1$ metabolism was leaf age. The metabolism of [$^3$H]$GA_1$ was slower in discs from mature leaves as compared to that in young leaves of shoot apical regions (Table III; Figs. 2 and 3). [$^3$H]$GA_8$-glu was by far the main metabolite in young and mature leaves (Fig. 3), although in young leaves the other two metabolites were present in significant amounts. Evidently, the hydroxylation of [$^3$H]$GA_1$ and [$^3$H]$GA_8$ in young leaves occurs faster than the glucosylation of [$^3$H]$GA_8$ to [$^3$H]$GA_8$-glu, indicating that the demands of growth may limit the supply of glucose for glucosylation in young leaves.

An interesting feature of the experiments reported here was the very rapid rate of metabolism obtained when [$^3$H]$GA_1$ of high specific activity was used in experiments with young leaves (Fig. 2). Other workers (1, 2, 6, 14) have reported low rates of conversion of [$^3$H]$GA_1$, probably because hormone of relatively low specific activity was used and because, in some instances, it was applied to mature, expanded leaves. Endogenous GAs are usually present in leaf tissues at extremely low concentration about $10^{-3}$ to $10^{-1}$ $\mu$g/g fresh weight) and the capacities of enzyme systems to metabolize GAs are likely to be correspondingly small. The specific activity of hormone used therefore is of considerable importance in GA metabolism studies and application of low specific activity [$^3$H]$GA_1$ results in low levels of GA turnover. As part of the experiments reported here, we tried to establish the presence of $GA_1/GA_3$ in extracts of young leaves by gas chromatography. Although insufficient $GA_1/GA_3$ was extracted to make identification positive, the evidence indicated occurrence of a $GA_1/GA_3$-like compound(s) at a concentration of about $10^{-2}$ $\mu$g/g fresh weight. In the pulse-labeling experiment (Fig. 2), about $10^{-2}$ $\mu$g/g fresh weight was taken up in 3 hr and more than 70% of it was metabolized within the next 12 hr, indicating that any $GA_1$-like substances present in young maize leaves undergo rapid turnover in the space of 12 hr. In view of the occurrence of an endogenous pool of GAs in maize, it is not unlikely that metabolism of $GA_1$ proceeds at an even faster rate than is indicated by the data. Finally, it is well established that young leaves are much richer in GAs than older ones; therefore the much greater metabolism of $GA_1$ detected in young, as compared to old leaves is undoubtedly real. Despite this apparent difference, our results fail to discriminate in any significant way between the metabolism of [$^3$H]$GA_1$ in dwarf and normal tissues, and we conclude that [$^3$H]$GA_1$ metabolism is not directly involved in dwarfism in *d-5* maize.

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