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Radioactive Gibberellin A_5 and Its Metabolism in Dwarf Peas

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ABSTRACT

Radioactive gibberellin A_5 (^{3}H-GA_5) was synthesized from gibberellic acid. When it was applied to dwarf peas grown in the dark, an average of 3% was converted to another acid gibberellin within 48 hours. The biological activity of the metabolite did not account for the response to applied GA_5. GA_5 is therefore assumed to be biologically active *per se*. ^{3}H-GA_5 did not appear to form a stable complex with a macromolecule in pea shoots. When injected into dwarf pea pods, ^{3}H-GA_5 was readily metabolized by maturing seed to more water-soluble substances and to two other acidic compounds. This metabolism continued even throughout germination of the seed without reconversion of the metabolites to GA_5. It is concluded that "bound" GA_5 plays no part in the germination of dwarf pea seeds.

Dwarf peas contain two major fractions with gibberellin-like activity. The chromatographic and biological properties of one resemble those of gibberellin A_1 while the chromatographic and biological properties of the other are similar to those of gibberellin A_5 (8, 11) and its dihydro derivative, gibberellin A_{20} (13, 16), which has been isolated by Komoda *et al.* (12) from the pods of a tall variety of peas. Radioactive GA_1[1] has been synthesized and administered to dwarf peas in an attempt to determine the cellular component with which it reacts to effect a physiological response (6, 10). It was presumed that the hormone must react with a macromolecule, probably a protein, since only proteins would have the specificity to recognize physiologically active gibberellins as opposed to inactive ones that are sterochemically similar. No stable macromolecule-GA_1 (6, 10) complex was found, but Ginzburg and Kende (unpublished data) demonstrated by means of equilibrium dialysis that a protein fraction from peas reversibly binds this gibberellin. The binding appears to be of noncovalent nature and the protein or proteins are present in such low concentration that purification has not yet been possible. We have prepared radioactive GA_5 in the hope that it may be bound more strongly or by more receptors. Jones and Lang (8) found that both endogenous gibberellins are readily extracted from the tips of dwarf pea shoots, but that only the GA_1-like factor diffuses from the tips. One explanation for this finding is that the GA_5-like factor is bound more effectively than the GA_1-like factor in the apical tissue.

In this initial paper we report the metabolism of ^{3}H-GA_5 in pea shoots and in maturing pea seed. Metabolism of GA_5 in dwarf pea shoots was studied in order to determine whether it is active *per se* or through conversion to the GA_1-like compound. Clearly it was of importance to confirm direct biological activity before looking for "receptor" molecules. The metabolism of GA_5 in maturing seed was studied in order to complement similar work by Barendse *et al.* using ^{3}H-GA_1 (2). The major point of interest was whether ^{3}H-GA_5 became bound in the maturing seed and then reconverted to free GA_5 at the onset of germination, as is the suggested fate of ^{3}H-GA_1 in the same tissue (2).

MATERIALS AND METHODS

Synthesis of ^{3}H-GA_5. Gibberellic acid in methanol was methylated by diazomethane. The crystallized product (I, Fig. 1, 140 mg) in a solution of 5 ml of ethyl acetate and 0.1 ml of pyridine plus 140 mg of 2% palladized barium carbonate was reduced under an atmosphere of tritium and hydrogen[2] to yield gibberellin A_1-3,4-^{3}H methyl ester (II, Fig. 1) and the monomethyl ester of a dicarboxylic acid (III, Fig. 1) (7). The two compounds were dissolved in ethyl acetate and partitioned against 5% $NaHCO_3$ while methyl ^{3}H-GA_1 remained in the ethyl acetate phase from which it was crystallized with unlabeled methyl GA_1 (specific activity 430 mc/mmole). ^{3}H-GA_1-methyl ester was then converted to ^{3}H-GA_5 by a modification of the procedure described by MacMillan and Pryce (14). Methyl ^{3}H-GA_1 (4 mg) was tosylated by toluene-*p*-sulphonyl chloride (30 mg) in 0.5 ml of pyridine over 4 days at 25° (IV, Fig. 1). Thereafter the mixture was poured into 10 ml of ethyl acetate, and the solution was washed with 2 N HCl, water, 5% $NaHCO_3$, and water. The ethyl acetate was evaporated, and the residue was taken up in 3 ml of collidine and left overnight in the dark at 25°. Insoluble *N*-tosyl collidine chloride was filtered off, and the filtrate was refluxed for 7 hr to yield ^{3}H-GA_5-methyl ester (V, Fig. 1). After evaporating the collidine, the residue was taken up in ethyl acetate and washed with $NaHCO_3$, HCl, and water. The ethyl acetate was then evaporated, and the residue was dissolved in 2 ml of tetrahydrofuran to which were added 5 ml of 3 N NaOH. The mixture was stirred vigorously and heated under reflux at 70° for 7 hr. The aqueous phase was removed, and another 5 ml of 3 N NaOH were added to the tetrahydrofuran and again refluxed with stirring. Five aqueous extractions obtained in this way contained most of the radioactivity from the organic phase. They were pooled, acidified, and extracted with ethyl acetate. Radioactive GA_5 in this extract was purified by thin layer chromatography with washed kieselguhr (17) and the solvent system benzene-acetic acid-water (8:3:5, v/v) (15). Tritiated GA_5 was eluted from the appropriate chromatographic zone and cocrystallized with unlabeled GA_5 to a constant specific activity of 113 mc/mmole. The purity and identity of ^{3}H-GA_5 was checked by gas-liquid chromatography of its methyl ester ac-

[1] Abbreviations: GA_1, GA_5, and GA_{20}: gibberellins A_1, A_5, and A_{20}, respectively.

[2] The tritiation was performed by New England Nuclear Corporation.

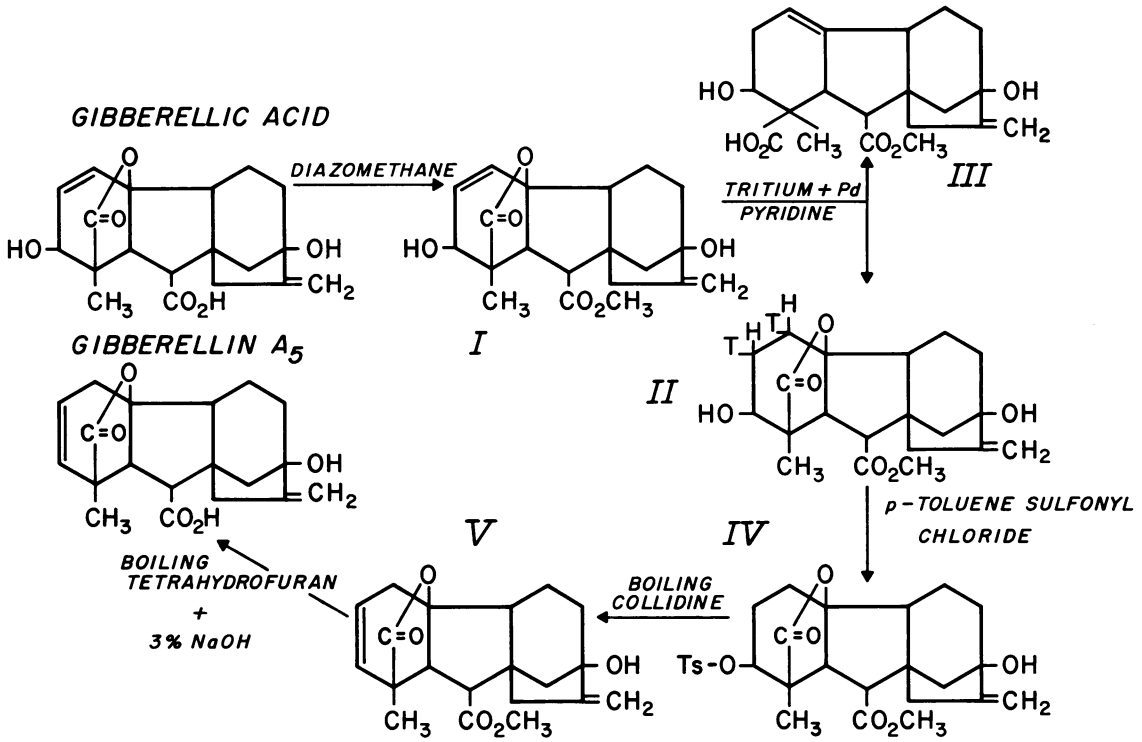

FIG. 1. Synthesis of ^{3}H-GA_5 from gibberellic acid.

cording to the method of Cavell *et al.* (3) with a column of 2% QF-1. A single sharp peak was obtained which had the retention time of and cochromatographed with a GA_5-methyl ester standard. When the effluent was monitored for radioactivity, a single peak was recorded which again correlated with the retention time of unlabeled methyl GA_5. The sample of ^{3}H-GA_5 was stored at −20° in acetone, where it appears to be stable. After 9 months ^{3}H-GA_5 and its methyl ester chromatograph on thin layers as single peaks of radioactivity associated with the respective standard. During tritiation of GA_3 to GA_1 two tritium atoms are incorporated at the 3- and 4-positions (II, Fig. 1). When GA_1 is converted to GA_5, some loss of radioactivity is probable, particularly at position 3. To test this, 40 mg of ^{3}H-GA_1 methyl ester were repeatedly crystallized (specific activities 216, 225, and 228 μc/mmole) and converted to GA_5 which after three separate crystallizations gave specific activities of 150, 151, and 168 μc/mmole. Since the specific activity of the ^{3}H-GA_5 is approximately three-quarters that of the starting material, it is possible that the tritium atoms are added randomly at positions 3 and 4 during the reduction and that 1 tritium at position 3 is lost upon removal of the tosyl group.

Growth and Treatment of Pea Seedlings. Dwarf peas (*Pisum sativum* L. cv. Progress No. 9) were grown in the light or dark for 5 days at 25° and then treated with 5-μl droplets of a solution of ^{3}H-GA_5 or of unlabeled GA_1 or GA_5 as described before (10). In some experiments the peas were treated with Amo 1618 (150 mg/liter in Hoagland's solution) to reduce the natural gibberellin level in the seedlings (1, 4). The height of peas treated with unlabeled gibberellins was measured 3 days after gibberellin application. Peas treated with ^{3}H-GA_5 (0.0066 to 0.165 μg per plant ≡ 2,000–50,000 cpm) were harvested 1 or 2 days after treatment. The shoots were rinsed in 50% methanol, and the apical halves were excised and frozen in liquid nitrogen. Between 15 and 33% of the applied radioactivity was removed in the methanol washes.

Extraction Procedure. Twenty-four or 48 frozen shoots were extracted by methanol, and the extracts were processed as described before (10). Three fractions were assayed for radioactivity: the acid-ethyl acetate phase, which contained free gibberellins; the aqueous phase, generally assumed to contain gibberellins covalently bound to more polar compounds; and the tissue residue left after methanol extraction. Radioactivity was measured in a liquid scintillation spectrometer as described earlier (10). Quench corrections were made when necessary by an external radioactive standard.

Treatment and Growth of Excised Pea Pods. The procedure described by Barendse *et al.* (2) was followed except that pods were injected with ^{3}H-GA_5 10 or 14 days after anthesis (0.066 μg per pod ≡ 20,000 cpm). Seeds from pods injected 10 days after anthesis were harvested and extracted 2, 4, 9, 14, and 21 days after injection. Seeds harvested 21 days after injection were removed in their pods from the agar at 14 days and left to dry at 25° for 7 days. Seeds from pods cultured and injected 14 days after anthesis were harvested 14 days after injection. Mature seeds were germinated in the dark at 24° on bacteriological agar (1%). Only 10% of the seeds from pods injected 10 days after anthesis germinated. Those that did germinate were harvested 5 days after radicle emergence. Ninety-five per cent of the seeds from pods cultured 14 days after anthesis germinated and were harvested 2 or 5 days after radicle emergence. Shoots and roots of germinated seed were separated from cotyledons, and all were extracted separately.

Chromatography. The metabolism of ^{3}H-GA_5 was followed by thin layer chromatography of the acid-ethyl acetate phase with Silica Gel G and the following solvent systems: solvent A, chloroform-ethyl acetate-acetic acid (60:40:5, v/v) (18); solvent B, benzene-*n*-butanol-acetic acid (70:25:5, v/v) (9); solvent C, diisopropyl ether-acetone-acetic acid (90:30:1, v/v) (19). Standards of GA_1 and GA_5 were chromatographed with the extracts. Their positions were determined under ultraviolet light after spraying the chromatograms with 5% (v/v) H_2SO_4 in ethanol and heating at 105° for 10 min (5). The distribution of

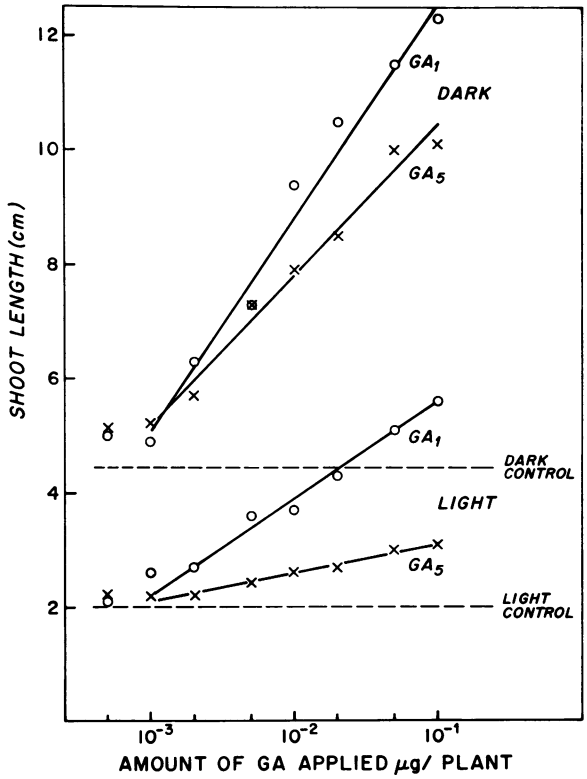

FIG. 2. Growth response of dark- and light-grown dwarf pea shoots, treated with Amo-1618 (150 mg/liter), 3 days after applying different quantities of GA_1 and GA_5. Each point represents the mean length of 12 shoots. The gibberellin was applied in a 5-μl drop of 0.05% Tween-20 solution. Amo-1618 was used to increase the response of dark-grown peas to gibberellin treatment. It was applied to light-grown peas for control purposes only.

Table I. *Percentage Distribution of Radioactivity in Extracts of Light- and Dark-grown Dwarf Pea Shoots 24 and 48 hr after Applying Different Amounts of ^{3}H-GA$_5$*

Forty-eight shoots treated with 0.006 μg of ^{3}H-GA_5 per plant were extracted per treatment but only 24 were extracted for any other treatment. The peas were grown in half-strength Hoagland solution with or without Amo 1618 (150 mg/liter).

Amount of ^{3}H-GA$_5$ Applied	Conditions	Time after GA$_5$ Application	Acid-Ethyl Acetate	Aqueous	Residue	Total Activity Extracted
μg/plant		hr	%	%	%	cpm
0.033	Dark +	24	91.8	7.3	0.9	136,107
	Amo	48	90.2	8.4	1.4	177,427
0.165	Dark	24	93.3	4.9	1.8	575,942
		48	92.1	5.9	1.0	740,922
	Light	24	94.3	4.9	0.8	826,560
		48	89.8	8.5	1.7	752,847
0.006	Dark	24	88.0	10.7	1.3	68,981
		48	84.3	13.5	2.2	66,406
	Light	24	89.3	9.5	1.2	58,236
		48	90.5	8.4	1.1	58,560

radioactivity over the chromatogram was determined by scraping zones of the silica gel into vials containing Bray's scintillation fluid and measuring the radioactivity in a scintillation counter. The term "chromatographed twice" means that after developing a chromatogram in the normal manner, it was air dried and rechromatographed in the same solvent in the same direction.

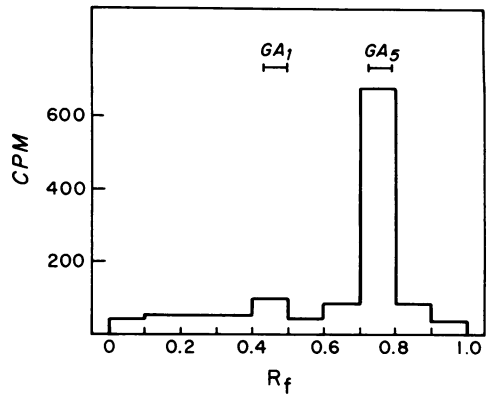

FIG. 3. The distribution of radioactivity in the acid-ethyl acetate phase of an extract of dark-grown pea shoots made 2 days after treatment with 0.033 μg of ^{3}H-GA_5 per plant. The extract was chromatographed on Silica Gel G in benzene-*n*-butanol-acetic acid (70:20:2, v/v).

Fractionation of Subcellular Particles. Two hundred 6-day-old dark-grown pea shoots treated the day before with ^{3}H-GA_5 (0.033 μg per plant), were chopped into small pieces in the cold. This tissue was then ground with a cylindrical mortar and pestle (6) in 40 ml of ice-cold tris-HCl buffer (0.05 M, pH 7.5) containing 0.4 M sucrose, 0.001 M $MgCl_2$, 0.01 M KCl, and 0.04% mercaptoethanol. The homogenate was filtered through four layers of cheesecloth and one layer of Miracloth (6). The filtrate was fractionated by differential centrifugation, and the pellets were resuspended in tris buffer, centrifuged again, and assayed for radioactivity. The high speed supernatant (150,000*g*) was sampled for radioactivity and then saturated with ammonium sulfate. The resultant precipitate was centrifuged, and the pellet was resuspended in 35 ml of tris buffer and again precipitated by saturating the solution with ammonium sulfate. The second precipitate was pelleted and dissolved in 5 ml of fresh buffer.

RESULTS

Response of Light- and Dark-grown Pea Shoots to GA_5 and GA_1. In earlier studies it was shown that dwarf pea shoots were more responsive to GA_1 than to GA_5 when the shoots were grown in the light (11). Therefore, applied GA_5 may be active only through conversion to a GA_1-like factor, the conversion being inhibited in the light. Since it was important to determine whether or not GA_5 was active *per se*, the response of both light- and dark-grown pea shoots to low concentrations of GA_1 and GA_5 was studied in more detail. The percentage of GA_5 that would have to be converted to GA_1 in order to account for the biological activity of GA_5 can be calculated from the growth response curves which are shown in Figure 2. When 0.006 μg and 0.033 μg of ^{3}H-GA_5 were applied to dark-grown pea plants, a minimum of 50 and 30% of the GA_5, respectively, would have had to be converted to GA_1 or a similar factor to account for the growth response observed. Figure 2 also shows that light reduced the response to both gibberellins, but more to GA_5 than to GA_1; 0.1 μg of GA_5 was 4 times less effective than GA_1 in the dark and 30 times less effective in the light.

Fate of ^{3}H-GA_5 in Pea Seedlings. Five-day-old dwarf pea seedlings were treated with different amounts of ^{3}H-GA_5 and harvested 24 or 48 hr later. The distributions of radioactivity between the acid-ethyl acetate, aqueous, and residue phases are shown in Table I. The distribution was independent of treatment except perhaps for a negative correlation between radio-

Table II. *Percentage of Radioactivity in Acid-Ethyl Acetate Phase Associated with ^{3}H-GA_1-like Compound*

Light- and dark-grown dwarf pea shoots were extracted 24 or 48 hr after application of different amounts of ^{3}H-GA_5. The amount of radioactivity associated with the GA_1-like compound was monitored on thin layers of Silica Gel G with solvent system A or a modification of B: benzene-*n*-butanol-acetic acid (70:20:2, v/v). GA_5 and GA_1 were separated by more than two R_F units in both systems. The data from applying 0.033 μg per plant are the means of two separate experiments.

Amount of ^{3}H-GA_5 Applied	Light Treatment	Radioactivity in GA_1 Region	
		24 hr	48 hr
μg/plant		%	%
0.006	Light	0.52	1.12
	Dark	1.64	2.72
0.033	Light	0.52	0.50
	Dark	3.60	4.50
	Dark + Amo	1.65	3.17
0.165	Light	0.32	0.94
	Dark	1.56	1.25

Table III. *Biological Activity in Extracts of GA_5-treated and Untreated Pea Shoots*

Gibberellin A_5 (1 μg per plant) was applied to 48 Amo-treated (250 mg/liter) dark-grown pea shoots. They were extracted 2 days later as were 48 pea shoots grown on Amo-1618 in the absence of GA_5. The acid-ethyl acetate phases were chromatographed twice in system A, and the biological activity in each R_F zone was assayed on etiolated Amo-treated (150 mg/liter) peas. The biological response in the GA_1 and GA_5 regions was expressed as micrograms of GA_5 equivalents.

Treatment	GA_1 region	GA_5 region
	μg GA_5 equivalents	
Control	0.88	0.26
GA_5-treated	2.00	11.80
GA_5-treated minus control	1.12	11.54

activity in the aqueous phase and the amount of ^{3}H-GA_5 applied. Approximately 90% of the radioactivity was in the acid-ethyl acetate phase, and nearly all of it cochromatographed with a GA_5 standard in systems A and B (Fig. 3). Only one other peak of radioactivity was detected, and in systems A and B it was associated with a GA_1 standard (Fig. 3). The percentages of counts associated with this conversion product, under the different treatments, are noted in Table II. Clearly more ^{3}H-GA_5 was converted in the dark than in the light, but the conversion never exceeded 8% in the dark or 1.5% in the light. Applying less ^{3}H-GA_5 did not affect the percentage of conversion; a reduction in the natural level of gibberellins by Amo-1618 also had no effect.

Biological Activity of ^{3}H-GA_5 Metabolite. The chromatographic properties of the ^{3}H-GA_5 metabolite from pea shoots resembled those of GA_1. The percentage conversion of GA_5 to the metabolite was low in all treatments, but if the metabolite was biologically very active it could possibly account for the activity of GA_5. To test this possibility, 96 etiolated plants were first grown on Amo-1618 (250 mg/liter). After this, half of the plants were treated with ^{3}H-GA_5 (1 μg per plant). Two days after GA treatment, the plants of both groups were extracted, and the acid-ethyl acetate phases were chromatographed twice in system A. The 10 R_F zones were eluted with water-saturated ethyl

Table IV. *Distribution of Radioactivity in Subcellular Fractions of ^{3}H-GA_5-treated Pea Shoots*

Two hundred pea shoots treated with ^{3}H-GA_5 (0.033 μg per plant) were extracted 24 hr after treatment in tris-HCl buffer (0.05 M, pH 7.5) containing 0.4 M sucrose, 0.001 M $MgCl_2$, 0.01 M KCl, and 0.04% mercaptoethanol. The high speed supernatant (150,000*g*) was saturated with ammonium sulfate, and the resultant precipitate was centrifuged down, resuspended in buffer, again precipitated by saturating the solution with ammonium sulfate, and finally resuspended in 5 ml of buffer.

Centrifugal Force	Time	Major Component	Activity
g	*min*		*cpm/fraction*
100	10	Nuclei	300
1,000	10	Chloroplasts	400
10,000	10	Mitochondria, broken chloroplasts	1,600
40,000	10	Membranes, endoplasmic reticulum, Golgi bodies	1,000
150,000	120	Ribosomes	170
		Remaining soluble fraction	615,000
		Ammonium sulfate precipitate	100,750

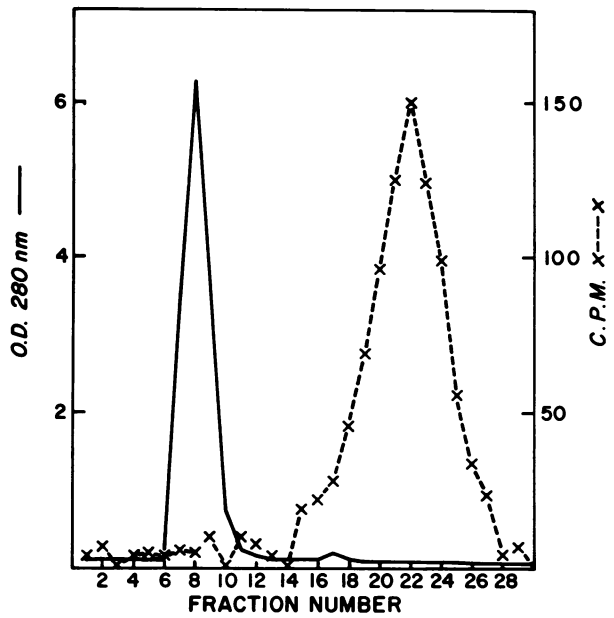

FIG. 4. Chromatography of the material precipitated by ammonium sulfate from the high speed supernatant of 200 dark-grown, ^{3}H-GA_5-treated shoots (24 hr after applying 0.033 μg per plant) on a column of Sephadex G-10 (38 cm long, 12 mm diameter). The column was eluted with tris-HCl buffer (0.05 M, pH 7.5), and 2.5-ml fractions were collected.

acetate, the eluant was evaporated, and the residue was taken up in 0.1 ml of Tween-20 solution (0.02%). The solutions were bioassayed on Amo-treated, dark-grown peas by applying 5-μl drops to eight plants per R_F fraction. The responses in the GA_1 and GA_5 regions of the chromatograms were expressed as micrograms of GA_5 equivalents (Table III). The biological response to extracts of peas not treated with GA_5 represents the level of endogenous gibberellins after Amo-1618 treatment. When the activity of extracts of GA_5-treated peas was corrected for the natural gibberellin content (GA_5-treated minus control),

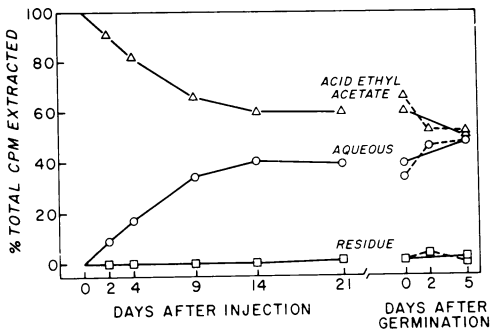

FIG. 5. Distribution of radioactivity between the acid-ethyl acetate, aqueous, and residue phases of extracts of maturing and germinating seeds. The extra data for germinated seeds (dotted line) resulted from pods injected with ^{3}H-GA$_5$ (0.066 μg in 10 μliter of 0.05% Tween-20 solution) 14 days after anthesis and cultured for 14 days before seed germination. Other data resulted from peas in pods injected 10 days after anthesis. Shoots of 2-day-old seedlings were approximately 0.5 cm long and those of 5-day-old seedlings approximately 6 cm long.

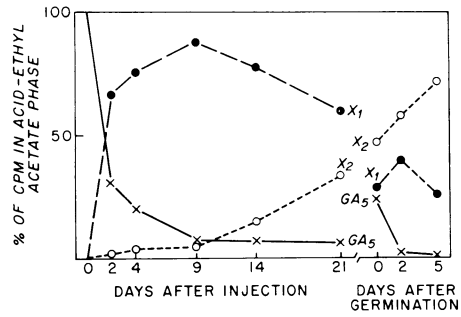

FIG. 6. Distribution of radioactivity between ^{3}H-GA$_5$ and its two acidic metabolites in the acid-ethyl acetate phases of extracts of developing pea seeds and seedlings. Data from maturing seeds were obtained from seeds injected with ^{3}H-GA$_5$ (0.066 μg in 10 μliters of 0.05% Tween-20 solution) 10 days after anthesis while those for germinating seeds were obtained from seeds injected 14 days after anthesis.

Table V. *Distribution of Radioactivity in Extracts of Germinating Seed*

Pods were cultured and injected with 10 μl of ^{3}H-GA$_5$ (0.06 μg per pod) 14 days after anthesis. Peas harvested 14 days later were germinated in the dark at 24° on sterile bacteriological agar (1%). Seeds, cotyledons, shoots, or roots were extracted; and the distribution of radioactivity between the acid-ethyl acetate, aqueous, and tissue phases as well as within the acid-ethyl acetate fraction was determined.

Time after Radicle Emergence	Total Radioactivity Extracted	Percentage of Total Extracted Radioactivity					
		Acidic	GA$_5$	X$_1$	X$_2$	Aqueous	Tissue
days	*cpm*						
0 (seed)	26,077	66.2	15.8	18.5	31.7	32.6	1.2
2 (cotyledons)	23,988	54.4	1.0	22.0	31.4	42.3	3.3
2 (shoots)	1,949	13.3	1.5	6.2	5.3	85.9	0.7
2 (roots)	907	43.0	3.2	22.5	17.3	57.0	0.0
5 (cotyledons)	20,255	53.3	0.6	14.0	38.6	46.7	0.0
5 (shoots)	1,477	25.1	2.9	16.3	5.8	72.1	2.8
5 (roots)	1,272	57.4	2.5	26.0	28.4	41.8	0.8

the response to the fraction in the GA$_5$ region was 10-fold greater than the activity of the fraction cochromatographing with GA$_1$. Therefore, the GA$_1$-like metabolite accounts only for a small portion of the response to applied GA$_5$.

Attempted Extraction of ^{3}H-GA$_5$ Macromolecule Complexes. Methanol extracts of pea shoots treated with ^{3}H-GA$_5$ contained all but 1 to 2% of the radioactivity (Table I). Since proteins are methanol-insoluble, it is probable that little of the ^{3}H-GA$_5$ was left in association with protein after methanol extraction. However, since methanol denatures protein, ^{3}H-GA$_5$-treated pea shoots were homogenized in buffer, and the homogenate was fractionated by differential centrifugation and Sephadex chromatography. Less than 1% of the total extracted radioactivity was associated with subcellular particles (Table IV). In contrast, the material precipitated from the high speed supernatant by ammonium sulfate (100% saturation) accounted for approximately 16% of the extracted radioactivity. To test whether this radioactivity was firmly bound to large molecules, another batch of ^{3}H-GA$_5$-treated peas was extracted, the extract was centrifuged at 150,000g, and the remaining solution was saturated with ammonium sulfate. The precipitate that formed was resuspended in fresh tris buffer, and a sample of it was chromatographed on a Sephadex G-10 column. The elution profiles of optical density and radioactivity are shown in Figure 4. The radioactivity was retarded on the column whereas any ^{3}H-GA$_5$ in a stable complex with a protein would have been excluded from the Sephadex matrix and eluted in fractions 7 to 10. Therefore, the radioactivity precipitated by ammonium sulfate must have been loosely bound to large molecules.

Fate of ^{3}H-GA$_5$ in Maturing and Germinating Seeds. Figure 5 shows the distribution of radioactivity extracted from maturing and germinating pea seeds. During seed maturation increasing amounts of radioactivity became associated with the aqueous phase, and this trend continued throughout germination. In addition, ^{3}H-GA$_5$ was metabolized to two other acidic compounds. For convenience, one is designated X$_1$ and the other X$_2$. The three compounds (X$_1$, X$_2$, and GA$_5$) were best separated from one another on thin layers of silica gel by developing the chromatograms twice in solvent A. Compound X$_2$ remained near the origin (between R_F 0.0 and 0.2), X$_1$ was eluted between R_F 0.3 and 0.5, and GA$_5$ was eluted between R_F 0.7 and 0.8. In systems A and B, X$_1$ had an R_F value similar to that of GA$_1$, but in solvent system C it remained near the origin while GA$_1$ moved to R_F 0.2 to 0.3. The distribution of radioactivity between these compounds with time is represented in Figure 6. In young seeds ^{3}H-GA$_5$ was rapidly metabolized to X$_1$, which in turn appeared to be converted to X$_2$. During germination the same pattern of conversion from GA$_5$ to X$_1$ and X$_2$ was observed. The distribution of radioactivity between the root, shoot, and cotyledons of germinating seeds and within each of these organs is shown in Table V. It is apparent that the level of ^{3}H-GA$_5$ in the root and shoot was exceedingly low compared with the radioactivity in the seedlings as a whole, and that there was no selective accumulation of ^{3}H-GA$_5$ in either roots or shoots.

DISCUSSION

When ^{3}H-GA$_5$ was applied to pea shoots, it underwent conversion to one other gibberellin-like compound. If the metabolite was GA$_1$, or a gibberellin of similar biological activity, at least 50 and 30% conversion of GA$_5$ would have been required in order to account for the response of etiolated dwarf peas to 0.006 μg and 0.033 μg of GA$_5$ per plant, respectively. In practice, conversions between 1 and 8% were observed, which are insufficient to account for the growth response to GA$_5$ unless the metabolite is much more active than GA$_1$. When ^{3}H-GA$_5$-treated peas were extracted and the acid-ethyl acetate fraction

was chromatographed, the biological activity in the GA_5 region was considerably greater than in the region of the metabolite. If the metabolite had been the biologically active compound, the response to the GA_1 region should have been at least as great as that to the GA_5 region. Similarly, since the dose response curves of dark-grown plants to GA_1 and GA_5 converge with decreasing gibberellin concentrations, the conversion of ^{3}H-GA_5 to the GA_1-like factor should have increased when the GA_5 dose was lowered. The conversion of GA_5 to its metabolite was, however, independent of the hormone concentration.We therefore propose that GA_5 is active *per se*, rather than being a gibberellin precursor.

Light had no effect on the distribution of radioactivity between the acid-ethyl acetate, aqueous, and residue phases. Light did, though, reduce the conversion of ^{3}H-GA_5 to the other biologically active compound. Since GA_5 appears to be active *per se*, the growth inhibition by light cannot be explained by reduced conversion of GA_5 to an active hormone. This conclusion is supported by the fact that the levels of the endogenous GA_1- and GA_5-like factors remain unchanged when dwarf peas are exposed to light (8, 11).

Maturing pea seeds metabolized ^{3}H-GA_5 more rapidly than did the shoots. The metabolism continued throughout seed maturation and subsequent germination. Thus, there is no reason to believe that any of the metabolites represent a storage form of GA_5 from which GA_5 is released during germination. This is in contrast to the results obtained from similar experiments with ^{3}H-GA_1 (2) since during germination a small amount of GA_1 appeared to be released from a bound form produced during seed maturation.

No stable complex between ^{3}H-GA_5 and a macromolecule could be isolated. The cell organelles prepared from ^{3}H-GA_5-treated pea shoots accumulated less than 1% of the total radioactivity, but the material precipitated from the high speed supernatant by ammonium sulfate accounted for 16% of the total extracted radioactivity. Whether or not the radioactivity was ^{3}H-GA_5 bound to specific gibberellin receptors remains to be determined, but the association between the radioactivity and the precipitate was not strong enough to prevent dissociation and separation of the micro- and macromolecular components when chromatographed on a Sephadex G-10 column. Thus it seems that, for GA_5 as for GA_1, the interaction with the receptor is of a loose, possibly noncovalent nature.

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