STUDIES IN PLANT METABOLISM. V. THE METABOLISM OF RADIOACTIVE 2,4-D IN ETIOLATED BEAN PLANTS[1,2,3]

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Radioactive 2,4-dichlorophenoxyacetic acid (2,4-D) has been used to study the translocation and metabolism of this chemical in plants by several workers (1, 2, 3, 4, 10, 11). Holley et al (2) and Holley (3) using carboxyl-C^{14}-labeled 2,4-D demonstrated the presence of free 2,4-D and two radioactive water soluble, ether insoluble organic acids in bean plant homogenate. Jaworski and Butts (4), using both carboxyl- and α-methylene-C^{14}-labeled 2,4-D, found that two major radioactive compounds in addition to unchanged 2,4-D were present in an 80 % alcohol extract of the stems of bean plants after treatment on the leaf with radioactive 2,4-D. One of the major radioactive compounds was found to be a complex containing 2,4-D. The formation of this complex was suggested to be a detoxification mechanism in bean plants.

Mitchell and Brown (5) found that 2,4-D was not translocated from the leaves of young bean plants which had been depleted of carbohydrates. Rice (7) was able to demonstrate that even though transport of 2,4-D from carbohydrate-depleted leaves did not occur, entry of this chemical into the leaf did take place. It has been further shown by Rohrbaugh and Rice (8), and Weintraub and Brown (9) that carbohydrate-depleted bean plants can be induced to move 2,4-D stimulus from the leaves by applying sucrose, glucose, fructose, maltose and galactose to the leaves. Later, Mitchell et al (6) using normal plants demonstrated that the translocation of 2,4-D was increased by application of sugar solution externally to the leaves. Addition of boric acid to the sugar solution will increase the rate of translocation of 2,4-D by 50 % as compared with the sugar solution alone. They found that glucose was the most effective sugar, followed by sucrose and fructose.

The present experiments were undertaken in an attempt to obtain direct information concerning the effect of sugars and other chemicals on the translocation and metabolism of 2,4-D in etiolated plants.

EXPERIMENTAL METHODS

Sixty bean plants (*Phaseolus vulgaris* var. Black Valentine) were grown in individual pots in a dark box for a period of 12 days, and a second group of 8 plants was grown under greenhouse conditions for the same length of time. The temperatures of the greenhouse were 70 to 75° C during the daytime and approximately 60° C during the nighttime.

The plants which had been grown under greenhouse conditions had fully unfolded primary leaves and were of normal height, 5 to 6 inches. On the other hand, the etiolated plants were much elongated (7 to 8 inches height) with underdeveloped leaves. The size of the primary leaves of the etiolated plants were much smaller and the petioles were much shorter as compared to those of the normal plants. The trifoliate leaves of both normal and etiolated plants were still folded in the terminal buds.

Four plants were selected at random to comprise a group of each of the 16 treatments. All plants were treated with 20 μg of carboxyl-C^{14}-labeled 2,4-D acid per plant. A 95 % ethanol solution containing 0.1 % radioactive 2,4-D and 0.5 % Tween-20 was used. 2,4-D solution was applied to the base of one primary leaf by means of a microsyringe. In order to eliminate the difficulty of immersing a proper amount of leaf area into the solution, the following technique was used. A thread, 2 inches in length, ran through the midrib of the treated leaf (approximately 1 cm from the point received 2,4-D treatment), with the lower end dipping into a vial containing 5 ml of one of thirteen solutions. Through capillary action, the treated leaf was getting a continuous supply of the chemical from the solution. The vials were filled daily with the proper solutions. The solutions supplied to the leaves contained 0.025 % sulfanilamide for the purpose of minimizing microbial growth. All plants, except the normal ones, were kept in the dark for 4 days following treatment. All manipulations were carried out at night under diffused red light.

Four days after treatment, each group of plants was harvested by cutting off the plant level with the soil. The plants were sectioned into leaves and stems (including petioles and terminal buds), homogenized separately in a Waring blendor with 80 % ethyl alcohol for 1 minute and transferred quantitatively to suitable volumetric flasks.

The radioactivity of the extracts was determined by the direct plating method. Five-tenth ml samples were evaporated to dryness in 1 inch stainless steel cupped planchets and counted with a thin mica window G-M counter (1.9 mg/cm^2) and a Tracerlab "64" scaler. In every case an accuracy of 5 % or better was realized. For the identification of 2,4-D and other radioactive compounds, the extract was applied to a spot about 7 cm from the end of strips of Whatman #1 filter paper (1 × 22 in.) by means of a fine eye dropper. Two strips were made in each case. All chromatograms were developed in a n-butanol-propionic acid-water (12 : 5.6 : 8) system for 12 hours. A descending chromatographic technique was used.

[1] Received January 17, 1955.

[2] This work was supported by a grant from the Atomic Energy Commission.

[3] Approved for publication as Technical Paper No. 894 by the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Agricultural Chemistry, Oregon State College, Corvallis, Oregon.

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After developing, the strips were air dried at room temperature and cut into 1-cm sections starting from the original spot. The radioactivity of each section was determined directly. The relative percentage concentrations of compounds containing C^{14} in the 80 % alcohol extracts were computed (1, 4).

Results and Discussion

The data on the radioactivity recovered from the leaves and stems of all 16 groups of bean plants are presented in table I. In the etiolated plants, no radioactivity was found in the stem extract from plants which received no supplement revealing that 2,4-D was not translocated to the stem. In the normal plants, approximately 35 % of the applied activity was translocated from the leaf. The accumulation of C^{14} in the bean stems was increased with an exogenous supplement of sugar solution to the treated leaves. This observation confirms the work of Rohrbaugh and Rice (8) and of Weintraub and Brown (9), that the movement of 2,4-D from the leaves of carbohydrate-depleted bean plants can be induced by applying certain sugar solutions to the leaves even though the plants were kept in darkness. Sucrose and glucose were most effective among the carbohydrates tested, followed in order by melibiose, fructose, cellobiose and galactose. Gentiobiose, arabinose, succinate, glucose 1-phosphate and phosphoglycerate were relatively ineffective. The non-specificity with respect to sugar suggests that transport of radioactive 2,4-D is facilitated by food movement in general rather than by any specific combination with particular metabolite. Since both glucose and glucose-1-phosphate are common constituents in plant tissue, the ineffectiveness of glucose-1-phosphate in particular suggests that 2,4-D may inhibit the enzyme system involved for the conversion of glucose-1-phosphate to glucose in treated plants. From a separate identical experiment, using P^{32}-labeled glucose-1-phosphate, we were able to demonstrate the presence of radioactivity beyond the point where the thread entered the leaf. A small amount of radioactivity was also noted in the petioles and the terminal buds of the plants. It is believed in this case that glucose-1-phosphate was taken up by the plants by this method even though it is known that glucose phosphate esters do not penetrate readily the intact cells.

The average values of chromatographic results of 80 % alcohol extract of bean leaves are represented in table II. Due to the low radioactivity found in most stem extracts, only four groups were analyzed chromatographically. In general, two major radioactive spots were found in all chromatograms. One radioactive spot, having a R_f value of 0.84 to 0.88 was free 2,4-D and the other spot, R_f 0.44 to 0.46, was a 2,4-D complex. Three additional radioactive compounds were found to be present in many of the extracts but in very low concentrations. Their respective R_f values were 0.22 to 0.28, 0.36 to 0.38, and 0.65 to 0.68. In this experiment, the R_f value (0.44) of 2,4-D complex (unknown 1) was found to be somewhat higher than the value reported previously (4). A slight increase of the amount of water in the solvent composition and the use of a different batch of filter paper strips may be responsible for the change of R_f value.

Under identical conditions, no significant difference in the rate of reaction between 2,4-D and plant substrate (formation of unknown 1) in the leaves of etiolated and normal bean plants was observed. It is believed therefore, that the metabolism of radioactive

Table I

Absorption and Translocation of C^{14} in Etiolated Bean Plants Treated with 20 μg Radioactive 2,4-D and Supplemented with Different Chemical Solutions

Plant used	Treatments	Activity recovered in leaves	Activity recovered in stems	Total recovery	Translocation and accumulation in stems
		$\times 10^3$ cpm	$\times 10^3$ cpm	%	%
Normal	None	65.6	60.5	74	35.2
Normal	0.025 % sulfanilamide	84.2	50.4	79	29.4
Etiolated	None *	122.7	0.0	96	0.0
"	0.025 % sulfanilamide *	128.7	0.8	101	0.62
"	1 % sucrose	150.0	11.7	94	6.80
"	1 % glucose	155.0	5.5	94	3.20
"	1 % fructose	150.1	2.2	89	1.28
"	1 % galactose	163.9	1.7	97	0.99
"	1 % arabinose	170.0	0.5	99	0.29
"	1 % cellobiose	143.8	1.9	85	1.11
"	1 % melibiose	125.5	2.6	75	1.51
"	1 % glucose 1-PO_3	159.3	0.5	93	0.29
"	1 % ph-glycerate *	113.9	0.2	89	0.16
"	1 % succinate	173.5	1.1	102	0.64
"	1 % indoleacetic acid	163.5	0.8	96	0.47
"	1 % gentiobiose	156.8	0.9	92	0.52

Each group consisted of 4 plants.
* These groups consisted of 3 plants.
(1 μg 2,4-D = 2160 cpm)

TABLE II

PERCENTAGE DISTRIBUTION OF MAJOR RADIOACTIVE COMPOUNDS FOUND IN 80 % ALCOHOL EXTRACTS OF LEAVES AND STEMS FROM BEAN PLANTS TREATED WITH CARBOXYL-C^{14}-LABELED 2,4-D AND SUPPLEMENTED WITH DIFFERENT CHEMICAL SOLUTIONS

PLANT USED	TREATMENT	PLANT PART	R_f VALUES OF RADIOACTIVE SPOTS	
			COMPLEX OF 2,4-D 0.44–0.46	FREE 2,4-D 0.84–0.88
			%	%
Normal	None	leaf	24	68
Normal	0.025 % sulfanilamide	leaf	24	73
Etiolated	None	leaf	25	71
"	0.025 % sulfanilamide	leaf	33	56
"	1 % sucrose	leaf	29	63
"	1 % glucose	leaf	33	62
"	1 % fructose	leaf	30	66
"	1 % galactose	leaf	24	67
"	1 % arabinose	leaf	23	72
"	1 % cellobiose	leaf	27	51
"	1 % melibiose	leaf	28	60
"	1 % glucose 1-PO_3	leaf	31	53
"	1 % ph-glycerate	leaf	24	67
"	1 % succinate	leaf	24	69
"	1 % indoleacetic acid	leaf	20	71
"	1 % gentiobiose	leaf	35	55
Normal	None	stem	68	23
Normal	0.025 % sulfanilamide	stem	74	17
Etiolated	1 % sucrose	stem	45	43
Etiolated	1 % glucose	stem	38	57

All values are average of duplicate runs.

2,4-D will take place equally well in both etiolated and normal plants.

Mitchell and Brown (5) have reported that no stem curvature occurred in starch-depleted bean plants which were treated with 2,4-D on the primary leaf and kept in darkness. If the treatment were made on one side of the stem, the curvature developed within 24 hours. They explained that the failure of the stem of starch-depleted bean plants to develop curvature was due to the insufficient amount of 2,4-D translocated to the stem. In the present experiment, no stem curvature was observed in all etiolated plants. For the same reason, the failure of etiolated plants to develop a stem curvature might also be due to the insufficient amount of 2,4-D in the stem. However, to clarify this question, eight etiolated plants were raised in the same manner. Radioactive 2,4-D solution (20 μg per plant) was applied to the terminal bud, to the stem or to both. Again, no stem curvature was noted 4 days after treatment. At this time, the plants were harvested and the stems were homogenized with 80 % ethanol. Chromatographic results revealed that this extract contained 33 % of the applied 2,4-D as a 2,4-D complex and the remaining was free 2,4-D. The failure of the stems of etiolated plants to respond to 2,4-D treatment could not therefore, be explained either by the lack of 2,4-D in the stem or at a vital site of the plant, or by the absence of 2,4-D complex. The formation of a 2,4-D complex is independent of the photosynthetic processes as well as the influence of a number of exogenous metabolites. It also appears that the occurrence of a physiological response by 2,4-D treatment is dependent on the presence of certain enzyme systems requiring activation by light.

SUMMARY

A study has been made on the translocation and metabolism of carboxyl-C^{14}-labeled 2,4-D by etiolated bean plants. It was found that the growth regulator was absorbed by the leaves of etiolated plants. The movement of the 2,4-D molecule or 2,4-D complex from the leaf to the stem in etiolated plants may be induced by applying a sugar solution to the leaves. Sucrose and glucose were most effective among the chemicals tested.

Radioactive 2,4-D is metabolized in the leaves of etiolated plants as well as in the normal plants. Under identical conditions, no significant difference in the rate of reaction between 2,4-D and plant substrate (formation of 2,4-D complex) in the leaves of etiolated and normal bean plants was observed.

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PHOTOSYNTHESIS AND RESPIRATION OF THREE BLUE-GREEN ALGAE[1,2]

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Reported herein is a study of certain salient features of photosynthesis and respiration in three blue-green algae, *Anabaena variabilis*, *Anacystis nidulans*, and *Nostoc muscorum* G. Histories and brief descriptions of the three species are included in a previous report (2) which presented methods of culture and growth characteristics. Of the various blue-green algae available in pure culture the species used were chosen because they suspend readily to give homogeneous suspensions which submit to ease of manipulation. The objectives of the study are two-fold: first, to obtain information on metabolism of the blue-green algae, a group which has received only cursory attention; and secondly, to develop a base of information which would allow use of a blue-green alga as a reliable experimental organism for studies on the process of photosynthesis. Because of the simplicity of their structure the blue-green algae appear especially desirable for studies on photosynthesis pursued to subcellular levels.

METHODS

Cellular material of *Anabaena variabilis* and *Anacystis nidulans* was obtained from bacteria-free cultures grown in a continuous-culture apparatus (6). The culture medium was medium C, described completely in a previous report (2), containing per liter 0.25 gm $MgSO_4 \cdot 7\,H_2O$, 1.0 gm K_2HPO_4, 0.025 gm $Ca(NO_3)_2 \cdot 4\,H_2O$, 1.0 gm KNO_3, 0.165 gm sodium citrate, 0.004 gm $Fe_2(SO_4)_3 \cdot 6\,H_2O$, and 1.0 ml of an A_5 trace element solution; pH 7.3 to 7.5 when aerated with 0.5 % CO_2 in air. The cultures were maintained at 25 or 39° C and illuminated by tungsten lamps. Pure cultures of *Nostoc muscorum* G (strain obtained from G. C. Gerloff) were grown with the same medium and aerating gas in large test tube cultures in baths held at 25° C and illuminated by 20-watt daylight fluorescent lamps. For all cultures rate of growth was controlled by light intensity. However, since the illumination was multidirectional and the effective light intensity not easily interpretable, a more useful description of the previous history of the cells is provided by the specific growth rate, k, which will be expressed in $\log_{10}$ units per day.

Photosynthesis measurements were made by Warburg manometry in a water bath with glass bottom thermostated at 25 or 39° C. Illumination was provided by a bank of tungsten lamps operated at 120 ± 1 volts from a voltage stabilizer. Light intensity could be varied by use of Jena NG neutral filters attached to the bottoms of the vessels by holders which also masked out stray light. Respiration measurements were made in conical flasks containing 2.0 ml cell suspension, 0.25 ml KOH in the center well, and 0.5 ml of buffer or buffer plus substrate in the sidearm.

In preparation for manometric experiments cells were centrifuged out from a known volume of suspension, washed in the buffer to be used, and taken up in buffer. Because of its small size (ca $1.6 \times 2.2\ \mu$) *Anacystis nidulans* does not sediment as easily as other algae; $3300 \times g$ for 6 minutes in an angle centrifuge were required for complete sedimentation. Cell quantity was determined routinely in terms of packed cell volume. Aliquots of original cell suspension were centrifuged out, transferred to calibrated capillary tubes, and centrifuged at $2400 \times g$ for one hour in an International centrifuge. Packed cell volume of all three species attained a minimum volume after 45 minutes. The relation between cell volume and dry weight was determined separately upon several differ-

[1] Received January 19, 1955.
[2] Aided by Grant G259 from the National Science Foundation.
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