SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON SOLUBLE NUCLEOTIDES & NUCLEIC ACID OF SOYBEAN SEEDLINGS [1, 2]

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One of the most marked biochemical changes in plants known to accompany 2,4-D[4] treatments is the increase in nucleic acid. Rebstock et al (33) found the nucleic acid content to double in the stems of bean plants after 2,4-D treatment. Similar increases in the RNA content of 2,4-D-treated cucumber plants were reported by West et al (40); 2,4-D treatment was also shown to alter the RNA content of excised tissue both in cucumber and in corn (40). It has been proposed that auxin action is linked to nucleic acid metabolism (36), and that an alteration of nucleic acid metabolism is involved in the abnormal growth and development of 2,4-D-treated plants (33). Work by Marrè and Forti (24) has shown that auxins initiate a large increase in ATP in pea stem sections within 30 minutes after treatment. More recently Ormrod and Williams (29) have shown that 2,4-D treatment causes a rapid rise in soluble organic phosphates, suggesting an increase in such compounds as ATP (since inorganic phosphate is usually incorporated into organic compounds via oxidative phosphorylation). RNA is apparently involved in such processes as protein synthesis (3), oxidative phosphorylation (12), and ion absorption (19, 38), processes known to be affected by 2,4-D (9, 16, 28, 35, & 37). Soluble nucleotides are involved in such essential biological processes as oxidative phosphorylation (20), amino acid metabolism (8), carbohydrate transformations (14), and lipide syntheses (15). Therefore it seemed that additional basic information on the effects of 2,4-D on nucleotide metabolism was needed.

These data are results of experiments undertaken to study some relationships of 2,4-D to nucleotide metabolism. The results suggest that changes in nucleotide metabolism may underlie the growth aberrations induced by 2,4-D. However, more basic knowledge of the normal growth processes will be necessary before the role of 2,4-D as an auxin or as a herbicide can be fully clarified.

Materials & Methods

Soybean seeds (Hawkeye variety) were lightly dusted with the fungicide Spergon (U.S. Rubber Co.) and planted in 8.5 × 13 inch pyrex baking dishes between layers of vermiculite moistened with tap water and germinated at 28° C in the dark. In experiments where whole plants were used, the seedlings were sprayed with 15 ml of 5×10^{-4} M 2,4-D per tray of seedlings (ca. 200 plants) after 60 hours of germination. The 2,4-D was twice recrystallized from ethanol and placed in solution as the potassium salt (pH 6.0). Comparable seedlings which were not sprayed with 2,4-D were used as controls in all experiments. In studies where sections of hypocotyl were used, the sections were cut from seedlings which had been germinated for 72 hours as described above.

Unless otherwise stated in figures and tables, sections of hypocotyl tissue were incubated in a solution consisting of 0.002 M potassium dihydrogen phosphate (neutralized to pH 6.0 with ammonium hydroxide), 0.5 percent sucrose, and 0, 10, or 500 ppm 2,4-D.

For analysis of protein and nucleic acid and estimates of soluble nucleotide, tissue samples were ground in an ice-jacketed Potter-Elvehjem tissue homogenizer for 2 to 3 minutes in 5 ml of 0.5 M sucrose. The cell debris and nuclei were removed by centrifuging the homogenate at 1000 × g for 5 minutes at 0° C. Aliquots of the cleared homogenates were treated with equal volumes of ice-cold 10 % trichloroacetic acid, and the precipitate was sedimented by centrifuging at 2000 × g for 5 minutes. The pellet was washed by resuspension and resedimentation in 10 % trichloroacetic acid, dissolved in 0.1 N NaOH, and aliquots were analyzed for protein by the method of Lowry et al (21).

Additional aliquots were removed from the cleared homogenate and treated with an equal volume of 0.4 N perchloric acid for nucleic acid and nucleotide analyses. The precipitate was sedimented as above, and the pellet was washed by resuspension and resedimentation in 0.2 N perchloric acid. The combined supernatant solutions were used for estimation of soluble nucleotide by referring the 260 to 290 mμ absorption difference to an AMP standard curve. The centri-

[1] Received July 23, 1960.

[2] Contribution from the Department of Agronomy, University of Illinois. This research was supported in part by Grant G-3358, National Science Foundation and represents a portion of a thesis submitted by J. L. Key for the Ph.D. degree.

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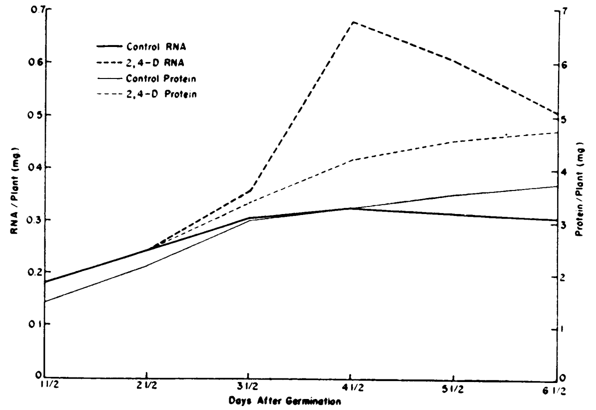
[4] Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; RNA, ribonucleic acid; ADP, adenosinediphosphate. Other abbreviations as footnotes in tables.

fuge pellet was next extracted twice with 2-ml portions of ethanol-ether-chloroform (2:2:1) to remove lipides. The defatted pellet was extracted with 0.5 N perchloric acid for 30 minutes at 70° C, centrifuged, and the supernatant solution used for determining RNA by referring the 260 to 290 mμ absorption difference to a standard curve with yeast RNA.

In experiments where ADP-C^{14} incorporation into RNA was studied, 3 g of the designated tissue were incubated at 28° C in 20 ml of solution (see footnote to table III) containing 1 μc of ADP-8-C^{14} (1.25 μc/mg ADP). After the tissue sections were blotted and weighed, the samples were homogenized in deionized water, made to a volume of 20 ml, filtered through glass wool and centrifuged for 5 minutes at 1000 × *g*. A 0.1 ml aliquot of the supernatant fluid was removed and plated for determination of total ADP uptake. (No attempt was made to determine what form the C^{14} entered the tissue, but is assumed to be ADP-C^{14}.) Additional aliquots were removed, and the procedures described above were followed for RNA extraction. One milliliter of the KOH-neutralized perchlorate-free (potassium perchlorate being relatively insoluble at 0° C) extract was plated for determining ADP incorporation into RNA. Counting efficiency was routinely checked by adding known amounts of radioactivity as ADP-C^{14} to comparable aliquots from "cold" tissue homogenates. The efficiency of counting was 10 % whether made on the crude supernatant for total uptake or on the RNA extract for incorporation.

A more exact analysis for soluble nucleotides was made by the procedures of Cherry and Hageman (6). Briefly the methods were as follows: Sixty-gram samples of hypocotyl tissue were homogenized for 4 minutes in an Omnimixer in 120 ml of cold 0.6 N perchloric acid. The homogenate was centrifuged at 20,000 × *g* for 15 minutes at 0° C. The supernatant solution was filtered through glass wool and neutralized to pH 6.8 with cold 4.0 N KOH. After 30 minutes at 4° C the solution was filtered, and the perchlorate-free extract was placed on a column (1.2 × 40 cm) of Dowex-1×10 (200–400 mesh, formate form) ion-exchange resin. Nucleotides were eluted from the column by means of a formic acid gradient followed by a formic acid-ammonium formate gradient. The eluate was collected in 5 ml volumes in a Rinco automatic fraction collector. The concentration of nucleotide was estimated by measuring the 260 mμ absorption of the contents in each tube in a Beckman DU spectrophotometer.

The contents of all tubes from each peak were combined and purified for identification of the component nucleotides. Co-chromatography on paper in Pabst solvents (31) and ultraviolet absorption characteristics were used to identify the components present in each purified fraction. Standard nucleoside-5′-phosphates were co-chromatographed with each unknown fraction. The nucleotides were located on the developed chromatograms with a Mineralight lamp. The ultraviolet-quenching spots were eluted with sodium phosphate buffer from the chromatograms which had been developed in Pabst solvent III, and the ultraviolet absorption spectra were read between 220 and 300 mμ at pH 2, 7, and 11 for both known and unknown compounds. In addition 2 mg each of standard Pabst AMP, GMP, UMP, ADP, UDP, and ATP (5′-phosphates) were added separately to extracts of the hypocotyl tissue and column chromatographed to confirm the elution position of these compounds. The presence of DPN was confirmed by adding sodium hydrosulfite to the fraction which was believed to contain DPN, the oxidized DPN being reduced to DPNH which gave a second absorption peak at 340 mμ.



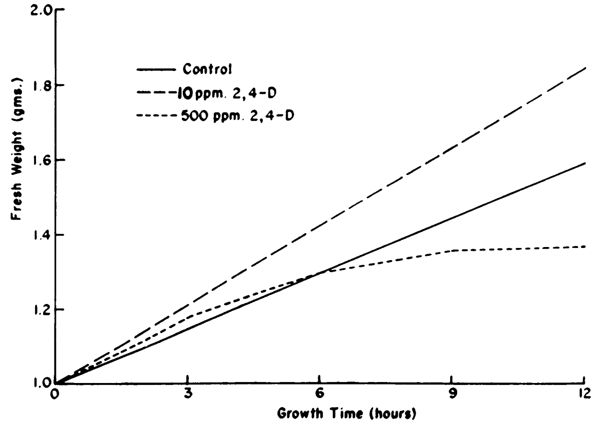


FIG. 1. Changes in RNA and protein accompanying normal and 2,4-D-induced growth of soybean seedlings. Analyses were made on triplicate samples.

FIG. 2. Growth of excised soybean hypocotyl sections accompanying 2,4-D treatment. 1-cm hypocotyl sections were taken just back of the cotyledons and incubated in a solution consisting of 0.002 M NH_4KPO_4 (pH 6.0), 0.5 % sucrose and 0, 10, or 500 ppm 2,4-D. Fresh weights were measured at 3 hour intervals for 12 hours. Values represent averages from two experiments each with duplicate samples.

TABLE I

DISTRIBUTION OF NUCLEIC ACID & PROTEIN IN SOYBEAN HYPOCOTYL AS FUNCTION OF MATURITY & 2,4-D TREATMENT*

SECTION	TREATMENT	RNA		PROTEIN		SOLUBLE NUCLEOTIDE	
		Mg/g F.W.	Mg/ SECTION	Mg/g F.W.	Mg/ SECTION	Mg/g F.W.	Mg/ SECTION
0.0–0.5 cm	Control	2.80	0.038	30.7	0.43	0.44	0.006
	2,4-D	3.07	0.052	31.6	0.53	0.49	0.009
0.5–1.5 cm	Control	1.29	0.042	15.4	0.51	0.31	0.013
	2,4-D	0.76	0.058	8.4	0.64	0.26	0.020
1.5–2.5 cm	Control	0.58	0.020	6.6	0.23	0.21	0.007
	2,4-D	0.56	0.038	5.5	0.37	0.22	0.015
2.5–3.5 cm	Control	0.42	0.016	5.6	0.22	0.18	0.007
	2,4-D	0.80	0.096	6.8	0.38	0.24	0.014

* Successive sections of 3.5-day-old control and 2,4-D-treated soybean hypocotyls were taken for analyses beginning just back of the cotyledons. 2,4-D-sprayed plants were treated at 2.5 days. Data are averages of two closely duplicating experiments.

RESULTS

PROTEIN & NUCLEIC ACID: The general morphological and growth responses to 2,4-D (5×10^{-4} M in the experiments reported) were essentially those often reported, and are described in a previous paper (16). Elongation of root and shoot of treated intact plants was almost completely inhibited, apparently due to inhibition of both cell division and cell elongation (34). Normal meristematic activity was resumed, at least in part, above and below the cotyledons between 2 and 3 days after treatment with 2, 4-D. Root growth which is dependent upon food reserves from the cotyledons (39) did not show this recovery. Disorganization of the phloem resulting from uncontrolled cell division (10, 34) might account for lack of recovery in the root tissue. Tissue proliferation in the lower hypocotyl and root became apparent between 48 and 72 hours after treatment with 2,4-D.

The changes in protein and nucleic acid per plant (less cotyledons) accompanying 2,4-D-induced growth abberations (fig 1), are in general agreement with published results (33, 35, 40). The changes with time are of special interest here. After 3.5 days of growth there were no marked changes in protein or RNA in control seedlings, although the slight increase in protein and decrease in RNA were found reproducible. In contrast the RNA content doubled in 2,4-D-treated plants between 24 and 48 hours after treatment. The peak in RNA content occurred just prior to the time proliferation in the lower hypocotyl became apparent, suggesting a rather direct interaction of 2,4-D-induced RNA synthesis and cell proliferation. The increase in RNA seems not to be a simple increase in cytoplasm since the protein/RNA ratio decreased very markedly in treated seedlings, especially at 48 hours after treatment. As the plants recovered (4.5–6.5 days), there was a noticeable shift in the protein/RNA ratio back toward normality. Similar analyses were made separately on hypocotyl and root tissue and revealed that the effects were obtained in both tissues, although the changes were of greater magnitude in the hypocotyls (18).

It was of interest to attempt to localize the RNA changes so far as stage of cell development was concerned. In order to do this sections were cut successively from the hypocotyls, starting with the hypocotyledonary hook as the 0.0 to 0.5 cm section and proceeding down the hypocotyl to obtain sections with increasing mean cell age. As shown in table I the largest increase in RNA occurred in the mature tissue (2.5–3.5 cm) which would have rapidly proliferated in another 24 to 48 hours. The increase in RNA in the first three sections (0–2.5 cm) was essentially proportional to the radial enlargement which these cells had undergone. The increase in soluble nucleotide which occurred following 2,4-D-

TABLE II

FRESH WEIGHT, NUCLEOTIDE, NUCLEIC ACID, & PROTEIN CHANGES ACCOMPANYING GROWTH OF EXCISED SOYBEAN HYPOCOTYL SECTIONS*

TREATMENT	INCREASE IN FRESH WT %	RNA Mg/g F.W.	PROTEIN Mg/g F.W.	SOLUBLE NUCLEOTIDE Mg/g F.W.
Initial	...	2.35	19.1	0.47
Control	53.7	2.07	17.4	0.41
10 ppm 2,4-D	86.2	2.05	17.9	0.40
500 ppm 2,4-D	38.0	2.19	18.9	0.34

* Details of experiments are given in footnote of figure 2. Average of three experiments.

treatment is of interest and will be discussed more fully in the material which follows.

Similar studies were extended to excised tissue-section growth where West et al (40) had also shown 2,4-D to affect RNA metabolism. Figure 2 shows the effect of 10 and 500 ppm 2,4-D on the growth of the excised hypocotyl tissue. (The apical cm section of the hypocotyl was used in these experiments.) The initial promotion of growth by 500 ppm 2,4-D (small but very reproducible) followed by complete inhibition is of interest. Growth stimulation followed by inhibition as a result of auxin treatment has been reported previously (1, 23, 30), and West (41) reported a comparable stimulation and inhibition of respiration. As shown in table II, growth of excised hypocotyl sections was associated with the catabolism of both RNA and protein. The growth-promoting concentration of 2,4-D had no observed effect on RNA and protein metabolism, whereas the inhibitory concentration of 2,4-D either blocked this catabolism or induced a greater resynthesis of these constituents. The presence of ammonium ion and sucrose would provide materials for synthesis. Solutions remained clear with no evident bacterial contamination.

Some preliminary experiments were run in an attempt to see if herbicidal concentrations of 2,4-D had inhibited the normal catabolism of RNA or if the synthetic processes had been speeded up relative to the breakdown. Excised sections were incubated in ADP-C^{14} in the presence of 0, 10, and 500 ppm 2,4-D, and the incorporation of ADP into acid-insoluble nucleic acid was measured, (table III).

Experiment A was run under conditions about comparable to those of table II: that is with 0.002 M

TABLE III

EFFECT OF 2,4-D ON INCORPORATION OF ADP-C^{14} INTO RNA OF EXCISED SOYBEAN HYPOCOTYL TISSUE*

TREATMENT & TISSUE	% INCREASE FR WT	SOLUBLE NUCLEOTIDE	RNA	ADP-C^{14} UPTAKE & INCORPORATION		
		mg/g Fr Wt		c/m/g Fr Wt UPTAKE	c/m/g Fr Wt RNA	% INCORPORATION
EXPERIMENT A						
Tip—initial	...	0.44	2.75	...	...	...
Control	45.7	0.43	2.28	14,580	1,790	12.3
10 ppm 2,4-D	71.0	0.40	2.57	16,060	2,080	12.5
500 ppm 2,4-D	35.7	0.37	2.66	9,760	815	8.4
Base—initial	...	0.23	0.57	...	...	...
Control	3.4	0.20	0.58	6,020	790	13.2
10 ppm 2,4-D	10.3	0.23	0.66	6,830	1,710	25.0
500 ppm 2,4-D	0.7	0.19	0.60	5,010	415	8.3
EXPERIMENT B						
Tip—initial	...	0.45	3.02	...	...	...
Control	54.2	0.39	2.25	17,700	2,440	13.8
10 ppm 2,4-D	70.0	0.42	2.20	18,800	3,310	17.6
500 ppm 2,4-D	22.9	0.24	2.13	12,950	2,490	19.2
Base—initial	...	0.20	0.56	...	...	...
Control	7.9	0.20	0.56	12,725	2,380	18.8
10 ppm 2,4-D	16.0	0.20	0.60	14,400	3,775	26.0
500 ppm 2,4-D	3.0	0.11	0.62	12,200	4,900	39.5
EXPERIMENT C						
Control tip —I	...	0.48	3.11	...	...	...
F	35.2	0.38	2.59	17,450	2,182	11.9
2,4-D tip —I	...	0.54	3.52	...	...	...
F	18.5	0.45	3.04	14,170	1,645	11.8
Control base —I	...	0.22	0.57	...	...	...
F	6.2	0.20	0.56	6,377	688	11.0
2,4-D —I	...	0.23	0.42	...	...	...
F	22.3	0.21	0.55	7,035	1,248	16.9

* Conditions for experiment A: 3 g of tissue were incubated for 10 hours in a solution containing 1 % sucrose, 0.002 M KH_2PO_4 buffer neutralized to pH 6 with NH_4OH, 1 μc ADP-8-C^{14}, and 0, 10, or 500 ppm 2,4-D as designated. Conditions for experiment B were same as for A except that tissue was incubated for 15 hours in 0.004 M buffer. Tip and base refer to the tip 1 cm of the hypocotyl just below the cotyledons and the 2 to 3 cm-section, respectively. In experiment C, the tissue was incubated under identical conditions to experiment B except that the solution contained no 2,4-D. 2,4-D in the table refers to sections which were excised from plants which had been sprayed 24 hours previously with 5 × 10^{-4} M 2,4-D as described in text. I and F refer to samples analyzed at beginning of experiment and after incubation, respectively. Data are averages of two closely duplicating experiments.

potassium dihydrogen phosphate buffer (neutralized to pH 6 with ammonium hydroxide) and for 10 hours. Under these conditions, 2,4-D maintained or increased the level of RNA in the tissue. The growth-promoting concentration of 2,4-D enhanced the absorption of ADP relative to the control, while the herbicidal concentration depressed absorption. The proportion of the absorbed ADP incorporated into RNA was increased by 10 ppm 2,4-D in the basal section, but not in the tip section. The high concentration of 2,4-D not only inhibited the absorption of ADP, but also inhibited the incorporation into RNA of that which was absorbed.

As shown in experiment B, however, if the buffer concentration is doubled and the time of incubation extended to 15 hours, a markedly different result is obtained. Here the herbicidal concentration of 2,4-D increased the percentage of absorbed ADP which was incorporated into RNA. Since it appears that high salt concentrations can accelerate RNA metabolism by endogenous enzymes (13), the different result may come largely from the increased buffer. A detailed study will be necessary to clarify these divergent results.

To obtain information relative to ADP incorporation under conditions more nearly comparable with those in the rest of this investigation, tip and basal hypocotyl sections were excised from control and 2,4-D-sprayed plants, and analyzed for nucleotides before and after floating for 10 hours on 0.002 M buffer containing C^{14}-labeled ADP. In the tip section the 2,4-D pretreatment decreased the amounts of growth and ADP absorbed. The percentage of absorbed ADP entering the RNA was unchanged, however. In the basal section, the 2,4-D pretreatment increased expansive growth, ADP absorbed, and the percentage incorporated into RNA. In addition, this section had a net increase in RNA, just as would have occurred in situ (fig 1). The sucrose and ammonium ion probably did not supply needed substrate as well as translocation from the cotyledons, thus reducing the absolute gain in RNA.

As has already been pointed out (40), these data show definite differences between the effects on nucleic acid metabolism induced by 2,4-D in intact plants and in excised plant parts. The data also indicate that 2,4-D has some marked and basic effects on nucleic acid metabolism and on the acid-soluble nucleotide fraction of 2,4-D-treated sections and seedlings. Since alterations of nucleotide metabolism seemed basic to the response to 2,4-D, it seemed of considerable interest to characterize the soluble nucleotide changes which accompanied 2,4-D treatment.

SOLUBLE NUCLEOTIDE: Since the separation and identification of soybean seedling nucleotides had apparently not been done, it was necessary to first separate and identify the component nucleotides of normal soybean seedlings before being able to evaluate the changes induced by 2,4-D.

The elution chromatograms obtained from mature

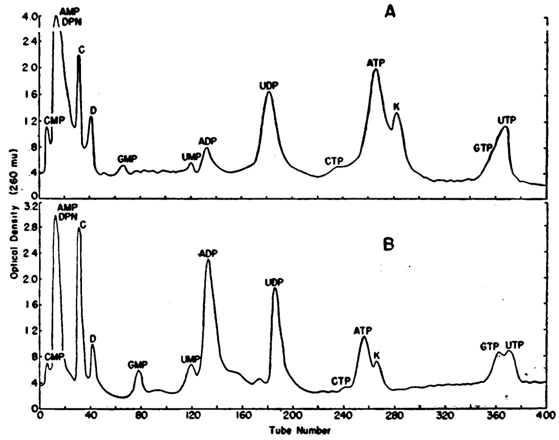


FIG. 3. Elution chromatogram of nucleotides. A. From 50 g of mature, hydrated soybean seed. B. From 50 g of control 2.5-day-old soybean hypocotyl.

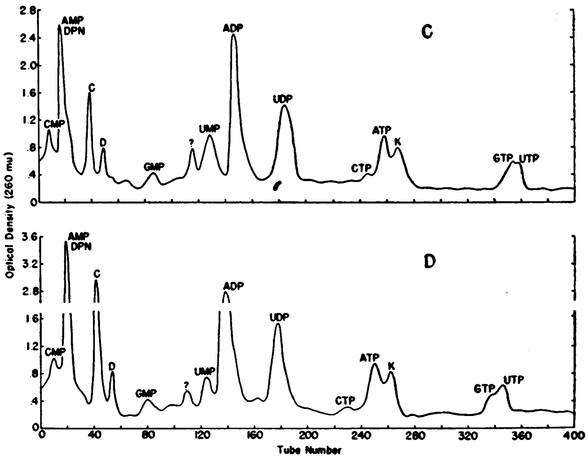


FIG. 4. Elution chromatogram of nucleotides from 50 g of 3.5-day-old soybean hypocotyls. C. Control. D. 2,4-D-treated.

soybean seeds and soybean hypocotyl tissue (control & 2,4-D-treated) are shown in figures 3, 4, and 5. Excellent separation of the nucleotide components with symmetrical peaks was obtained in most cases. The data presented in table IV were used as the primary means of identification of the individual nucleotides so designated in figures 3, 4, and 5. In addition to the data shown in table IV similar ultraviolet absorption data were obtained at pH 2 and pH 11 and mobility characteristics in other solvents checked. These data are not reported because of complete agreement with the data shown in table IV. In the case of ATP identification, enrichment of an extract with 2 mg of adenosine-5′-triphosphate was the only technique used which clearly distinguished between adenosine-5′-triphosphate and the component present in peak K. Otherwise identical data suggest that the component present in this fraction may be another form of ATP. In addition to the identification of

Table IV
Identification of Nucleotide Components From Elution Chromatograms*

Compound		R.F.**	250/260***	280/260	λ Max.
CMP	K	0.70	0.85	0.95	273
	UK	0.69	0.87	0.91	275
AMP	K	0.22	0.78	0.12	260
	UK	0.24	0.77	0.17	260
DPN	K	0.24	0.76	0.09	260
	UK	0.27	0.81	0.08	260
GMP	K	0.38	1.13	0.67	252
	UK	0.38	1.10	0.63	252
UMP	K	0.66	0.67	0.34	262
	UK	0.67	0.69	0.37	262
ADP	K	0.32	0.74	0.11	260
	UK	0.31	0.76	0.12	260
UDP	K	0.74	0.70	0.34	262
	UK	0.73	0.71	0.32	262
CTP	K	0.80	0.86	1.37	275
	UK	0.78	0.88	1.40	274
ATP	K	0.37	0.78	0.14	260
	UK	0.37	0.80	0.17	260
"K"	UK	0.36	0.79	0.20	260
GTP	K	0.52	1.14	0.66	252
	UK	0.53	1.12	0.64	252
UTP	K	0.73	0.76	0.40	261
	UK	0.72	0.77	0.43	262

* C, A, G, and U represent cytidine, adenosine, guanosine and uridine. respectively. MP, DP, and TP represent mono-, di-, and triphosphate nucleotides, respectively. DPN is diphosphopyridine nucleotide.

** R.F.'s were determined in Pabst Solvent III using authentic samples of nucleoside-5′-phosphates (K) and material from the designated peaks (UK).

*** All optical density values reported were determined at pH 7.0 from eluates of nucleotide spots from chromatograms developed in solvent III.

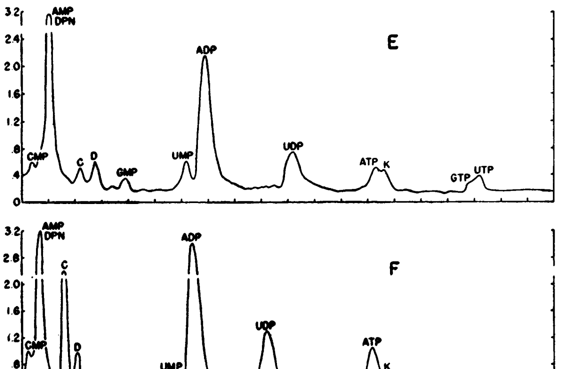


Fig. 5. Elution chromatogram of nucleotides from 50 g of 4.5-day-old soybean hypocotyls. E. Control. F. 2,4-D-treated.

the nucleotides shown in table IV, the component present in peak C of the elution chromatograms has been recently identified as ascorbic acid (17). The component in peak D is an artifact of isolation in that it represents a breakdown product of ascorbic acid (18).

The data indicate a general increase in soluble nucleotides in 2,4-D-treated seedlings and a maintenance of this high level, whereas the quantity of nucleotide decreased with age of control seedlings. The changes noted in adenine nucleotides and ascorbic acid deserve special attention. Quantitative changes in ADP and ATP are shown in table V. Within 3 hours after treatment with 2,4-D the ATP content of the hypocotyl had increased about 45 %. Apparently this increase in ATP was not associated with a corresponding decrease in AMP and ADP. Although the quantity of AMP is not shown in table V because of the presence of DPN in the same fraction, recent work (17) has shown that the oxidized pyridine nucleotide content does not change with 2,4-D treatment, thus it can be concluded that 2,4-D had no effect on the concentration of AMP (figs 3, 4, & 5). The increase in ATP noted here is consistent with

Table V
Effect of 2,4-D Spray Treatment on Level of Adenine Nucleotides in Soybean Hypocotyls*

Treatment	O.D. Units/50 g tissue**		
	ADP	ATP	Total
Control (63 hr old)	106	47	153
2,4-D (63 hr old)	106	68	174
Control (84 hr old)	90	33	123
2,4-D (84 hr old)	130	47	177
Control (108 hr old)	93	10	103
2,4-D (108 hr old)	147	55	202

* Absorbance read in $HCOONH_4$-HCOOH. The base line absorption was subtracted from each reading before the units of nucleotide were calculated. One O.D. unit is that amount of nucleotide which will give unit optical density in 1 ml of solution in 1 cm Beckman cuvettes. Values reported are averages from closely duplicating experiments.

** O.D. values determined at 260 mμ.

the work of Marrè and Forti (24), which showed a large increase in ATP in auxin-treated pea stem sections shortly after treatment. The high level of ATP was maintained for only a short time, again in agreement with the work of Marrè and Forti.

The increase in ascorbic acid (peak C) following 2,4-D treatment (figs 4 & 5) has been discussed in greater detail elsewhere, (17). However, in view of the work of Marrè et al (24, 25) and Prochazka's group (from Bentley, 2), the results obtained here are suggestive of a close relationship between auxin action and ascorbic acid metabolism.

Discussion

The data reported here lend further support to the view that the ultimate action of auxin may be related to nucleotide-nucleic acid metabolism. The major quantitative changes in RNA (fig 1) occur several hours after some effects of 2,4-D have become apparent. However, with the techniques used, only quantitative changes in RNA were measured. It is possible that 2,4-D may initiate changes in specific kinds of metabolically active RNA which in turn could mediate auxin effects. More refined techniques are obviously needed to adequately characterize the RNA from auxin-treated tissue. The results do strongly suggest that the 2,4-D-induced increase in RNA underlies the abnormal proliferation of the mature tissue. The experiments using ADP-C^{14} also show that 2,4-D induces changes in RNA metabolism which are not necessarily reflected in quantitative changes (see table III). Recently, Masuda (27) has proposed that auxin promotes cell elongation by causing an increase in the cation binding capacity of RNA at the protoplasmic surface which would allow for loosening of cell walls because of calcium removal from cell wall pectic substances. As already pointed out, Skoog (36) has proposed a mechanism of auxin action involving nucleic acids. Certainly, more work is needed to clarify possible auxin-nucleic acid relationships as a mechanism of auxin action.

In addition to the effects of 2,4-D on RNA, certain changes in the soluble nucleotide fraction were noted. In general, the 2,4-D treatment caused the maintenance of a higher concentration of many of the nucleotides (see figs 4 & 5). Of most interest are the changes in adenine nucleotides noted in table V. In view of the early increase in ATP noted in table V and the data of Marrè and Forti (24), an explanation of the respiratory burst following auxin treatment based solely on activation of endergonic processes which utilize ATP thus giving more adenylate acceptor (4, 5,11) seems to need some modification. As pointed out by Marrè and Forti (24), the shift in the ATP-ADP ratio indicates first an increase in ATP as a result of activation of oxidative phosphorylation followed, in turn, by activation of endergonic processes. Marrè et al (24, 25) attribute this activation either to the effect of auxin on the oxidation-reduction state of the ascorbic acid-glutathione system or to a direct interaction of auxin with the dehydrogenase enzymes. In view of the effects of 2,4-D on increasing the concentration of ascorbic acid and soluble and protein sulfhydryl in soybean seedlings (17), such an activation of the respiratory system is conceivable.

Another possible explanation of the auxin-induced respiratory burst could lie at the adenylate level. If it is assumed that the level of adenylate acceptor normally limits respiration (4, 5, 11), an increase in adenine nucleotide would presumably result in an enhanced rate of respiration. The data reported in table V and figures 4 and 5 suggest that such an increase has occurred. It has been reported previously (16) that mitochondria isolated from 2,4-D-sprayed soybean seedlings contain considerably more soluble nucleotide than mitochondria from comparable control tissue; also, the oxygen uptake by isolated mitochondria from 2,4-D-treated seedlings was not enhanced by adding AMP.

Summary

Some studies on the effects of 2,4-D on soluble nucleotide and nucleic acid metabolism of soybean seedlings and excised hypocotyl sections are reported. Large increases in RNA content of treated plants were found, the maximum concentration being obtained just prior to initiation of cell proliferation in the more mature tissue. The protein-RNA ratio decreased markedly up to 48 hours after treatment, after which time there was a decrease in RNA and an increase in the ratio. Alterations of ADP-C^{14} incorporation into RNA were effected by 2,4-D treatment.

Treatment with 2,4-D also induced an increase in acid-soluble nucleotides of soybean seedlings. These nucleotides were separated and isolated by ion-exchange chromatography and identified. Marked changes in adenine nucleotides were obtained, and these changes are discussed in relation to auxin-induced respiratory increases.

Some changes in ascorbic acid concentration following 2,4-D treatment are discussed.

Acknowledgment

The authors wish to express their appreciation for the very valuable technical assistance of Mrs. Mary Fisher.

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