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## EFFECTS OF INDOLEACETIC ACID ON THE UTILIZATION OF ACETATE-1-C<sup>14</sup> BY PEA STEM SLICES<sup>1</sup>

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Studies on the utilization and evolution of acetaldehyde by plant tissues suggest that indoleacetic acid (IAA) may influence the partition of pyruvate and related products of glycolysis into various metabolic pathways (5, 6). Boroughs and Bonner (1) tested this possibility in their study of the effects of IAA on the metabolism of acetate-1-C<sup>14</sup> by *Avena coleoptiles*. Except for a very appreciable stimulation of incorporation of activity into the non-cellulosic polysaccharides by IAA, they found no notable response to the auxin. It was demonstrated later that in wheat roots IAA significantly affects incorporation of activity from C<sup>14</sup>-labeled pyruvate and acetate into organic acids, sugars, polyuronide hemicelluloses, pectic substances, lipides and cellulose (7, 8). Studies by Perlis (9), however, on the utilization of acetate-1-C<sup>14</sup> by pea stem slices revealed experimental effects of doubtful significance.

Recent work in this laboratory indicates that effects of IAA on the metabolism of acetate by pea stem tissue can be demonstrated conclusively in experiments involving pre-treatment of the slices with IAA followed by brief exposure to the labeled substrate (4). Experiments of this sort are described below.

### MATERIALS AND METHODS

Pea seedlings (var. Alaska) were grown in the dark at 20° C on sphagnum saturated with 0.025 M CaCl<sub>2</sub> and were used for the experiments 8 days after the seeds were planted. Preliminary experiments had shown that stem sections from peas grown in this manner exhibit a more impressive growth response to IAA than those from seedlings given a more complex nutrient solution. Transverse slices, 2 mm in length, were cut from a section of the stem extending from about 2 to 12 mm below the epicotyl arch. The slices were washed, then centrifuged in a perforated container at a low speed to remove adhering water.

Acetate-1-C<sup>14</sup> having a specific activity of 6.7 millicurie/millimole was the source of carbon 14. This material was available as the potassium salt and was used in this form for most of the experiments. For

use in two experiments the salt was converted to the calcium salt by passing a solution of it through a column containing the calcium form of Duolite C-3. Most of the experiments of this study involve a pre-treatment period during which the stem tissues were incubated in various non-radioactive solutions. For pre-treatment the solutions and tissues were placed in Erlenmeyer flasks of one liter capacity and these were placed on a shaker to effect aeration of the solution. During the period of exposure to radioactive acetate the slices and solutions were in closed containers (Pyrex gas washing bottles, no. 31760). A short piece of capillary tubing replaced the fritted glass aerator usually found in this assembly. The outlet tube was attached to the inlet tube of a second gas washing bottle. This bottle, containing 0.1 N NaOH to trap CO<sub>2</sub>, was equipped with a fritted glass aerator. Carbon dioxide-free air was supplied for the aeration of the slices. Fresh weights of tissues used in the experiments ranged from 3.25 to 7.0 g.

After exposure to radioactive acetate the washed slices were extracted with boiling, 95 % ethanol and then with an equal volume of boiling, 70 % ethanol. Three extractions with each solvent mixture were made and a total volume of 250 ml of extractant was used for each sample. This is the 1st step of a fractionation procedure which is outlined in figures 1 and 2.

Fractionation of the ethanol extract (fig 1) involved removal of the ethanol, after the solution was adjusted to pH 9.0 with NaOH, by vacuum distillation, acidification and extraction of the aqueous residue with ether for 72 hours by a method essentially that described by Isaacs and Broyer (2) and separation of the lipides from the organic acids in the ether extract by addition of water and titration of the acids with NaOH to pH 8.5. The ethereal solutions were dried down on a warm water bath and the residue taken up in chloroform. Activity in the chloroform solution was determined by the technique of direct plating, as were all other determinations of activity reported here. Details of the counting procedure are given below. The aqueous solution from the ether extract was brought to dryness on a hot water

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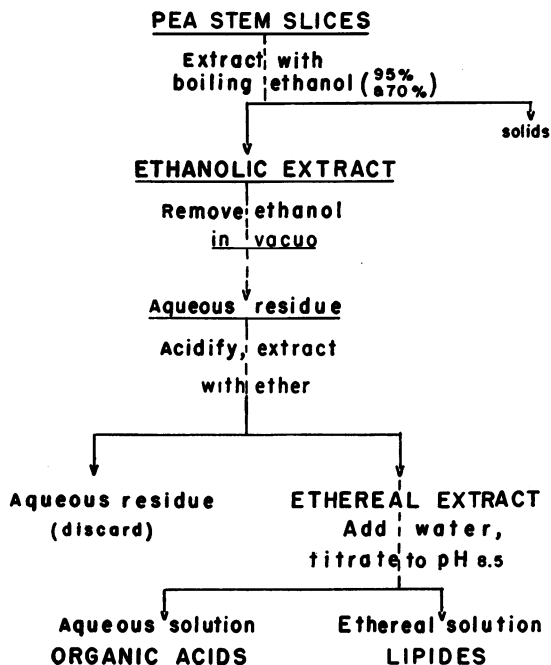


FIG. 1. Procedure for fractionation of pea stem slices. Separation of materials in the ethanolic extract.

bath. This residue was redissolved in water and aliquots assayed for activity.

The ethanol-extracted slices were placed in a Servall tissue homogenizer, covered with cold water and homogenized at 14,000 rpm for 5 minutes (fig 2). During the period of homogenization, the stainless steel vessel containing the tissue was immersed in an ice bath. Particulate and fibrous material of the homogenate was separated from a very cloudy, colloidal preparation by means of glass wool. Solids retained on the glass wool were resuspended in water and the mixture filtered once more through glass wool. These two aqueous preparations were combined, labeled "water extract," and assayed for radioactivity. This extract includes materials hydrolyzed by Takadiastase and by a hemicellulase of fungal origin. There is no solubilization of activity when the mixture is incubated with Pectinol R-10, a commercial pectinase preparation, suggesting the absence of pectic substances.

Isolation of the pectic substances of the cell wall materials was effected by repeated extraction of the solids held by the glass wool with 0.05 N HCl, followed by the precipitation of the pectic substances from the acid extract with ethanol, a procedure recommended by Kertesz (3). Each sample was extracted four times with 15 ml of 0.05 N HCl held at 80° C for 30 minutes. After each extraction the mixture was filtered through glass wool. To the pooled extracts enough 10% HCl was added to make the concentration of the acid equal to 0.05%. Precipitation of the pectic substances was accomplished by the slow addition of two volumes of 95% ethanol. Sepa-

ration of the pectic substances from the alcohol was made by centrifugation. The centrifugate was re-suspended in an ethanol : water mixture (2 : 1, v : v) and recentrifuged three times to remove free acid. Finally, the washed pellet was suspended in water and aliquots of this preparation were assayed for activity.

Solids remaining after the extraction of the pectic substances were washed with water, dried and covered with 5 ml of 4% NaOH and allowed to stand for 48 hours in a sealed desiccator, otherwise empty. The alkaline solution containing polyuronide hemicelluloses was filtered off the solid material with suction, and the residue resuspended in water and refiltered three times. Glass wool served as a filter. The combined extracts were neutralized with weak HCl then made barely alkaline with NaOH. Activity was determined on aliquots of these solutions.

Radioactivity in the samples was determined by a solid-counting technique. Because of the high specific activity of the acetate used it was possible to count each sample at infinite thinness. The planchets were counted in a windowless flow counter.

Activities of the various groups of compounds in the auxin-treatments except for the alcohol extracts have been calculated on the basis of an absorption of carbon-14 equal to that of the respective non-auxin-treated controls. In his work with pea stem slices, Perlis (9) found that the alcohol extracts reflect relative rates of C<sup>14</sup>-absorption in a given pair of experimental treatments with an accuracy of about 5%. For this reason and because determination of the C<sup>14</sup>-absorption by difference is not feasible for experiments of 30 minutes and less where only 10 to 15%

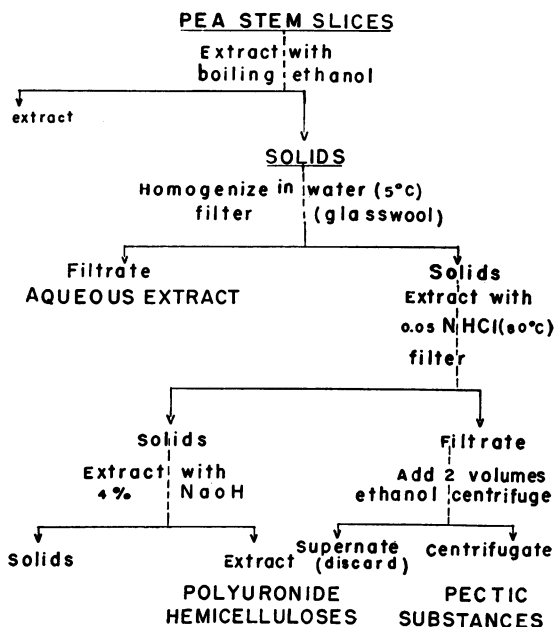


FIG. 2. Procedure for fractionation of pea stem slices. Separation of cell wall constituents.

of the supplied isotope is taken up by the tissues, the alcohol extracts have been used as measures of relative  $C^{14}$ -absorption. One exception to this general rule is to be found in the data of table IV. Because two treatments of this experiment (7 and 8) were supplied with a much higher level of acetate-1- $C^{14}$  than the others, results of these two treatments are calculated on the basis of the alcohol extract of a control given the lower level of IAA (treatment 5).

### RESULTS

Effects of IAA on the utilization of acetate-1- $C^{14}$  by pea stem slices in four hours are shown in table I. In view of the inhibitory effects of calcium on growth and the probability that these effects involve the pectic substances it appeared that inclusion of a calcium salt in the test solutions might accentuate effects of auxin on this class of compounds. All treatments of this experiment include  $CaCl_2$  at 0.005 M. Acetate-1- $C^{14}$  was supplied as the calcium salt. Addition of IAA, adjusted to pH 4.7 with  $Ca(OH)_2$ , to the basal solution enhances growth and the incorporation of carbon-14 into the organic acids and  $CO_2$  appreciably, inhibits incorporation into the lipides, and has insignificant effects on syntheses of other groups of compounds assayed.

Addition of KCl to the  $CaCl_2$ -Ca-acetate mixture produces small increases in growth and incorporation of acetate into organic acids. When IAA is added along with the KCl the growth increment is very appreciably enhanced but the only effects of possible significance are increases in the activities of the respiratory  $CO_2$  and the lipides. For the last experimental treatment the IAA concentration was raised to  $2.5 \times 10^{-4}$  M, with the other salts as in the previous treatment. This level of IAA, obviously quite high, more than doubles the growth rate of the slices, though the growth promotion is less than that elicited by the lower level of auxin. Absorption of carbon-14, as revealed by the activity of the alcohol extract, is somewhat greater than that for the lower level of IAA. The most impressive effect of the higher level of auxin is that it inhibits incorporation of carbon-14 into the pectic substances. An inhibitory effect on

the formation of  $C^{14}O_2$  of about 28 % may be of significance.

These results are, in their rather unimpressive nature, quite similar to those obtained by Perlis (9). Search for a more revealing technique led to the experiment summarized in table II. This and subsequent experiments involve incubation of the slices in a variety of non-radioactive solutions prior to the exposure of the slices to acetate-1- $C^{14}$ . In this experiment the slices were pre-treated for 225 minutes in acetate only, in acetate plus IAA at  $2.5 \times 10^{-5}$  M and in acetate plus IAA at  $2.5 \times 10^{-4}$  M. These three samples and three not given the pre-treatment were then incubated for 30 minutes in acetate-1- $C^{14}$  with each batch of slices exposed to the level of IAA it received in the pre-treatment.

Both levels of IAA promote growth (table II), the higher level having the greater effect in this experiment. Growth for the 30 minutes when they were in the radioactive solutions is reported for the slices not given the pre-treatment. For the other samples, growth occurring in the pre-treatment period is reported.

There is an impressive response to IAA in the pre-treated tissues so far as utilization of acetate-1- $C^{14}$  is concerned. Incorporation of activity into the organic acids is more than doubled at the higher level of IAA, and it is increased by about 50 % at the lower concentration. Incorporation of activity into the pectic substances and to a lesser degree into the water extractable materials is affected by both concentrations. Inhibited conversions of acetate to hemicelluloses, lipides, and  $CO_2$  are produced by the higher level of auxin. The lower level, however, enhances these conversions.

Effects of IAA on the untreated pea stem slices in 30 minutes are not as dramatic as those in the pre-treated tissues, though some appear to be of significance. Incorporations into pectic substances, water extractable materials, organic acids, and  $CO_2$  are enhanced appreciably by both levels of auxin. Other effects seem to be of doubtful significance.

In another experiment the effects of pre-treatment time were considered (table III). For this experiment

TABLE I

INCORPORATION OF  $C^{14}$  FROM CALCIUM ACETATE-1- $C^{14}$  BY PEA STEM SLICES IN 4 HR. TOTAL ACTIVITIES.  $\times 10^4$  CPM \*

No.	KCl mM/l	IAA $\mu$ m/l	FRESH WT % GAIN	ALCOHOL EXTRACT	PECTIC SUBST.	LIPIDES	WATER EXTRACT	ORGANIC ACIDS	$CO_2$
1	0	0	9	5,200	324	160	150	376	854
2	0	25	23	4,790	323	122	151	563	1,100
3	10	0	11	5,290	325	159	148	442	906
4	10	25	33	5,700	359	187	157	479	1,100
5	10	250	29	5,124	206	173	180	437	794

Each treatment included 7 g of pea stem slices which were incubated for 4 hr at 25° C in 100 ml of a basal solution containing  $(CH_3C^{14}O)_2Ca$ ,  $6 \times 10^{-5}$  M;  $CaCl_2$ ,  $5 \times 10^{-3}$  M; and having 0.54  $\mu$ c of activity per ml. Each solution was adjusted to pH 4.7 with  $Ca(OH)_2$  (No. 3), or KOH (Nos. 4 and 5) or HCl (Nos. 1 and 2).

\* Values for the pectic substances, lipides, water extract, organic acids and  $CO_2$  for each IAA-treated sample have been calculated on the basis  $C^{14}$ -absorption equal to that of the appropriate control. Activities of the alcohol extracts serve as measures of the relative amounts of  $C^{14}$  absorbed.

TABLE II

UTILIZATION OF ACETATE-1-C<sup>14</sup> BY PEA STEM SLICES IN 30 MIN. EFFECTS OF PRE-TREATMENT WITH UNLABELED ACETATE AND IAA. TOTAL ACTIVITIES.  $\times 10^3$  CPM \*

No.	PRE-TREATMENT	IAA $\mu\text{m}/\text{l}$	FRESH WT % GAIN	ALCOHOL EXTRACT	PECTIC SUBST.	HEMI-CELLULOSE	WATER EXTRACT	LIPIDES	ORGANIC ACIDS	CO <sub>2</sub>
1	None	0	3	5,170	280	220	154	1,130	1,100	1,090
2	None	25	4	4,780	320	239	279	898	1,180	1,570
3	None	250	5	5,790	364	220	225	1,097	1,460	1,260
4	KAc	0	7	6,300	320	190	346	809	1,120	871
5	KAc, IAA	25	25	6,960	248	232	272	1,010	3,290	945
6	KAc, IAA	250	27	7,660	120	129	244	648	4,690	707

For the pre-treatment 4.5 g samples of pea stem slices were incubated in 200 ml of solution containing CH<sub>3</sub>COOK,  $4 \times 10^{-4}$  M and IAA as indicated above for 3.5 hr at 24° C.

For the carbon 14-treatment the slices were incubated for 30 min in 50 ml of a solution containing  $1 \times 10^{-4}$  M CH<sub>3</sub>C<sup>14</sup>OOK, IAA as indicated above, and 0.9  $\mu\text{c}$  of activity per ml. All solutions were adjusted to pH 4.7 with KOH or H<sub>2</sub>SO<sub>4</sub>.

\* Values for pectic substances, hemicelluloses, water extract, lipides, organic acids and CO<sub>2</sub> for the IAA-treated tissues have been calculated on the basis of C<sup>14</sup>-absorption equal to that of the appropriate control. Values for the alcohol extracts serve as measures of the relative C<sup>14</sup>-absorption.

only one level of IAA was employed,  $25 \times 10^{-4}$  M, and this promoted growth in each of the pre-treatment periods. Exposure of the slices to acetate-1-C<sup>14</sup> was limited to 15 minutes.

Most impressive is the inhibition of incorporation into the pectic substances. This effect becomes progressively greater as the pre-treatment periods are extended and eventually activity in the control tissues exceeds that of the IAA-treated by more than 6 fold. As with other entities considered here incorporation of activity into the pectic substances of the control tissues shows a similar trend suggesting that the endogenous auxin of the slices affects their activities in a manner like that of the exogenously supplied IAA. Incorporation of activity into hemicelluloses, water extractable materials, and lipides is inhibited to a very appreciable degree by pre-treatment with IAA. Activities in the organic acids and in the CO<sub>2</sub>, except for the longest pre-treatment period, are much en-

hanced by the auxin. Each of these effects is clearly discernible in the tissues that have received only 15 minutes of pre-treatment. As in the previous experiment, certain effects seen in the tissues not given the pre-treatment are reversed in the pre-treated tissues. This is true for pectic substances and organic acids but not for the hemicelluloses, lipides and CO<sub>2</sub>.

In another experiment effects of pre-treatments with calcium acetate, CaCl<sub>2</sub> and KCl are compared (table IV). Indoleacetic acid is present at a concentration of  $5 \times 10^{-4}$  M in this experiment, and even at this high level promotes growth. It is seen, however, that this level of auxin inhibits absorption of radioactive acetate as indicated by the values for the alcohol extracts. These inhibitions range from 23 % for the KCl pre-treatment to 32 and 35 % for the calcium acetate and CaCl<sub>2</sub> treatments respectively. Auxin-inhibition of incorporation of activity into the alcohol extract, hemicelluloses, and water-extractable

TABLE III

UTILIZATION OF ACETATE-1-C<sup>14</sup> BY PEA STEM SLICES IN 15 MIN. EFFECT OF PRE-TREATMENT TIME. TOTAL ACTIVITIES.  $\times 10^3$  CPM \*

No.	PRE-TREATMENT MIN	IAA $\mu\text{m}/\text{l}$	FRESH WT % GAIN	ALCOHOL EXTRACT	PECTIC SUBST.	HEMI-CELLULOSE	WATER EXTRACT	LIPIDES	ORGANIC ACIDS	CO <sub>2</sub>
1	0	0	..	2,430	149	180	378	142	256	286
2	0	250	..	2,690	164	169	294	156	221	445
3	15	0	9	2,400	131	190	429	204	435	235
4	15	250	12	3,070	95	129	320	136	679	295
5	105	0	10	4,270	88	218	442	169	1,250	120
6	105	250	23	4,730	37	128	234	99	2,120	149
7	225	0	10	4,670	64	177	410	79	965	74
8	225	250	25	5,250	7	92	78	29	1,540	66

For the pre-treatment 3.25 g of pea stem slices were incubated in 200 ml of a solution of CH<sub>3</sub>COOK,  $4 \times 10^{-4}$  M and pH 4.7, for the periods indicated.

For the C<sup>14</sup>-treatment the samples were transferred to 50 ml of a solution containing  $1 \times 10^{-4}$  M CH<sub>3</sub>C<sup>14</sup>OOK, adjusted to pH 4.7 with H<sub>2</sub>SO<sub>4</sub> and having 0.9  $\mu\text{c}$  of activity per ml.

Concentrations of IAA indicated apply to both treatments.

\* Values for the pectic substances, hemicelluloses, water extract, lipides, organic acids and CO<sub>2</sub> for the IAA-treated tissues have been calculated on the basis of a C<sup>14</sup>-absorption equal to that for the appropriate control tissues. Activities of the alcohol extracts serve as measures of the relative C<sup>14</sup>-absorption.

TABLE IV

UTILIZATION OF ACETATE-1-C<sup>14</sup> BY PEA STEM SLICES IN 15 MIN. EFFECTS OF VARIOUS PRE-TREATMENT SOLUTIONS. TOTAL ACTIVITIES.  $\times 10^8$  CPM\*

No.	PRE-TREATMENT	IAA $\mu\text{m}/\text{l}$	FRESH WT. % GAIN	ALCOHOL EXTRACT	PECTIC SUBST.	HEMI-CELLULOSE	WATER EXTRACT	LIPIDES	ORGANIC ACIDS	CO <sub>2</sub>
1	None	0	..	1,360	68	79	426	313	157	984
2	None	500	..	1,360	81	101	265	338	176	646
3	Ca(Ac) <sub>2</sub>	0	6	1,470	13	15	88	52	309	169
4	Ca(Ac) <sub>2</sub>	500	24	1,000	9	2	24	38	389	584
5	CaCl <sub>2</sub>	0	7	1,980	62	51	230	343	271	205
6	CaCl <sub>2</sub>	500	20	1,290	7	9	46	105	581	688
7	KCl	0	11	5,070	41	49	207	173	303	30
8	KCl	500	27	3,900	6	68	61	118	371	40

For pre-treatment 3.75 g of pea stem slices were incubated 225 min at 24° C in 200 ml of (CH<sub>3</sub>COO)<sub>2</sub>Ca, CaCl<sub>2</sub> or KCl with IAA as indicated above. Each salt was used at  $5 \times 10^{-4}$  M and each solution was adjusted to pH 4.7 with Ca(OH)<sub>2</sub> (1 through 6), KOH (7 and 8), and HCl as needed.

For carbon 14-treatments 1 through 6 the slices were incubated 15 min in 50 ml of a solution containing (CH<sub>3</sub>C<sup>14</sup>OO)<sub>2</sub>Ca,  $6 \times 10^{-5}$  M, with IAA as indicated above and the pH adjusted to 4.7 as for the pre-treatment solutions, and having 0.54  $\mu\text{c}/\text{ml}$  of activity. Solutions for C<sup>14</sup>-treatments 7 and 8 differed in that CH<sub>3</sub>C<sup>14</sup>OOK was used at  $1 \times 10^{-4}$  M, 0.9  $\mu\text{c}/\text{ml}$ .

\* Values for pectic substances, water extract, hemicelluloses, lipides, organic acids and CO<sub>2</sub> of the IAA-treated tissues and No. 7 have been calculated on the basis of C<sup>14</sup>-absorption equal to that of the appropriate control. Treatments 1 and 2 are controls for 2 and 4 respectively, 5 is the control for 6, 7 and 8. Activities of the alcohol extracts serve as measures of the relative C<sup>14</sup>-absorption.

materials is significantly greater in the tissues pre-treated with calcium acetate than it is in those given CaCl<sub>2</sub>. It may be of greater importance, however, that the acetate ion itself profoundly inhibits syntheses of pectic substances, hemicelluloses, water-extractable materials and lipides. Enhancement of organic acid synthesis by IAA is greater in the CaCl<sub>2</sub> treatment, and the effect on the activity in the CO<sub>2</sub> is about equal in the two calcium pre-treatments. Perhaps the most interesting difference between the calcium pre-treatments and that with KCl relates to activity in the respiratory CO<sub>2</sub>. There is much less activity in the CO<sub>2</sub> produced by the KCl-treated tissues, and the enhancement of CO<sub>2</sub> formation from the acetate by auxin is far less for these tissues.

Auxin-effects on the slices used without pre-treatment differ from those of the previous experiment (table III) so far as two entities are concerned, or-

ganic acids and respiratory CO<sub>2</sub>. In the experiment of table III, where the added cation is potassium, IAA inhibits incorporation into organic acids and enhances it for CO<sub>2</sub>. In the experiment of table IV where calcium is the added cation the reverse occurs.

Effects of pre-treatment in KCl or CaCl<sub>2</sub> alone followed by exposure to radioactive acetate with and without IAA were determined in an experiment summarized in table V. No dramatic effects are to be seen in this experiment though some of the differences in activity appear to be significant. Absorption of acetate-1-C<sup>14</sup> is appreciably increased by IAA in the tissues pre-treated with KCl, but this is not true for the CaCl<sub>2</sub>-treated slices. Contrary to the effect one obtains when untreated slices are used, there is here a small inhibition of incorporation into the pectic substances by IAA. Inhibitory effects on lipide synthesis are of a similar order of magnitude. Incorporation

TABLE V

UTILIZATION OF ACETATE-1-C<sup>14</sup> BY PEA STEM SLICES IN 15 MIN. EFFECTS OF IAA AFTER PRE-TREATMENT WITH KCl OR CaCl<sub>2</sub> ONLY. TOTAL ACTIVITIES.  $\times 10^8$  CPM \*

No.	PRE-TREATMENT	IAA $\mu\text{m}/\text{l}$	ALCOHOL EXTRACT	PECTIC SUBST.	WATER EXTRACT	LIPIDES	ORGANIC ACIDS	CO <sub>2</sub>
1	KCl	0	4,580	52	302	788	2,610	289
2		250	6,330	48	239	671	3,380	256
3		0	6,480	92	387	860	4,130	253
4	CaCl <sub>2</sub>	250	6,750	78	369	780	3,690	219

For pre-treatment 4 g of pea slices were incubated at 26° C for 225 min in 100 ml of KCl or CaCl<sub>2</sub> at  $5 \times 10^{-4}$  M.

For the C<sup>14</sup>-treatment the tissues were incubated for 15 min at 26° C in 50 ml of a solution containing CH<sub>3</sub>C<sup>14</sup>OOK at  $1 \times 10^{-4}$  M, having 0.9  $\mu\text{c}/\text{ml}$  of activity and adjusted to pH 4.7 with HCl.

\* Values for pectic substances, water extract, lipides, organic acids and CO<sub>2</sub> of the IAA-treated tissues have been calculated on the basis of C<sup>14</sup>-absorption equal to that of the appropriate control. Activities of the alcohol extracts serve as measures of the relative C<sup>14</sup>-absorption.

ration of  $C^{14}$  into the organic acids is enhanced by IAA in the KCl treatment (30%) and inhibited in the  $CaCl_2$  treatment (11%).

### DISCUSSION

It is apparent from the pre-treatment studies that the utilization of acetate by pea stem slices is profoundly influenced by IAA. Two aspects of these experiments suggest reasons for the failure of studies involving simultaneous exposure to acetate- $1-C^{14}$  and IAA to reveal similar differences.

The first of these relates to the fact that in a number of the groups of compounds analyzed the responses to auxin in the 1st period of 15 minutes are the opposite of those observed in the 2nd period. This is true, for example, for experiment III with respect to incorporation in pectic substances, lipides, and organic acids. Incorporation for each is promoted in the 1st period and inhibited for each in the 2nd period after 15 minutes of pre-treatment with cold acetate and IAA. With simultaneous exposure to IAA and radioactive acetate one obtains an average of these opposing effects resulting in the appearance of no effect or the occurrence of a smaller one.

Secondly, it is well known that prolonged exposure to labeled metabolites commonly leads to a random distribution of the label. This effect is clearly indicated in tables II and III, in which results of exposures to acetate- $1-C^{14}$  of 30 and 15 minutes are reported. Appreciably smaller experimental effects are produced by the longer incubation in the labeled material. An even more random distribution appears to be characteristic of the experiment in which the tissues were in the radioactive solution for four hours (table I). As the label becomes more randomly distributed, syntheses of cellular constituents by processes not affected by auxin mask those that are.

It may be stated as a general summary of this study that IAA inhibits the incorporation of radioactivity from acetate- $1-C^{14}$  into pectic substances, hemicelluloses and lipides, but enhances incorporation into organic acids and  $CO_2$ . Competition between synthetic pathways utilizing the acetate appears to be concerned. Exogenously supplied IAA augments the rate at which acetate is drawn into pathways of acid metabolism and probably in this manner reduces the availability of the labeled substrate for syntheses of lipides and polysaccharides.

### SUMMARY

Two experimental techniques have been used in a study of the effects of IAA on the utilization of acetate- $1-C^{14}$  by pea stem slices. The first and less revealing technique involves the simultaneous presen-

tation of IAA and acetate- $1-C^{14}$  to the tissues. In slices so treated for four hours with calcium as the sole basic cation, IAA ( $2.5 \times 10^{-5}$  M) stimulates incorporation of activity into respiratory  $CO_2$  and organic acids and inhibits it for lipides. With the addition of KCl incorporation into the lipides is stimulated to a small degree but the other results are not appreciably different. With KCl and IAA at  $2.5 \times 10^{-4}$  M incorporation into the pectic substances is inhibited. A greater part of the study is concerned with the effects of pre-treatment with unlabeled potassium acetate or other salts plus and minus IAA, followed by brief exposure of the tissue to acetate- $1-C^{14}$ . A typical response to 105 minutes of pre-treatment and 15 minutes of  $C^{14}$ -treatment is a strong inhibition of incorporation into pectic substances, lipides and water extractable materials and a marked stimulation of incorporation into organic acids, and respiratory  $CO_2$  by IAA at a growth-promoting concentration of  $2.5 \times 10^{-4}$  M. In the presence of potassium salts the effect of IAA is much greater on organic acids than on  $CO_2$ ; with calcium the effect of IAA is about the same on these two entities. It appears that IAA promotes entry of acetate into pathways of organic acid metabolism and thereby reduces the availability of this substrate to other systems.

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