AUXIN TRANSPORT, AUXIN METABOLISM AND AGEING.

H. VEEN

Centrum voor Plantenfysiologisch Onderzoek, Wageningen

SUMMARY

Transport and metabolism of auxin have been studied in explants of *Coleus*.

An ageing period preceding a transport experiment causes a decrease in the amounts of radioactivity transported.

A pre-treatment with auxin during the ageing period maintains the transport on the original level.

The formation of various auxin metabolites is affected by the ageing process.

1. INTRODUCTION

The role of auxin as an abscission-regulating hormone has been investigated extensively. In bean explants the time of application of auxin directly affects the abscission rate (RUBINSTEIN & LEOPOLD 1963). The authors conclude from their experiments that there are two stages: a first stage in which the leaf abscission is inhibited by auxin, and a second in which abscission is promoted by the same auxin concentration. JACOBS (1968) discusses the reality of this second phase in relation to starvation-contamination problems. Jacobs urges a re-examination of the promotive effects of auxin in the second phase, as in sterile cultured *Coleus* explants no promotion of the abscission could be found, regardless of the position of the applied auxin (JACOBS *c.s.* 1965). It is, in general, assumed that the delaying effect of auxin on leaf abscission acts via a growth induction. As long as there is an elongation of the petioles no abscission will take place (JACOBS *c.s.* 1964).

When basipetal auxin transport decreases in aged explants (Rubinstein and Leopold's phase 2), it is likely that this will result in a suboptimal auxin concentration in the petiole tissue, which has as a consequence the speeding of the abscission. It is therefore valuable to gather data on auxin transport in these aged explants.

The relationship between petiole age and immobilization activity has recently been investigated (VEEN & JACOBS 1969). From an analysis of tissue extracts from petiole segments of increasing age it was concluded that young tissue immobilizes auxin to a lesser extent than older, more mature tissue does. No differences were found between mature and senescent tissue.

In the present paper attention will also be paid to the metabolic turn-over of auxin in fresh and in aged explants.

2. MATERIALS AND METHODS

Explants were taken from vegetative *Coleus rhenaltianus* plants, earlier described in detail by GORTER (1964). Two different types of explants were used; Group A: the explants comprised a node with 5 mm pieces of stem above and below the node and two petiole stumps of 5 mm each (see *fig. 1*); Groups B and C: the explants comprised a node with 8 mm pieces of stem above and below the node and two petiole stumps of 5 mm each. From the apical and basal stem parts in Groups B and C 3 mm were cut off after an ageing period of 17 hours. During this period plain agar blocks were applied to the apical and basal cut ends of the explants of Group B.

OSBORNE *c.s.* (1968) found an enhancement of auxin transport after a pre-treatment with auxin. Therefore the ageing period of 17 hours in our experiments was replaced by a period in which stable NAA in a concentration of 10^{-5} M was applied at the apical proximal cut end of the explant (Group C). Plain agar blocks were applied to the basal end of the explants of Group C. In all agar blocks, donor as well as receivers, a phosphate buffer (Na_2HPO_4-KH_2PO_4) of 0.01 M was used to control the pH (pH = 5.6). Transport experiments were carried out with naphthylacetic acid-1-^{14}C (NAA-1-^{14}C) with a specific activity of 8.27 mc/mmole, purchased from Radiochemical Centre, Amersham, England. Immediately after the explants were cut (Group A) or after an ageing period either without or with stable NAA (Groups B and C, respectively) NAA-^{14}C was applied in agar blocks to the apical proximal end of the explant, see *fig. 2*. Plain agar blocks were placed at the basal proximal ends. In these transport experiments the phosphate buffer was used in both donor and receiver blocks.

During the 17-hour ageing-period as well as during the 5-hour transport-period, the explants were placed horizontally in petri dishes on small foam-plastic cushions. The dishes remained in darkness at 20° C; 15–20 explants were used for each treatment. The purity of the NAA-^{14}C was checked by thin-layer chromatography. No impurities were present in the stock solution. After a 5-hour transport period the donor and receiver blocks were removed and count-

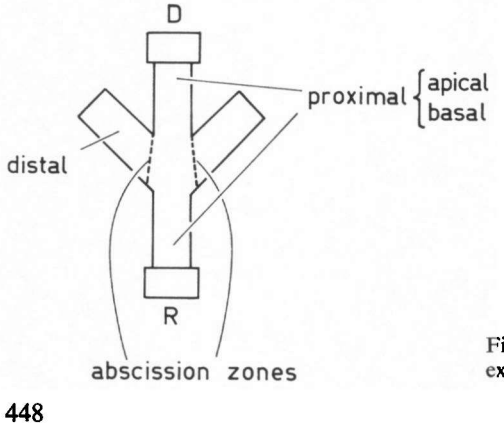

Fig. 1. Scheme of an explant used in the experiments.

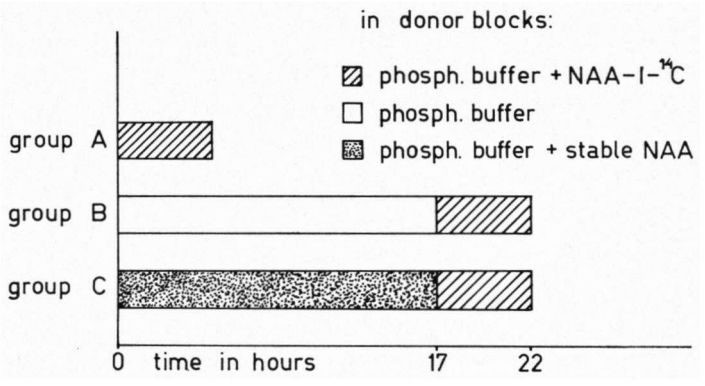

Fig. 2. Histogram representing the three different treatments within the experiments.

ed in a Nuclear-Chicago liquid scintillation spectrometer as described earlier (VEEN 1967). One minor deviation was introduced: the blocks were transferred into vials with 2 ml ethanol 50 per cent instead of vials with 2 ml water and 1 ml ethanol 96 per cent. After the transport period the tissue was frozen in dry ice and afterwards extracted with hot acetonitrile (70–80 °C) for about 8 hours. The final 12 ml extract was reduced in volume to precisely 5 ml. From this 5 ml extract a sample of 1 ml was taken in order to count the acetonitrile soluble material. The remaining 4 ml was evaporated to dryness and the residue was taken up in 0.5 ml acetonitrile and spotted onto thin-layer plates. Thin-layer chromatography on silica gel was used in combination with autoradiography and liquid scintillation counting to obtain a quantitative picture of the auxin metabolites in the acetonitrile-soluble fraction. This technique has been described in detail (VEEN, 1966, 1967). Quenching by chlorophyll was corrected by a procedure in which the channel ratio of an external standard was used.

Each experiment was repeated several times, and each treatment included 10–20 explants each. The data are presented as the mean values for each treatment with standard errors. Statistical methods follow SNEDECOR (1955). The significance of the difference between the treatments was checked by means of a t-test.

3. RESULTS

NAA-1-^{14}C was added to the donor blocks in two different concentrations, 10^{-5} and 10^{-6}M. The initial counts per minute in the donor blocks are indicated by D_0; D_5 and R_5 represent the radioactivity in donor and receiver blocks respectively after the 5-hour transport period. The difference between D_0 and D_5 is called net loss from the donor ($\triangle$ D). This value is expressed as a percentage of the initial amount. The radioactivity in receiver blocks is expressed as a percentage of $\triangle$ D. The total amount of radioactivity present in the acetonitrile tissue extract is expressed as a percentage of what has been lost from

the donor blocks minus the amount of radioactivity found in the receiver blocks.

The data for a typical experiment are presented in *table 1*. A comparison of the data for Groups A and B in *table 1* shows that an ageing period of 17 hours seriously decreases the amounts of ^{14}C transported basipetally. In all experiments a significant decrease in transport was observed ($P < 0.01$). In only one of four similar treatments was a significant difference found in the net loss from donor blocks.

Table 1. Transport of ^{14}C from NAA in freshly cut explants (Group A) and in aged explants (Groups B and C). During the ageing period of 17 hours auxin was applied to Group C at the concentration of 10^{-5}M. Transport period was 5 hours. Data are presented as counts per minute. Mean values of the different treatments with their standard errors are presented, showing the number of explants used in brackets. Transport experiments were carried out with two different donor concentrations, viz. 10^{-6}M NAA, $D_0 = 115 \pm 2$ cpm (10) and 10^{-5}M NAA, $D_0 = 1243 \pm 30$ cpm (10).

Treatment ageing period	Group A 0 hours	Group B 17 hours, no NAA	Group C 17 hours, with NAA
Donor Conc. = 10^{-6}M			
D_5	33 ± 2 (20)	30 ± 4 (15)	28 ± 2 (15)
$\triangle D$	82	85	87
% of D_0	71.3	73.9	75.6
R_5	22 ± 2 (20)	12 ± 1 (15)	19 ± 1 (15)
% of $\triangle D$	26.8	14.1	21.8
Tiss. extract	44	52	60
% of ($\triangle D$-R_5)	73.3	71.2	88.2
Donor Conc. = 10^{-5}M			
D_5	541 ± 22 (20)	343 ± 23 (15)	305 ± 20 (14)
$\triangle D$	702	900	938
% of D_0	56.4	72.4	75.4
R_5	59 ± 3 (20)	38 ± 3 (15)	80 ± 9 (15)
% of $\triangle D$	8.4	4.2	8.5
Tiss. extract	548	689	646
% of ($\triangle D$-R_5)	85.2	79.9	75.2

The data in Group C of *table 1* when compared with those of Group B indicate that a pre-treatment with auxin can fully compensate the decline of radioactivity in receiver blocks caused by the ageing. This experiment was repeated four times; in all cases a significant increase ($P < 0.01$) in amounts of auxin transported was observed. There were no significant differences between the net loss of radioactivity from the donor blocks in the different treatments. The total amounts of radioactivity recovered in the acetonitrile extracts are between 75 and 90 per cent of the total radioactivity present in the tissue. These values are much higher than those found earlier for IAA (VEEN & JACOBS 1969). This difference can be ascribed to the insensitivity of NAA to the action of an auxin-oxidase. No regular pattern between the recovery percentages of different treatments could be observed.

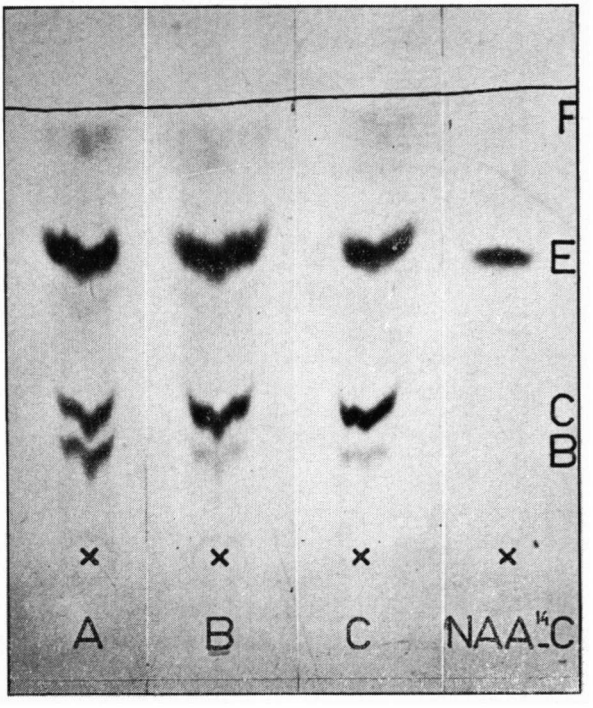

Fig. 3. A film plate, which had covered a thin-layer chromatogram, on which extracts from explants from Group A, B and C have been run.

Subsequently the metabolic turn-over of auxin in the tissue was studied. A film, which had covered a thin-layer chromatogram on which acetonitrile extracts of petiole segments of Groups A, B and C were spotted from a transport experiment with a donor concentration of 10^{-5}M is shown in *fig. 3*. The film shows the presence of at least four radioactive compounds on the thin-layer plate. Compound E has the same Rf-value as the control run of NAA-^{14}C and is apparently the auxin itself. Only one compound has been identified. Very probably compound C is identical with naphthylacetyl aspartic acid (VEEN 1966). The chemical nature of the other compounds has been postulated as follows (VEEN 1966):

Compound B: glucose ester of 8-hydroxynaphthylacetic acid, and F: naphthylacetyl-ß-D-glucose.

After removal of the film, the silicagel from areas with different Rf-values was scraped off and the radioactivity counted in the spectrometer. The activity present at different Rf-values is expressed as a percentage of the total amount of radioactivity chromatographed. The data are presented in *table 2*. They show that an ageing period of 17 hours preceding a transport period of 5 hours causes a decrease in the relative amounts of compound B, and a relative increase in compound C, whereas compounds E and F are still present in about the same amounts. This experiment was repeated with similar results. A pre-treatment with auxin during the ageing period caused a significant decrease of the relative amounts of compound E and an increase in the amounts of compound C.

Table 2. The relative amounts of various products of NAA metabolism at two different donor concentrations and after three different treatments. The data are given as percentages of the total amount of radioactivity chromatographed.
Group A: a 5-hour transport experiment without an ageing period; Group B: a 5-hour transport experiment after an ageing period of 17 hours; Group C: a 5-hour transport experiment after a 17 hours period in which stable NAA (10^{-5}M) was applied in the donor blocks. Donor concentration in the transport experiments was 10^{-5} and 10^{-6}M, respectively.

	Rf-values							
Area	0.0–0.05	0.05–0.15	0.15–0.25	0.25–0.36	0.36–0.58	0.58–0.72	0.72–0.86	0.86–0.98
Comp.			B	C		E		F
Donor Concentration 10^{-6}M								
Group A	0.0	0.0	7.3	15.0	3.8	68.5	1.7	3.8
Group B	0.0	0.0	1.6	24.5	3.5	60.5	2.7	7.2
Group C	0.0	1.0	2.3	41.2	4.3	46.1	2.5	2.6
Donor Concentration 10^{-5}M								
Group A	0.4	0.4	8.8	14.1	4.4	63.0	2.7	6.8
Group B	0.0	0.2	4.0	20.7	5.3	61.8	3.2	5.2
Group C	0.1	0.1	4.6	28.9	6.0	50.6	3.8	6.0

4. DISCUSSION

The data presented in this paper show that an ageing period of 17 hours is sufficient to cause a considerable decrease in the amounts of auxin transported. The results agree with those of OSBORNE *c.s.* (1968). These authors demonstrated a lesser movement of 2,4,5–tri-chlorophenoxyacetic acid-1-^{14}C (2,4,5-T) in aged bean explants compared with freshly cut explants. A substitution of plain agar blocks by blocks containing 2, 4, 5-T during the ageing period maintained the basipetal auxin transport on the original level, according to Osborne and co-workers. These results were obtained after distal application of the auxin, which means a basipetal transport. After proximal application of the auxin, ageing, or ageing in the presence of auxin, did not affect the movement of ^{14}C.

In our experiments presented in this paper only basipetal transport in the stem part of *Coleus* explants has been studied. There is, however, evidence that auxin transport characteristics are essentially the same for transport in stem parts or in petiole-stem parts of *Coleus* explants (GORTER & VEEN 1966).

It is not unlikely that the decrease in auxin transport in aged explants is one of the causal factors determining the speeding of abscission in Rubinstein and Leopold's second stage, as has been suggested earlier by Osborne and co-workers.

The similarity between transport of 2,4,5-T in aged bean and NAA in aged *Coleus* explants is striking. The question concerning the specificity of the observed phenomenon, however, cannot be answered yet.

Besides cytokinins auxins prevent ageing phenomena in isolated plant parts

(KARSTEN 1967). The restoration of auxin transport in aged explants which were pretreated with auxin must be considered as an expression of this effect.

Besides auxin transport, protein synthesis too is affected by an auxin pre-treatment during the ageing period. Osborne and co-workers gave data on the incorporation of ^{14}C-leucine into protein in aged bean explants and in explants which were pre-treated with auxin. It was shown that during the ageing period there is a fall in the rate of incorporation of labelled precursors in protein. This decline in protein synthesis can now be fully compensated by auxin application. Osborne and co-workers suggest that the polar transport system is regulated by the ability of the tissue to synthesize protein. Another phenomenon related to ageing is the change in the formation of auxin metabolites. Compound B is relatively less present in extracts from aged tissue than from freshly cut ones. A relative increase of compound C could be observed.

Earlier evidence has been given to identify compound C as naphthylacetyl aspartic acid (VEEN 1966).

SÜDI (1966) showed that the formation of the NAA-aspartate is inductive. This implies that a pre-treatment with stable NAA will cause an increase in the formation of this complex during the following 5-hour transport period. The data in *table 2* confirm the observations of Südi.

It is intriguing that in spite of the relatively lesser amount of "free auxin" in the tissue in aged explants pretreated with auxin, more auxin is transported into receiver blocks. It must be kept in mind, however, that there is no direct evidence that auxin transport and auxin metabolism are directly related. In recent years evidence has been given that auxin metabolism is independent of a physiological effect (i.e. inhibition of root growth, ANDREAE 1967). Andreae suggests that the site of inhibition lies external to the cytoplasma, either at the cell wall or cytoplasmic membrane.

HERTEL & FLORY (1968) showed that transport of auxin does not depend on the formation of a covalently bound complex. The authors assume that auxin transport and the primary action of auxin have one common critical step which might be located on the plasmalemma. It can be hypothesized now that a pre-treatment with auxin during an ageing period will result in two independent reactions: 1. stimulation of complex formation in cytoplasm and 2. stimulation of transport by influencing the characteristics of the plasmalemma structure. The observation of Osborne and co-workers that an auxin pre-treatment results in an increased protein synthesis might imply that in one of the reactions mentioned above, or in both, a protein synthesis is involved.

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